Lactic Acid Production from Kraft waste-pretreated Corn Cobs in Dairy Wastewater using *Lactobacillus plantarum* ATCC 14917: Process Modelling and Preliminary Scale-Up

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By

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BSc. Microbiology (Hons) (*cum laude*)

Submitted in fulfilment of the academic requirements for the degree of

Doctor of Philosophy

In Microbiology

School of Life Sciences

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Pietermaritzburg

South Africa

24 April 2024

PREFACE

The research contained in this thesis was completed by the candidate (Anthea Naomi David, 215025950) while based in the Discipline of Microbiology, School of Life Sciences, of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. The research was financially supported by the National Research Foundation (Grant number: 122080).

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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Signed: Professor Evariste Bosco Gueguim Kana (Supervisor)

Date: 24 April 2024

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Signed: Doctor Yeshona Sewsynker-Sukai (Co-supervisor)

Date: 24 April 2024

DECLARATION 1: PLAGIARISM

I, Anthea Naomi David, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) this dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) their words have been re-written but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

(vii) this dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

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Date: 24 April 2024

DECLARATION 2: PUBLICATIONS AND MANUSCRIPTS

This thesis represents a compilation of four published papers, two published book chapters and one manuscript (submitted). Details of contributions to publication that form part and/or include research presented in this thesis are provided within each publication and manuscript under the Credit Author Statement section. The student (Anthea Naomi David) contributed towards experimental work, data collection and manuscript preparation, under the guidance of Professor E.B. Gueguim Kana (Supervisor) and Doctor Y. Sewsynker-Sukai (Co-supervisor).

1. Sewsynker-Sukai, Y., David, A.N., Gueguim Kana, E.B., 2020. Recent developments in the application of Kraft pulping alkaline chemicals for lignocellulosic pretreatment: Potential beneficiation of green liquor dregs waste. *Bioresource Technology,* 306, 123225. (Chapter 2).
2. David, A.N., Sewsynker-Sukai, Y., Gueguim Kana, E.B., 2021. Development of Kraft waste-based pretreatment strategies for enhanced sugar recovery from lignocellulosic waste. *Industrial Crops and Products*, 174, 114222. (Chapter 4).
3. David, A.N., Sewsynker-Sukai, Y., Meyer, E.L., Gueguim Kana, E.B., 2023. Harnessing Artificial Neural Networks and large language models for bioprocess optimization: Predicting sugar output from Kraft waste-based lignocellulosic pretreatments. *Industrial Crops and Products*, 206, 117686. (Chapter 5)
4. David, A.N., Sewsynker-Sukai, Y., Gueguim Kana, E.B., 2022. Co-valorization of corn cobs and dairy wastewater for simultaneous saccharification and lactic acid production: Process optimization and kinetic assessment. *Bioresource Technology,* 348, 126815. (Chapter 6).
5. David, A.N., Sewsynker-Sukai, Y., Gueguim Kana, E.B., Novel Strategies for High-Efficiency Lactic Acid Production: Process Optimization and Scale-Up on Corn Cob

Waste and Supplemented Dairy Wastewater medium. Submitted to *Industrial Crops and Products* (Chapter 7).

6. Sewsynker-Sukai, Y., David, A.N., Laltha, M., Meyer, E.L., Gueguim Kana, E.B., 2023. Lignocellulosic biofuel production: Insight into microbial factories. In: Moodley, P., Ray, R.C., Gueguim Kana, E.B. (eds) Advances in Lignocellulosic Biofuel Production Systems. Woodhead Publishing. 203-228. (Appendix B).
7. Sewsynker-Sukai, Y., David, A.N., Rorke, D.C., Sanusi, I.A., Aruwajoye, G.S., Meyer, E.L., Gueguim Kana, E.B., 2023. Integrated biorefineries: The path forward. In: Moodley, P., Ray, R.C., Gueguim Kana, E.B. (eds) Advances in Lignocellulosic Biofuel Production Systems. Woodhead Publishing. 267-304. (Appendix C).

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Signed: Anthea Naomi David

Date: 24 April 2024

CONFERENCE CONTRIBUTIONS

1. David, A.N. Complete valorization of lignocellulosic and industrial wastes for lactic acid production: Process optimization, Scale-up and Kinetic assessment. College of Agriculture, Engineering and Science. Postgraduate Research and Innovation Symposium (PRIS). 8 December 2022. University of KwaZulu-Natal, South Africa. Oral Presentation (Online platform).
2. David, A.N. Complete valorization of lignocellulosic and industrial wastes for lactic acid production: Process optimization, Kinetic assessment and Artificial intelligence modelling. 19th International Symposium on Waste management and Sustainable landfilling (Sardinia symposium-Elsevier). 9-13 October 2023. Sardinia, Italy. Oral Presentation.
3. David, A.N. Complete valorization of lignocellulosic and industrial wastes for lactic acid production: Process optimization, Kinetic assessment and Artificial intelligence modelling. College of Agriculture, Engineering and Science. Postgraduate Research and Innovation Symposium (PRIS). 3 November 2023. University of KwaZulu-Natal (Coastlands Hotel), South Africa. Flash Presentation.
4. David, A.N. Development of Kraft waste-based pretreatments for simultaneous saccharification and lactic acid production in a minimally supplemented dairy wastewater medium: Process optimization, Scale-up and kinetic studies. College of Agriculture, Engineering and Science. Postgraduate Seminar. 8 November 2023. University of KwaZulu-Natal (PMB campus), South Africa. Oral Presentation.

Abstract

Microbial conversion of lignocellulosic biomass into value-added bioproducts like lactic acid (LA) is sustainable yet resource-intensive, characterized by low yields and costly operations. This study focused on the development of lignocellulosic LA bioprocesses through the valorization of agricultural, Kraft and dairy industrial waste. These wastes are low-cost, sustainable and discarded in abundance during raw material processing. Two complete waste-based pretreatment strategies: (1) steam-assisted combined GLD and PWW (SGLD-PWW), and (2) microwave-assisted combined GLD and PWW (MGLD-PWW) were developed and optimized using Response Surface Methodology (RSM) to enhance sugar release from corn cob waste (CCW). The CCW is non-food based, rich in carbohydrates, and geographically widespread. Artificial Neural Network (ANN) models were developed to predict glucose responses using experimental data from the Kraft waste pretreatment, followed by sensitivity analysis and comparative assessment with a Generative Artificial Intelligence (GAI) model like ChatGPT. Following pretreatment optimization, the Kraft waste pretreated CCW and supplemented dairy wastewater-based simultaneous saccharification and fermentation process (sDWW-SSF) was modelled and optimized using the RSM for LA concentration and conversion. The logistic and modified Gompertz models assessed the *Lactobacillus plantarum* ATCC 14917 cell growth and LA production kinetics for the: (1) supplemented DWW under SSF-microaerophilic (sDWW-$SSF_{microaerophilic}$), (2) supplemented DWW under SSF-anaerobic (sDWW-$SSF_{anaerobic}$), and (3) De Man, Rogosa and Sharpe medium modified with SGLD-PWW pretreated CCW instead of pure glucose under SSF-microaerophilic (mMRS-$SSF_{microaerophilic}$). Prior to scale-up, various buffer agents, pH changes, micronutrient supplementation and bioprocess types were evaluated for enhanced LA production and sugar utilization. Optimized conditions for LA production were assessed at 0.5 L with specific

mixing criteria: constant impeller tip speed (V_{tip}) and constant power input per unit volume (P/V), guiding subsequent scale-up to 5 L with kinetic analysis.

For the CCW pretreatment optimization, the SGLD-PWW (49.89% GLD, 118°C, 5 min) strategy resulted in a 32% and 40% higher reducing sugar and glucose yield, respectively, compared to the MGLD-PWW (48.70% GLD, 800 W, 9 min) strategy. The SGLD-PWW technology was thereafter selected for the SSF process optimization towards LA production.

The developed steam- and microwave-assisted ANN models showed high coefficient of determination (R^2) scores >0.95 for the observed and predicted glucose responses. Sensitivity analysis revealed high susceptibility to the stepwise variation in GLD concentration from 0% to 50% (>3.3-fold increase) and power intensity from 100 W to 900 W (>2.6-fold increase) in relation to its baseline value. Furthermore, the GAI model provided key insights that coincided with the study's contextual interpretations. These models offer a promising avenue to expedite labour-intensive wet lab experiments and enhance lignocellulosic pretreatment.

The optimized sDWW-SSF (25g/L CSL, 2 mL/L Tween 80 and 10% SL) process gave a LA concentration and conversion of 11.15±0.42 g/L and 18.90±0.75%, respectively. For the kinetic studies, the sDWW-$SSF_{microaerophilic}$ system observed slightly lower maximum specific growth rate (μ_{max}) (0.35 h^{-1}) and maximum potential LA concentration (P_m) (13.01 g/L g/L) values than the mMRS-$SSF_{microaerophilic}$ (μ_{max}= 0.64 h^{-1}, P_m= 14.01 g/L), but higher values than sDWW-$SSF_{anaerobic}$ (μ_{max}= 0.34 h^{-1}, P_m= 12.01 g/L). The negligible variations in the P_m values achieved for the sDWW-$SSF_{microaerophilic}$ system highlights its economic and resource-efficient attributes, mitigating reliance on complex media, freshwater and anaerobic conditions.

The SSF with $CaCO_3$ and MnO nanoparticle (sDWW-$SSF_{CaCO3(30)+MnO,\ pH5.5}$) achieved 31.12 g/L LA concentration and up to 46.27% sugar utilization at flask-scale. This contributed to a 64.17% (>2.7-fold) increase in LA concentration when paralleled to the sDWW-SSF system.

The 0.5 L bioreactor revealed 18.25% higher LA concentration and 40% reduced production time for constant P/V, conferring enhanced mixing efficiency in comparison to the constant V_{tip}. At 5 L scale with constant P/V, LA concentration peaked at 31.43 g/L with up to 43.55% sugar utilization, corresponding to 0.26 h^{-1} μ_{max} and 35.11 g/L P_m.

The major findings of this study underscore that leveraging waste residues from agricultural, Kraft, and dairy industries fosters interdisciplinary co-operation among these stakeholders for the comprehensive valorization of waste into high-value commodities. This strategy coincides with global sustainable development goals and effectively contributes to optimizing the food-energy-water (FEW) nexus. Thus, it reflects a tangible step towards achieving a circular bioeconomy and integrated framework for lignocellulosic bioprocesses, promoting environmentally friendly processes and economic viability.

Keywords: Artificial Intelligence modelling, Complete waste-based pretreatment, Kinetic modelling, Lactic acid production, Scale-up, Waste-based media formulation.

ACKNOWLEDGMENTS

All glory and honour to the One who is worthy, my Lord and Saviour Jesus Christ. For every good and perfect gift comes from Him. His abounding grace, mercy and love that surpasses all understanding has brought me through the most difficult parts of my life and has carried me through every storm when it seemed almost impossible to get through. My God is my ultimate source of strength and wisdom in everything I have accomplished.

To my supervisor Prof. Kana, your astounding knowledge, brilliant ideas and unending support has given me reason to believe in myself and has moulded my research capabilities. You have shown such humility and have always given importance to everyone in your research team regardless of the level of study. Thank you for trusting my judgement and making me feel as though I was capable enough. It was an honour to work under your supervision.

Yeshona, your caring nature, patience and guidance are more than I could have ever asked for. You have always tried your very best to motivate me when I felt like there was no light at the end of the tunnel. Your impeccable organization, attention to detail and knowledge in this field never ceases to amaze me. Your way around research has definitely shaped my thinking, research planning and writing style more than you will know. You have always guided me in not just my research but in many aspects of my life. You have surpassed all your roles as a supervisor, colleague and friend. Your insightful comments and knack for words have played a major role in the success of this thesis. It was an absolute pleasure to work with you and I could not have asked for a better mentor.

To Isaac, your assistance in the lab has been immeasurable. Thank you for always helping me even at the odd hours. I will carry your advice and motivation in research and life, always.

Many thanks to my Lab 117 colleagues for your advice and unconventional conversations that definitely livened up the dreary, unending days.

Pastor David and aunty Jane, your prayers have kept me afloat through my entire academic career and all of my life. For your unending love, blessings and constant check-ups, I am truly grateful!

To my family at Ephesus Gateway Church, thank you for your fervent prayers and encouragement.

To Shanel, your longstanding friendship is unmatched. I will always cherish our lunch dates that go on for hours since we always have way too much to say. Thank you for your support and love that I feel even when I don't see you as often. Salome, your friendship has surpassed all boundaries. Where would I possibly find a friend like you! I am eternally thankful for your unfailing support, encouragement, and pretty much everyday phone calls. You have always come to my aid whenever I needed you, especially those early mornings when I needed to be in the lab at literally the crack of dawn. To Naveshree, my sister/mother/friend. You play such a multifaceted role in my life. Thank you for always being in my corner.

To Kevalan, my knight in jeans and T-shirt, words cannot possibly express how much you mean to me. You have pushed me to be the best version of myself and stood by me in the toughest times. You've always been my number one supporter and believed in me when I didn't have the strength to believe in myself. You never complained to drive me to campus in the latest parts of the night or early mornings. You stayed with me through the nights in an uncomfortable, cold lab. Your belief in my abilities has kept me in line towards the goals I set for myself. You're always listening to my blabbering and never complain. You are simply the best friend I could have ever asked for. Your calm and realistic nature definitely balances out my impracticality. Thank you for being my person, the one whom my soul loves!

And most of all, my sincerest thanks to my family to whom I dedicate my every success. To my parents, brothers (Cameron and Dewayne) and their families, I would not have made it this far without your words of wisdom, encouragement and support even when I felt like my goals were unreachable. To my mummy, your love, Godly wisdom and spiritual counsel is by far the best gift you could have given me. Your strong faith in the Lord and your prayers have given me strength and carried me throughout my life and most especially when we experienced the greatest loss of our lives. Daddy, you are up in Heaven now, watching in the front row seat. You were a man of few words, but your nods of approval were definitely unmatched. Your words stuck like glue when you used to say, “This girl, what can she not do”. Those simple words have certainly pushed the boundaries of my achievements. Mum and Dad, I could not have done all of this without the foundations of your hard work and belief in my capabilities. From the inception of my academic career, you have been my scaffolding that I have always leaned on. Dad, how I long for you to be here to experience these moments with me.

Lastly, I would also like to acknowledge the National Research Foundation (NRF) for financially supporting funding this research and the University of KwaZulu-Natal for providing the facilities and necessary equipment to carry out this study.

This thesis is dedicated to Emmanuel Francis David, my daddy in heaven:

"It broke our hearts to lose you

But you didn't go alone

For part of us went with you

The day God called you home."

-Author unknown

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Note: This thesis represents a compilation of four published papers and two book chapters, whereby each chapter is an individual entity prepared as per the journals' specifications. Therefore, some repetition between chapters has been unavoidable.

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Chapter 7: Supplementary material

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LIST OF ABBREVIATIONS

FEW	Food-energy-water
LA	Lactic acid
PLA	Polylactic acid
LCB	Lignocellulosic biomass
GLD	Green liquor dregs
PWW	Paper wastewater
GL	Green liquor
BL	Black liquor
LAB	Lactic acid bacteria
SHF	Separate hydrolysis and fermentation
SSF	Simultaneous saccharification and fermentation
PSSF	Simultaneous saccharification and fermentation with prehydrolysis
MRS	De Man, Rogosa and Sharpe
CSL	Corn steep liquor
DWW	Dairy wastewater
RSM	Response Surface Methodology
CCW	Corn cob waste
TTA	Total titratable alkali
SL	Solid loading
EDTA	Ethylenediaminetetraacetic acid
AQ	Anthraquinone
SGL	Stimulated green liquor
SEM	Scanning electron microscopy

FTIR	Fourier transform infrared analysis
SGLD-PWW	Steam-assisted combined GLD and PPW
MGLD-PWW	Microwave-assisted combined GLD and PPW
MGLD-W	GLD in deionized water heated by microwave
SGLD-W	GLD in deionized water heated by steam
MW	Deionized water alone heated by microwave
SW	Deionized water alone heated by steam
MPWW	Paper wastewater alone heated by microwave
SPWW	Paper wastewater alone heated by steam
rsy	Reducing sugar yield
gy	Glucose yield
LAP	Laboratory analytical procedure
DNS	3,5-dinitrosalicylic acid
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
ADL	Acid detergent lignin
NREL	National renewable energy laboratory
F-value	Fisher-Snedecor distribution value
p-value	Probability value
R^2	Coefficient of determination
ANOVA	Analysis of variance
$sDWW\text{-}SSF_{microaerophilic}$	Supplemented DWW under the SSF bioprocess-microaerophilic
$sDWW\text{-}SSF_{anaerobic}$	Supplemented DWW under the SSF bioprocess-anaerobic
$mMRS\text{-}SSF_{microaerophilic}$	MRS medium modified with pretreated CCW under the SSF bioprocess-microaerophilic

X_0	Initial cell concentration
X_{max}	Maximum cell concentration
μ_{max}	Maximum specific growth rate
t_L	Lag time
$r_{p,m}$	Maximum lactic acid production rate
P_m	Maximum potential lactic acid concentration
rpm	Revolutions per minute
ADI	Arginine deiminase pathway
NAD	Nicotinamide adenine dinucleotide
NOX	NADH oxidase pathway
EMB	Embden-Meyerhof Parnas pathway
GAPDH	Glyceraldehyde triphosphate dehydrogenase
LDH	Lactate dehydrogenase reaction
AI	Artificial Intelligence
ANN	Artificial Neural Network
GAI	Generative Artificial Intelligence
BPS	Banana Pseudostem
LLM	Large Language Model
MAE	Mean Absolute Error
MAPE	Mean Absolute Percentage Error
MSE	Mean Squared Error
RMSE	Root Mean Squared Error
MLP	Multi-layered Perceptron
SE	Steam Explosion
BPNN	Back-Propagation Neural Network

MLR	Multiple Linear Regression
PLS	Partial Least-Square regression
BRNN	Bayesian Regularization Neural Network
SCGNN	Scaled Conjugate Gradient Neural Network
LMNN	Levenberg Marquardt Neural Network
ICP-OES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
TOC	Total Organic Carbon
NDIR	Non-Dispersive Infrared
sDWW-SSF$_{CaCO3(15)}$	sDWW-SSF supplemented with $CaCO_3$ (15g/L) at pH 4.8
sDWW-SSF$_{Na2HPO4.12H2O}$	sDWW-SSF supplemented with $Na_2HPO_4.12H_2O$ at pH 4.8
sDWW-SSF$_{KH2PO4}$	sDWW-SSF supplemented with KH_2PO_4 at pH 4.8
sDWW-SSF$_{NaHCO3}$	sDWW-SSF supplemented with $NaHCO_3$ at pH 4.8
sDWW-SSF$_{CaCO3(15)+MnO}$	sDWW-SSF supplemented with $CaCO_3$ (15g/L) and MnO at pH 4.8
sDWW-SSF$_{Na2HPO4.12H2O+MnO}$	sDWW-SSF supplemented with $Na_2HPO_4.12H_2O$ and MnO at pH 4.8
sDWW-SSF$_{NaHCO3+MnO}$	sDWW-SSF supplemented with $NaHCO_3$ and MnO at pH 4.8
sDWW-SSF$_{KH2PO4+MnO}$	sDWW-SSF supplemented with KH_2PO_4 and MnO at pH 4.8
sDWW-SSF$_{CaCO3(30)+MnO, pH 5.5}$	sDWW-SSF supplemented with $CaCO_3$(30 g/L) and MnO at pH 5.5
DWW-SSF$_{CaCO3(30)}$	sDWW-SSF supplemented with $CaCO_3$(30 g/L) at pH 4.8
sDWW-SSF$_{CaCO3, pH 5.5}$	sDWW-SSF supplemented with $CaCO_3$ at pH 5.5
sDWW-SSF$_{CaCO3+Mn2+, Mg2+, pH 5.5}$	sDWW-SSF supplemented with $CaCO_3$, $MnSO_4$, $MgSO_4$ at pH 5.5
sDWW-SSF$_{control}$	sDWW-SSF (no supplementation) at pH 4.8
sDWW-SSF$_{Mn2+ and Mg2+}$	sDWW-SSF supplemented with $MnSO_4$ (Mn^{2+}) and $MgSO_4$ (Mg^{2+}) at pH 4.8
sDWW-SSF$_{MnO}$	sDWW-SSF supplemented with MnO at pH 4.8

$sDWW\text{-}SSF_{pH\ 5.5}$	sDWW-SSF at pH5.5
sDWW-PSSF	Supplemented DWW under the PSSF bioprocess
$sDWW\text{-}PSSF_{CaCO3}$	sDWW-PSSF supplemented with $CaCO_3$ at pH 4.8
$sDWW\text{-}PSSF_{CaCO3+MnO}$	sDWW-PSSF supplemented with $CaCO_3$ and MnO at pH 4.8
$sDWW\text{-}PSSF_{PH\ with\ Tween\ 80,\ CaCO3}$	sDWW-PSSF supplemented with Tween 80 in prehydrolysis and $CaCO_3$ in fermentation at pH 4.8
$sDWW\text{-}PSSF_{PH\ with\ MnO,\ CaCO3}$	sDWW-PSSF supplemented with MnO in prehydrolysis and $CaCO_3$ in fermentation at pH 4.8
$sDWW\text{-}PSSF_{PH\ with\ MnO+Tween\ 80,\ CaCO3}$	sDWW-pSSF supplemented with Tween 80 and MnO in prehydrolysis and $CaCO_3$ in fermentation at pH 4.8
MDWW-SSF	sDWW-SSF supplemented with $CaCO_3$(30 g/L) and MnO at pH 4.8
V_{tip}	Constant impeller tip speed
P/V	Constant power input per unit volume
ROS	reactive oxygen species
MS	Malt sprout
rps	Revolutions per second
t_c	Circulation time
V_p	Pumping capacity
λ	Scale of turbulence

CHAPTER 1

General Introduction

1.1. Background

In an era of increasing environmental awareness and the urgent need for sustainable solutions, the focus on developing value-added bioproducts has gained significant momentum. These bioproducts offer a promising pathway to reduce dependence on non-renewable resources and address critical global challenges. The following discussion explores the importance of sustainable bioproducts, particularly lactic acid (LA), the innovative use of waste streams for bioproduct generation, and the optimization and scale-up methodologies that support bioprocess development.

1.1.1. The need for sustainable value-added bioproducts

Against the backdrop of a rapidly increasing global population that has surpassed 7.9 billion in 2022 and continues to grow to an estimated 9.6 billion by 2050 (International Energy Outlook, 2023), there is an escalating need to address the associated challenges of climate change, resource depletion and environmental degradation. This growing global concern has intensified the transition from a largely reliant non-renewable economy to one that encourages sustainable development, energy independence and effective management towards environmentally benign alternatives (Diaz et al., 2018). The food-energy-water (FEW) nexus (Figure 1.1) has exacerbated the global mandate to accelerate progress towards achieving sustainable development goals with the aim of alleviating macroeconomic, demographic and climatic pressures that are expected to rise in the short- and mid-term future (Bardazzi and Bosello, 2021). With current frameworks directed to single sector management, it is vital to identify and understand the interdependencies and interconnections among all three sectors in order to optimally manage the food, energy and water triad

(Valencia et al., 2021). In response to this pressing issue, the exploration of microbial-derived value-added bioproducts have emerged as a pivotal avenue of research. These bioproducts include biofuels (bioethanol, biodiesel, biohydrogen), biochemicals (lactic acid, itaconic acid, succinic acid) and biopolymers (polyhydroxyalkanoates, polylactic acid) among others (Diaz et al., 2018). Microorganisms, with their inherent ability to produce a diverse array of compounds through fermentation processes, present a promising solution to meet the global demand. As global systems strive to meet sustainability goals and address the challenges of climate change, the allegiance with microbial bioprocessing underscores a collective effort to forge a more stable and resilient future.

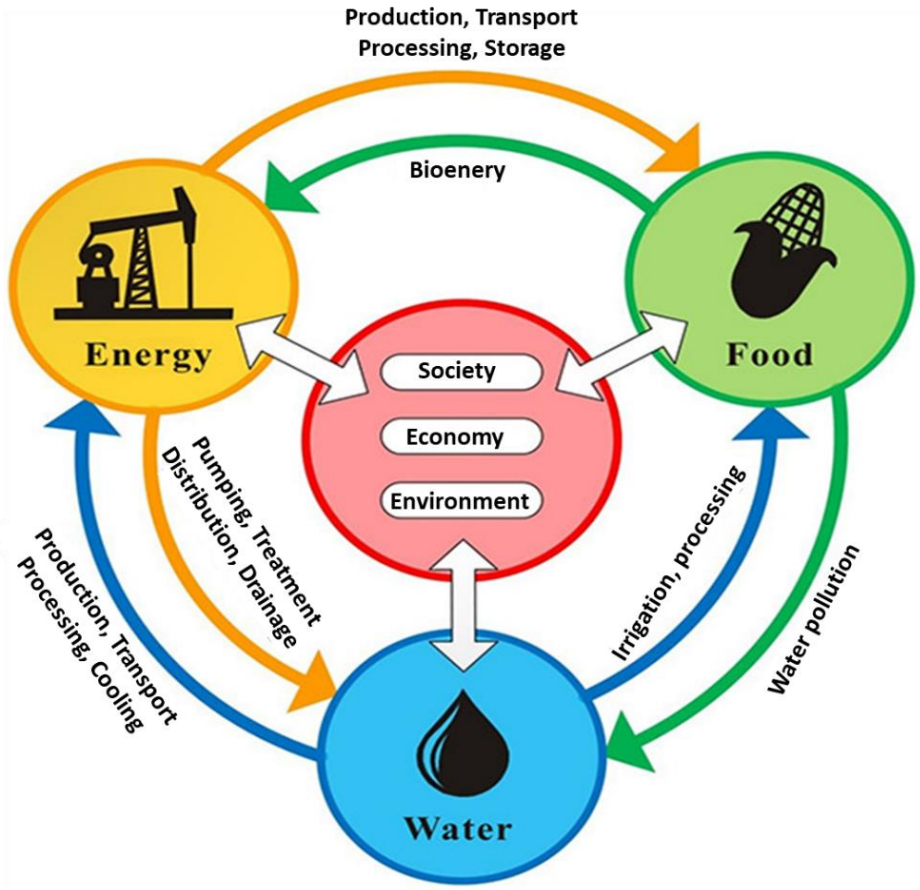

Figure 1.1. Overview of the interactions and interdependencies of the food-energy-water (FEW) nexus (adapted from Wen et al., 2021).

With a particular focus on compounds like LA, it has become a key player in this paradigm shift, holding significant potential as a versatile platform chemical with applications spanning from the food and pharmaceutical industries to biodegradable polymer production (Figure 1.2) (Nwamba et al., 2021). Biobased production of LA is preferred over chemical methods since it generates optically pure isomers (D-LA or L-LA), crucial for industries requiring specific isomeric forms. Unlike chemical synthesis, which produces a racemic mixture, necessitates costly enantio-separation and generates secondary waste, microbial fermentation is more cost-effective, environmentally friendly, and uses renewable resources, making it a sustainable and economically viable alternative (Abdel-Rahman et al., 2013). In order to support the growing demand for LA in industry, its overall projected annual revenue growth rate has significantly increased by 8.2% from 2024 to 2030 (Global Lactic Acid Market Size, Share and Trends Analysis Report, 2023) (Figure 1.3).

This discourse delves into the urgent need for sustainable microbially derived bioproducts, emphasizing the role of LA as a cornerstone in the development of green technologies, through its use in biodegradable materials and eco-friendly processes, thereby promoting circular bioeconomy within its niche. This focus is driven by the need to reduce reliance on fossil fuels, maintain food security standards and minimize environmental impact, making the adoption of a low cost, renewable, and non-food feedstock essential for the long-term supply of LA (Daful and Görgens, 2017). Recognizing the transition to green technologies, lignocellulosic biomass (LCB) stands out as promising substrates, offering both environmental and economic benefits. This sets the stage for investigating LCB as a key resource for microbially derived value-added products such as LA.

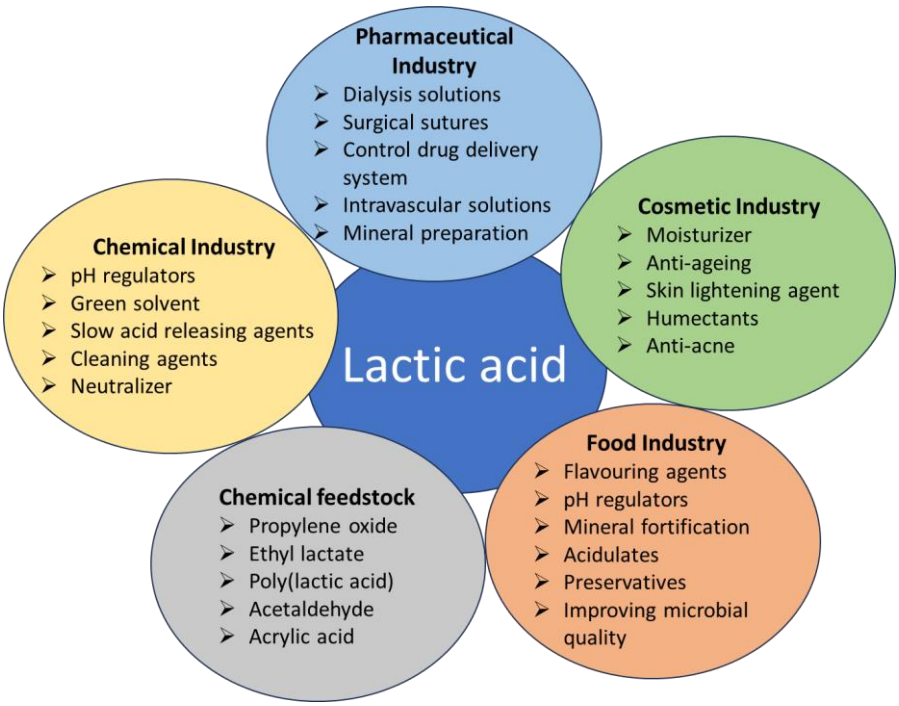

Figure 1.2. Biotechnological applications of lactic acid in various industrial sectors.

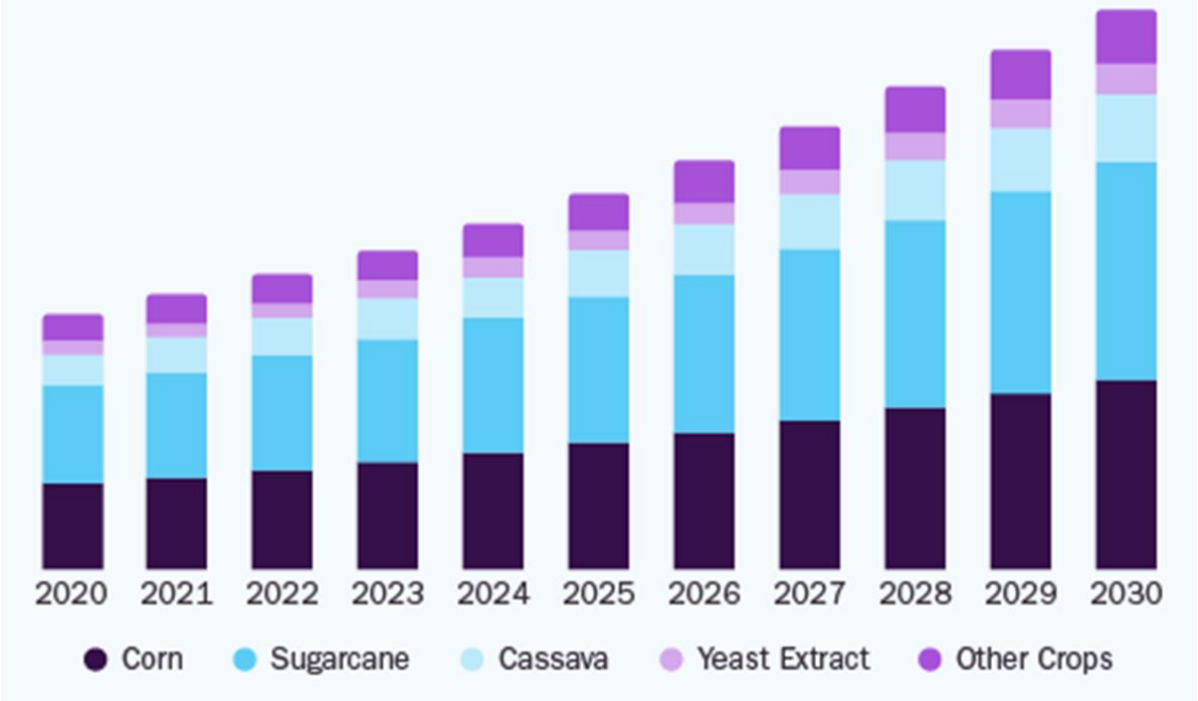

Figure 1.3. Global lactic acid production by raw material from 2020 to 2030 (adapted from the Global Lactic acid Market Size, Share and Trends Analysis Report, 2023).

1.1.2. Lignocellulosic biomass for value-added products

LCB serves as a prominent substrate over refined sugars from an economic and environmental point of view. In fact, its use also aids in land disposal reduction and valorization of waste residues for the production of marketable products without the risk of competing with food security (Bellasio and Sauer, 2015; Saini et al., 2015). Currently, LCB production stands at approximately 220 billion tons per annum, worldwide (Bansod et al., 2024), where only a small fraction has been used in non-food-based areas (Limayem and Ricke, 2012). Based on the input/output energy ratio, abundance and low cost (~50% lower), LCB is considered a superior substrate for commodity platform chemical production over other feedstocks such as starchy materials (FitzPatrick et al., 2010). Agricultural waste residues make up a major portion of LCB and these mainly comprise of corn, sugarcane, rice and wheat (Balan, 2014). Generally, the bulk of LCB consists of cellulose (38-55 wt%), hemicellulose (23-32 wt%) and lignin (15-25 wt%) with trace quantities of inorganic salts and extractive portions (resins, tannins, fatty acids) (McKendry, 2002). Some studies have explored lignocellulosic bioproduct generation and these include wheat straw (Cizeikiene et al., 2018), sugarcane leaf waste (Moodley and Gueguim Kana, 2019), giant reed (Jiang et al., 2020), rice straw (Chen et al., 2013), corn stover (Chen et al., 2020a), and corn cobs (Qiao et al., 2022), among others. Specifically, corn is one of the major cereal crops with an estimated global annual production of over 1 billion metric tons (USDA, 2024) and is used in the manufacture of human food, animal feed and products such as corn starch, adhesives and sweeteners (Ruan et al., 2019). The increased cultivation and exports of corn has led to the generation of non-food-based portions such as the leaves, husks, stalks and cobs, with the latter being the most abundant (Figure 1.4) (Sewsynker-Sukai and Gueguim Kana, 2018a). Corn cob waste (CCW) constitutes approximately 50% of the global corn production with a high energy density (4960–5210 MJ/Kg), rich carbohydrate content (38-45% cellulose, 26-35

% hemicellulose) and low lignin content (8-19%) (Potumarthi et al., 2012; Kim, 2018). Previous studies have demonstrated the microbial production of LA, bioethanol and biohydrogen among others, using CCW (Table 1.1). Nevertheless, lignocellulosic material such as CCW are protected by an amorphous matrix of hemicelluloses and lignin that is responsible for its highly recalcitrant structure (Daful and Görgens, 2017). The glucose rich polymer, cellulose, is embedded within the crystalline matrix, rendering enzymes inaccessible for hydrolytic attack. Lignocellulosic pretreatment is a crucial step in altering such properties in order to improve the amenability of enzymes to the cellulose microfibrils and hemicellulose matrices for microbial bioprocessing (Figure 1.5) (Kim et al., 2015).

Figure 1.4. Annotated image of the (A) corn plant (Adapted from Steil, 2019; modified) and (B) corn cobs (Adapted from Lovelyn, 2020; modified).

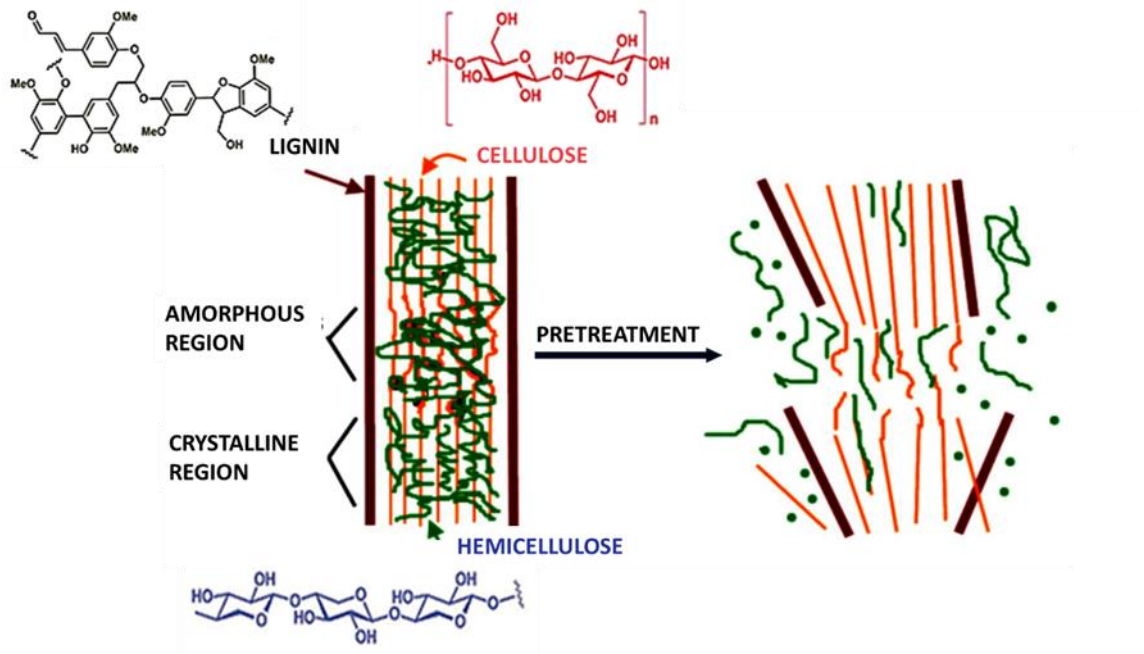

Figure 1.5. The effect of pretreatment on lignocellulosic biomass (Adapted from Muley and Boldor, 2017; modified).

Table 1.1. Microbial fermentation for biofuel and commodity chemical production using corn cob waste.

Bioproduct	Reference
Lactic acid	Wang et al. (2023)
Xylitol	Baptista et al. (2020)
Polyhydroxybutyrate	Stoica et al. (2018)
Biohydrogen	Li et al. (2022)
Biobutanol	Shanmugam et al. (2019)
Bioethanol	Sokan-Adeaga et al. (2024)
Malic acid	Li et al. (2021)
Butyric acid	Fu et al. (2022)

1.1.3. Current trends in lignocellulosic pretreatments and their limitations

Numerous technologies have been developed in an effort to mitigate the limitations of lignocellulosic waste-based commodity chemical production with minimal capital investments and increased product outputs. Pretreatment is the most crucial step that accounts for 40% of the total production expenses and sets the path for high yields, improved downstream applications and reduced capital costs (Saravanan et al., 2023). Various lignocellulosic pretreatment methods have been established and these include physical (grinding, steam explosion, microwave), biological (enzymes, microorganisms), chemical (acid, alkaline, inorganic salts) and combinations of these techniques (Chakraborty et al., 2024). Overall, strategies with the inclusion of chemicals display the highest efficacy since it has the ability to recover more than 75% of fermentable sugars as opposed to pretreatments employed without chemicals (Vasco-Correa and Shah, 2019). However, these pretreatment strategies are plagued by high energy requirements, undesirable inhibitor compounds and excessive costs due to the chemicals used, heating mechanisms, equipment and reactor vessel compatibility. Furthermore, with water being a finite resource, the water footprint within bioprocessing units have majorly impacted the sustainability and economic viability of lignocellulosic pretreatments (Kumar et al., 2017). As a result, the selection of a suitable pretreatment method using renewable and sustainable raw materials is imperative to enhance high sugar yields, minimize inhibitory compounds, reduce costs of post pretreatment operations and implement simple yet efficient reactor designs.

Recently, the use of chemical waste residues from industrial streams have been proposed as potential lignocellulosic pretreatment catalysts due to its economic and environmental benefits. The Kraft pulp and paper industry is one such processing unit that is highly established in the global market. These production plants generate large amounts of pulp with excessive waste in the form of green liquor dregs (GLD). According to Kinnarinen et al.

(2016), for every ton of pulp produced, the total annual production of GLD ranges approximately between 4 to 11 kg. Based on the characterization of GLD, it possesses alkaline species (Na_2CO_3 and Na_2S) that may represent an interesting replacement to conventional, expensive alkali-based pretreatments such as NaOH (Golmaei et al., 2018). Usually, GLD is landfilled after the Kraft paper and pulping process, as it contains high concentrations of calcite that remains in the filter lines causing blockages and hydraulic conductivity issues (Golmaei et al., 2018). Therefore, the retrieval of GLD from the Kraft paper and pulp waste lines (Figure 1.6) towards lignocellulosic pretreatment units may help overcome the large expenses attached to pretreatment chemicals, while relieving this industry of disposal costs. GLD consists of an array of elements and compounds that confer variable impacts on the chemical and structural characteristics of the biomass during pretreatment (Cheng et al., 2010; Gu et al., 2013). In particular, the combination of alkalic salts (Na_2CO_3 and Na_2S) aims to cleave the ester and glycosidic bonds within the cell wall matrix, engage in lignin rearrangement and modify the crystalline state of cellulose (Cheng et al., 2010; Geng et al., 2014). While using these individual alkalic salts (Na_2CO_3 or Na_2S) in lignocellulosic pretreatments is considered to be as effective as strong alkaline chemicals such as NaOH, it is important to note that a combination of alkalic salts (present within GLD) demonstrates a synergistic effect. This synergistic effect potentially surpasses the pretreatment outcomes achieved using solely NaOH (Rorke et al., 2021). In terms of the physical characteristics of GLD and its problematic disposal, many studies have focused on repurposing GLD to produce high value products. Examples of repurposing GLD include its use in fertilizers (Mäkelä et al., 2012), alkaline sealing barriers for acid mine drainage (Moyo et al., 2023), substitutes in cement products (Srivastava et al., 2024), and neutralizing agents (Cabral et al., 2008) among others. However, as it stands there are very few reports that have assessed the efficiency of GLD as a pretreatment catalyst (David et al., 2020; Rorke et al., 2021). Our

recent study provided a baseline on the development of a GLD pretreatment strategy for improved sugar release from CCW using fresh water (David et al., 2020) (Appendix A). Following our previous study on GLD (David et al., 2020), Rorke et al. (2021) optimized a surfactant-assisted GLD pretreatment for enhanced digestibility of paper mill sludge. Findings from both studies demonstrated the potential of GLD as a pretreatment alternative (David et al., 2020; Rorke et al., 2021).

Apart from the chemical cost in the lignocellulosic biorefinery production system, the water performance has also been considered to significantly impact the operations. Water is known to strongly influence the efficiency of thermochemical conversion and delignification of LCB within pretreatment units (Pelaez-Samaniego et al., 2024). Its extensive use raises concerns regarding the cost and sustainability within biorefinery processing systems. To resolve this, the use of industrial wastewater streams have been proposed to be coupled with pretreatment chemicals in order to omit the use of freshwater streams. One such abundant wastewater stream is that of the Kraft paper and pulp industry, which generates approximately 40% of the global industrial wastewater (Figure 1.6) (Toczylowska-Maminska, 2017). Therefore, the proposed concept of using the paper wastewater (PWW) effluent from the Kraft industry intends to manipulate every aspect of waste in the aim of transforming these materials to highly valuable commodities while decreasing production cost and alleviating the current water crisis. However, for optimal efficiency in this transformation, a focused emphasis on process optimization becomes indispensable. By strategically refining and enhancing each stage of the bioprocess, it does not only ensure resource efficiency but also unravels the full potential of this innovative “waste to wealth “concept, thereby contributing to sustainable and economic development.

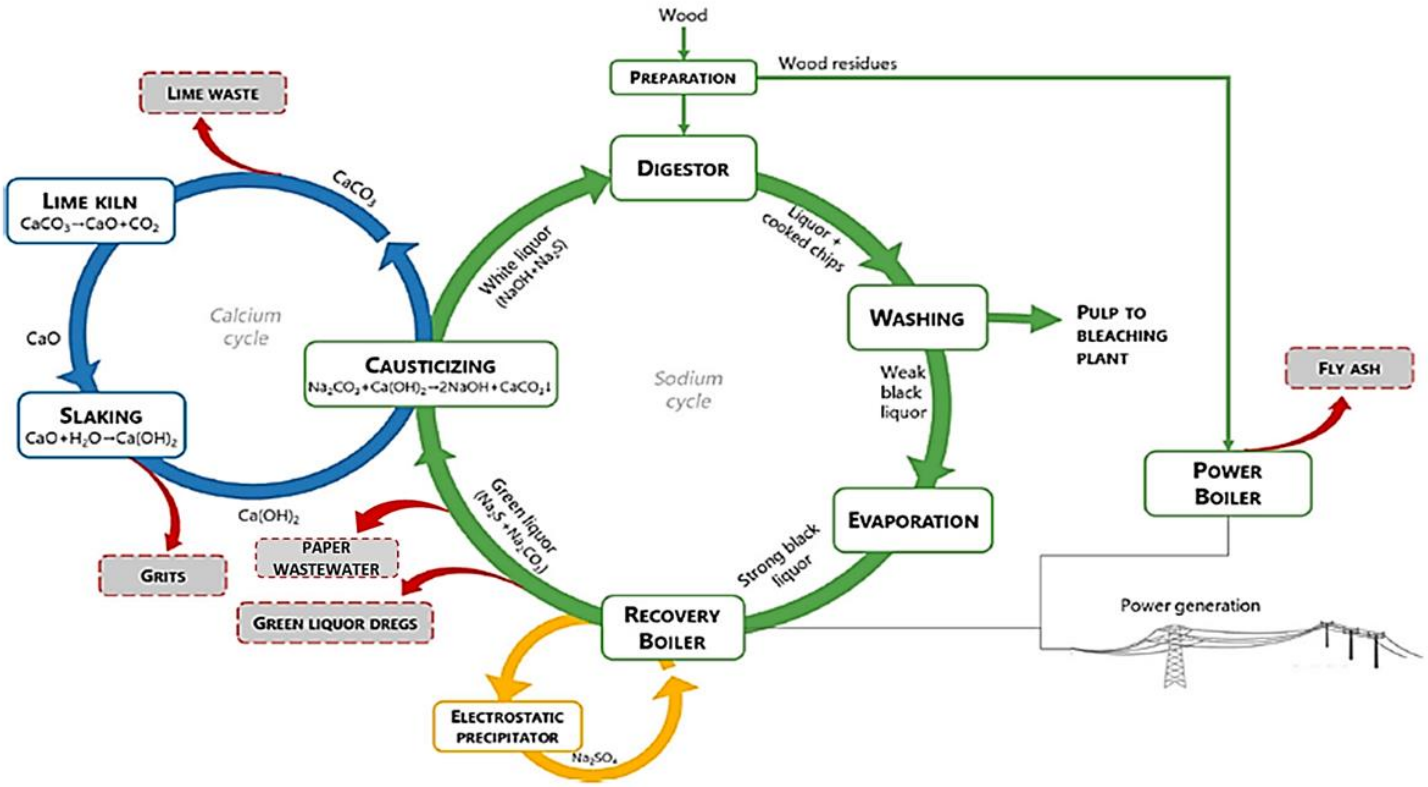

Figure 1.6. Overview of the Kraft paper and pulp chemical process (Adapted from Quina and Pinheiro, 2020; modified).

1.1.4. Artificial Intelligence modelling for lignocellulosic bioprocessing

Transitioning from the intricate details of LCB pretreatment, the pivotal role of Artificial Intelligence (AI) modelling tools such as Artificial Neural Networks (ANN) comes to the forefront in navigating the complexities of this process. Recognizing the need for a sophisticated modelling approach to optimize and predict the outcomes of Kraft waste-based pretreatments, ANNs stand out as a formidable solution due to their capacity to capture non-linear relationships and discern intricate patterns within vast datasets, positioning them as invaluable tools in the scope of sustainable bioprocessing (Sindhu et al., 2016). Unlike Response Surface Methodology (RSM) and other traditional statistical models, which are typically constrained by assumptions of linearity and require the specification of fixed relationships between variables, ANNs operate with strong adaptability and fault tolerance

(Ramírez-Brewer et al., 2024; Cheng et al., 2023). This flexibility enables ANNs to capture and process intricate, multidimensional interactions inherent in bioprocess systems without the need for predefined models or linear assumptions (Cheng et al., 2023). Consequently, ANNs deliver superior predictive accuracy and robustness, especially in scenarios where the complexity of the data challenges conventional modelling techniques. Their application not only enhances the precision of bioprocess optimization but also contributes to the development of more efficient and sustainable processes by effectively navigating the multifaceted variables involved in lignocellulosic bioprocessing systems. Moreover, the inclusion of sensitivity analysis in model development is essential for unravelling the key variables influencing the pretreatment outcomes. This analytical technique allows for a systematic exploration of the model's sensitivity to input variations, providing valuable insights into the relative importance of different parameters (Chen et al., 2020b). Interestingly, integrating Generative Artificial Intelligence (GAI) such as ChatGPT interpretation into the model development process adds a layer of knowledge that aids in bridging the gap between complex computational models and human understanding (Ray, 2023). GAI relies on deep learning techniques and neural networks to generate content that closely resembles human-generated outputs. By translating the sophisticated patterns, structures and computational outputs into accessible language, GAI systems contextualize and simplify model predictions, making them more understandable (Mathew, 2023). This capability facilitates decision-making, enhances communication among stakeholders, and ensures that the knowledge embedded in intricate models are not just confined to technical experts but can be broadly applied across various domains (Ray, 2023). In this way, GAI tools contribute to a more inclusive and effective utilization of advanced computational models, enhancing their practical impact and accessibility. This holistic approach not only contributes to the advancement of sustainable bioprocessing but also ensures the transparency

and interpretability of the developed models, fostering confidence in their real-world application.

1.1.5. Lignocellulosic lactic acid production: Bioprocess optimization, kinetic studies and scale-up

Lignocellulosic LA production is still in the infancy stage within the global markets, due to its reduced economic viability and low process efficiency as well as the ongoing challenges associated with scale-up processes. Its production presents many technological issues that occur at different stages of bioprocessing which hinders its large-scale implementation. One such outlook surrounding production is the choice of the LA producing microorganism, known as LABs and its specific strains. These selections are based on its enantiomeric configurations, desirability in industrial applications, high acid tolerance, enhanced yield and productivity, amenability to genetic engineering as well as nutritional requirements (Abedi and Hashemi, 2020; Macedo et al., 2020). LABs carbon metabolism drives homofermentative or heterofermentative pathways, with homofermentation preferred for its high optical purity (>99%) and yield whereby monosaccharides are converted almost solely to LA (theoretical yield of 1 g/g) (Martinez et al., 2013; Abedi and Hashemi, 2020). The respiratory needs of LABs can also influence their metabolic pathways, with anaerobic conditions favouring glycolysis for LA production (Sano et al., 2020). In contrast, aerobic conditions activate the minimal electron transport chain to promote a high energy metabolism for increased biomass growth and replication (Smetanková et al., 2012; Sano et al., 2020). Due to the mixed respiro-fermentative metabolism whereby microorganisms can switch metabolic routes upon availability of oxygen, understanding these mechanisms and oxygen requirements is crucial for designing efficient industrial-scale LA bioprocesses.

LABs require exogenous sources of carbon, nitrogen, phosphorus, vitamins and minerals for growth, due to their lack of several biosynthetic pathways (Chen et al., 2020c). Therefore, the fermentation medium should be specifically designed according to the nutritional requirements. Generally, LAB cultivation uses the commercial De Man, Rogosa and Sharpe (MRS) media since it contains three nitrogen sources (meat extract, peptone and yeast extract) along with several other micro and macro-nutrients (Chen et al., 2020c). Nevertheless, standard media components for LA fermentation contribute approximately 30% to the production costs (Tang et al., 2013). Therefore, intricate details about the nutritional requirements of LABs and the restrictions in the biosynthesis pathways due to unavailability of certain constituents is of utmost importance in order to mimic their natural niche (Van Niel and Hahn Hagerdal, 1999).

The LA fermentation medium should be economically practical, and productivity must not be affected as this could offset any cost reduced by medium optimization. The carbon and nitrogen sources within the fermentation media are valuable components that control the biosynthesis of LA. Nitrogen plays a vital role in synthesizing amino acids, proteins, and nicotinamide adenine dinucleotide (NAD), essential for cell functioning (Reitzer, 1987). Several cost-effective nitrogen sources, including soybean meal, ammonium chloride, urea, and corn steep liquor (CSL), have been explored as alternatives to traditional MRS media components (Thongchul et al., 2010; Liu et al., 2010; Li et al., 2016). CSL stands out for its rich organic nitrogen content and ability to replace yeast extract in LA fermentation. CSL not only provides essential nutrients but also offers economic feasibility by accounting for about one fifth of the cost of yeast extract (Tan et al., 2016). Another component of interest is the carbon source as it is the main energy substance selectively utilized by the microorganism and plays a key role in LAB metabolism, conferring growth and LA formation (Gäenzle, 2015). LCB has been categorized as a high energy, renewable, abundant substrate and its use

in industrial bioprocessing may address several economic and environmental implications. Even though nutritional supplementation in media formulation is imperative within fermentation processes, an avenue for its uptake into microbial cells is also extremely important. Tween 80, a non-ionic surfactant is a constituent in standard MRS media that is known for its association with microbial cell membrane permeability for enhanced penetration of nutrients into the cell (Taoka et al., 2011). Moreover, Tween 80 shows promise of multi-fold activities with regards to emulsification and improved enzymatic saccharification of lignocellulosic materials (Taoka et al., 2011; Zhang et al., 2018). In addition to the minimal media formulation, the high-water consumption also requires attention in bioprocessing units. As a result, several attempts have been made to either reduce water usage or omit it completely from the fermentation system. In light of these advancements, the dairy industry has been brought to the forefront due to the generation of large quantities of dairy wastewater (DWW) (~ 0.2-10 L of wastewater/L of processed milk) (Coelho et al., 2020). These wastewater streams are subjected to costly, energy exhaustive and environmentally non-viable treatments prior to disposal (Gogoi et al., 2021). Therefore, instead of costly treatment processes prior to polluting water bodies with DWW, creating an alternative path for application by substituting fresh water with DWW in fermentative processing may present a possible breakthrough for sustainable and economic development.

The aforementioned elements provide the foundational framework for minimally supplemented LA media formulation. However, it is crucial to delve into various physicochemical factors such as the effect of buffer agents, pH changes and micronutrient supplementation in addition to their intricate interactions to comprehensively understand and optimize the LA fermentation process. Buffer agents are crucial for LA fermentation since it maintains the overall pH stability within the optimal range of pH 4.5 to pH 6.5 for LAB growth and metabolic activity (Anagnostopoulou et al., 2022). Various compounds like

K_2HPO_4, $NaHCO_3$, $Na_2HPO_4 \cdot 12H_2O$, Na_3PO_4, and $CaCO_3$ serve as buffering agents to counteract excessive acidification caused by the production of LA. Validation of selected buffers through laboratory-scale experiments is essential prior industrial-scale applications. Micronutrient supplementation, particularly with $MgSO_4$ (Mg^{2+}) and $MnSO_4$ (Mn^{2+}), plays a crucial role in enhancing bacterial growth, biomass production, and overall LA fermentation performance. These micronutrients act as cofactors, boosting enzyme catalytic activity in biosynthetic pathways and improving enzyme-substrate binding affinity in carbohydrate metabolism (Yu et al., 2008, Lew et al., 2013). Apart from the abovementioned supplementations, nanoparticles such a manganese oxide (MnO) present ongoing research exploring novel possibilities for precise and efficient delivery of essential elements to LABs and improved efficiency in LA bioprocessing. Characterized by their nanoscale properties, nanoparticles offer high surface-to-volume ratios and unique surface features, facilitating enhanced adsorption and activation of reactant molecules (Sanusi et al., 2020).

LA production can be achieved through three bioprocess types: (1) separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and prehydrolysis with SSF (PSSF) (Carrillo-Nieves et al., 2017). Amongst these bioprocess types, the SSF process is being scrutinized as a viable option over the SHF process attributable to its reduced production costs, omission of separate saccharification steps, reduced risk of contamination and shorter fermentation times. The SSF process combines both the enzymatic hydrolysis and fermentation stage in a single bioreactor and enables a common working temperature for both processes while reducing the number of unit operations (Sewsynker-Sukai and Gueguim Kana, 2018b). Meanwhile, the PSSF bioprocess acting as an intermediate system, offers a compromise on the strengths of both the SHF and SSF processes, resulting in a shorter prehydrolysis step (6-24 hr) with increased saccharification efficiency due to the optimal temperatures (50°C) than the SHF followed by fermentation. Despite these advantages, the

prehydrolysis stages still necessitate a longer process duration and higher energy input compared to the SSF process, thereby diminishing its economic viability. Resultantly, SSF without prehydrolysis has been estimated to reduce the capital investment by more than 20% (Wingren et al., 2003). At the outset, this capital investment reduction already places the SSF process in a positive light given high costs associated with lignocellulosic LA production. However, the impact of the SSF and PSFF processes in terms of yield output, productivity, energy, and cost consumption remains a matter of debate (Zhu et al., 2015; He et al., 2016). Thus, emphasizing the integral role of bioprocess selection in optimizing and modelling the SSF and PSSF processes for LA production efficiency is imperative.

Based on the abovementioned components, studies have sparsely engaged in the optimization of a waste-based media formulation consisting of a nitrogen, carbon, and surfactant source for simultaneous saccharification and LA production. Response Surface Methodology (RSM) models are statistical analysis tools that use experimental designs and exploratory data to predict process outputs in relation to the input parameters presented (Kucharska et al., 2018). Like bioprocess type and optimization, kinetic modelling has been shown to play a fundamental role in predicting the behavioural patterns of microorganism growth and product formation for the bioprocess development trajectory towards scale-up. Kinetic models gather information from the experiments observations and quantitatively describe the systematic occurrences in response to changes in fermentation conditions. For instance, the logistic model is a substrate independent growth model that evaluates the changes in the microbial cell growth as a function of the growth rate, initial and maximum concentrations over time (Muloiwa et al., 2020). On the other hand. the modified Gompertz model has the ability to determine the production lag time, maximum production rate and maximum production concentration on a given substrate (Dodić et al., 2012). The predicted responses of these models assist in (i) enhancing product yield and productivity, (ii) managing resource

utilization, (iii) reducing undesired by-product formation, and (iv) minimizing cost and time, all while maintaining high product purity for industrial scale transformation (Kucharska et al., 2018).

When scaling up a bioprocess from flask to reactor, efficient mixing is vital for homogeneous nutrient distribution, gas dispersion, and microorganism dispersion with significant impact on mass transfer rates, heat transfer, and reaction kinetics (Bisgaard et al., 2021). Examining mixing regime parameters like constant power input per unit volume (P/V) and constant impeller tip speed enable scalability potential, enhances productivity, yield, and product quality. Constant P/V measures energy efficiency, informing reactor design for improved industrial-scale bioprocessing, while constant V_{tip} influences mixing, transfer processes, substrate utilization, and cell shear stress. Optimizing these parameters during scale-up is critical to maintain optimal process conditions, temperature regulation, and microbial integrity throughout the bioreactor.

1.2. Problem statement

LA is a simple hydroxy carboxylic acid that has been used as a precursor to generate multiple intermediate and commodity chemicals in the food, chemical, pharmaceutical and cosmetics industries (Choi et al., 2024). Therefore, its increasing demands coupled with economic growth pressures and environmental responsibility have stimulated the search for renewable and sustainable production strategies for LA. Technologies that produce LA from lignocellulosic derived sugars have gained attention as a result of stable feedstock supply, low cost and reduced carbon footprint (Raj et al., 2022). Despite LCB being a promising feedstock for biorefineries, its rigid and complex structure hinders its enzymatic bioconversion and microbial fermentation, thus hampering economic viability. To address

this issue, cost-effective pretreatment methods are being explored while addressing issues of high costs and resource consumption. More specifically, the application of Kraft paper and pulp waste such as GLD and PWW for pretreatment of CCW have been scantily reported in literature. Therefore, in an effort to develop a low cost and sustainable pretreatment method, a combined GLD and PWW pretreatment using two different heating mechanisms (microwave and steam) have been proposed.

With the imperative for kraft waste-based pretreatment of CCW, another crucial facet lies in comprehending the complex dynamics of glucose responses under various unique pretreatment conditions. To address this challenge, advanced modelling techniques such as ANN, sensitivity analysis, and GAIs like ChatGPT are crucial. These methodologies provide a sophisticated means to model and predict the glucose responses from Kraft waste pretreated CCW, with its ability to learn patterns and relationships as well as generate innovative and factually accurate insights based on the process data.

Furthermore, LA production is negatively impacted by the high cost associated with the routinely used commercial MRS media, impairing the economics of the system (Tang et al., 2013). In order to negate these excessive costs, the meticulous selection of cheap yet effective raw materials are desired for technoeconomic viability. There is a paucity of studies on the optimization of key input parameters that form part of a feasible media formulation for simultaneous saccharification and LA production. Factors such as substrate solid loading, nitrogen source concentration and surfactant concentrations, among others are key aspects that require optimization for enhanced LA concentration and LA conversion in SSF processes. Moreover, the use of industrial wastewater streams such as DWW instead of fresh water or other wastewater (WW) into the media formulation processes may reduce expenses and resources, while converting waste to wealth. Since DWW is a disposed stream from the dairy industry, its use in the media formulation for LA production is advantageous as it

recycles valuable components, provides a nutrient-rich environment inherent to LAB growth and may potentially reduce adaptation time for LABs. The abovementioned challenges necessitate the modelling and optimization of the simultaneous saccharification and LA fermentation process from a DWW-based medium with inputs of substrate solid loading (pretreated CCW), CSL concentration and Tween 80 concentration.

In the same context, the utilization of a basic media formulation, comprising a carbon source, nitrogen source, and surfactant in DWW, for LA production forms the base medium on which to build upon in order to enhance the product output. However, the LA fermentation process encounters a rapid decrease in pH due to product accumulation, leading to microbial and enzyme inhibition, along with an accumulation of substantial residual fermentable sugar. This phenomenon hinders the efficiency of the fermentation process and challenges the overall yield of LA. To address this issue, a comprehensive evaluation of physicochemical parameters such as addition of buffer agents, pH change and micronutrient supplementation is imperative to optimize the conditions for enhanced LA production and sugar utilization while maintaining microbial and saccharification enzyme activity. Furthermore, a comparative analysis between the SSF and PSSF bioprocesses is warranted to determine the most effective approach for mitigating pH-related challenges and improving overall fermentation outcomes.

Furthermore, there is a dearth of knowledge on the mathematical kinetic modelling of *Lactobacillus* species cell growth and LA formation. This information is essential for industrial scale-up of commodity chemicals such as LA since maintaining anaerobiosis incurs high cost. For this reason, it is essential to have an in-depth understanding of the SSF bioprocess kinetics with the aim of enhancing the feasibility and productivity of LA production.

The scale up of LA production using an optimized minimally supplemented waste-based media poses a significant challenge due to the lack of comprehensive studies on scalability criteria. While development of a waste-based medium show promise of sustainable LA production, there is a critical gap in understanding the impact of scale-up parameters such as constant V_{tip} and constant P/V. The lack of knowledge on some of these scalability factors hinders the assessment of the production process's scalability potential and will facilitate the transition from laboratory scale processes to industrial scale applications.

1.3. Aims and Objectives

This study aims to develop and optimize a lignocellulosic lactic acid (LA) bioprocess using Kraft pretreated corn cob waste (CCW) in a minimally supplemented dairy wastewater (DWW) medium.

To achieve this aim, the following specific objectives were undertaken:

(i) Development and optimization of two complete waste-based lignocellulosic pretreatments consisting of (1) a steam-assisted combined GLD and PWW (SGLD-PWW), and (2) microwave-assisted combined GLD and PWW (MGLD-PWW) pretreatment, to enhance the enzymatic saccharification of CCW.

(ii) Development of two Artificial Neural Network (ANN) models to predict glucose yield from steam- and microwave-assisted Kraft waste-based pretreatment of lignocellulosic wastes. Performing a sensitivity analysis of input variables on the glucose yield using the developed models and subsequently comparing its outcome with interpretations by a Generative Artificial Intelligence (GAI) model, ChatGPT.

(iii) Modelling and optimization of a simultaneous saccharification and fermentation (SSF) process using combined Kraft pretreated CCW (from objective ii) and supplemented DWW medium (sDWW-SSF) to achieve maximum LA concentration and LA conversion.

(iv) Investigation of the kinetics of *L. plantarum* ATCC 14917 cell growth and LA production for the optimized sDWW-SSF process under microaerophilic and anaerobic conditions, in conjunction with a SSF process using commercial De Man, Rogosa and Sharpe (MRS) medium (modified with pretreated CCW).

(vi) Exploration of various physicochemical parameters namely, buffer systems, pH changes, micronutrients supplementation and bioprocess types, to assess its effect on LA concentration and sugar utilization.

(vii) Development of preliminary scale-up experiments and kinetic analysis using the optimized bioprocess conditions from objective vi for LA production.

(viii) Lastly, assessment of the potential of the fermentation effluent as animal feed and biofertilizer using compositional and nutritional analysis, aiming to completely valorize the waste LA effluent.

1.4. Outline of thesis structure

This thesis consists of eight chapters presented in the research paper format as outlined in the thesis template by the College of Agriculture, Engineering and Science (CAES) of the University of KwaZulu-Natal, South Africa. Each experimental chapter is self-contained, consisting of an abstract, introduction for the study's motivation through literature, materials and methods, results and discussion, and conclusions. The development, screening and

application of novel waste-based pretreatment and fermentation strategies are key concepts in all chapters. A brief description of each chapter is as follows:

Chapter 1 provides a general outline of the present research and states the aims and objectives.

Chapter 2 presents an extensive review of literature on the potential application of Kraft paper and pulp waste residues such as green liquor dregs (GLD) and paper wastewater (PWW) as a chemical catalyst and fresh-water substitute, respectively, in lignocellulosic pretreatments.

Chapter 3 explores integrated biorefinery systems, covering aspects such as microbial lactic acid (LA) fermentation processes, the potential of low cost minimally supplemented media formulation, process optimization and artificial intelligence modelling, kinetic studies, and scale-up strategies.

Chapter 4 focuses on the development and optimization of two complete waste-based lignocellulosic pretreatment strategies, namely, (1) a steam-assisted combined GLD and PWW (SGLD-PWW), and (2) microwave-assisted combined GLD and PWW (MGLD-PWW) pretreatment to enhance sugar recovery from corn cob waste (CCW). The MGLD-PWW and SGLD-PWW pretreatments were modelled and optimized using the Response Surface Methodology (RSM) model with reducing sugar and glucose yields as the model responses.

Chapter 5 develops two Artificial Neural Network (ANN) models to predict glucose yields using data from available literature on steam- and microwave-assisted Kraft waste pretreatments under varied novel conditions. Moreover, an in-depth sensitivity analysis based on the developed ANN models, and a comparative assessment with Generative Artificial Intelligence such as ChatGPT were also conducted.

Chapter 6 models and optimizes the simultaneous saccharification and fermentation (SSF) processes using the SGLD-PWW pretreated CCW in a dairy wastewater (DWW) formulated medium (sDWW-SSF). The input parameters considered for the supplemented medium included varied concentrations of corn steep liquor (CSL) (10-30 g/L), Tween 80 (0-2 mL) and solid loading (10-20%). The developed model designated as sDWW-SSF gave LA concentration and LA conversion as the responses. Following sDWW-SSF process optimization, the kinetics of *Lactobacillus plantarum* ATCC 14917 cell growth and LA production were assessed using the logistic and modified Gompertz models, respectively, under microaerophilic ($sDWW\text{-}SSF_{microaerophilic}$) and anaerobic ($sDWW\text{-}SSF_{anaerobic}$) environments. Additionally, a third SSF process implemented the commonly used commercial De Man, Rogosa and Sharpe (MRS) medium, where pure glucose was replaced with pretreated CCW ($mMRS\text{-}SSF_{microaerophilic}$) for comparison with the DWW formulated processes.

Chapter 7 assesses the impact of various physicochemical parameters to enable complete sugar utilization and enhance LA production within the previously optimized DWW formulated medium under microaerophilic conditions (chapter 6) of the lignocellulosic LA bioprocess. These conditions included different buffer agents (K_2HPO_4, $NaHCO_3$, $Na_2HPO_4 \cdot 12H_2O$ and $CaCO_3$), examining the effect of pH changes (pH 4.8, pH 5.5), and supplementation with micronutrients ($MgSO_4$ and $MnSO_4$ or MnO). Additionally, two bioprocess types such as SSF and SSF with prehydrolysis (PSSF) were evaluated. Thereafter, the optimized bioprocess conditions for LA productions were selected for scale-up assessment at 0.5 L using constant impeller tip speed (V_{tip}) and constant power input per unit volume (P/V) as the mixing criteria. Experimental findings from the optimized LA output informed further incremental scale-up at 5 L, followed by kinetic assessment of *Lactobacillus*

plantarum ATCC 14917 microbial cell growth and LA production using the logistic and modified Gompertz models, respectively.

Chapter 8 incorporates the major findings from this study and provides conclusions and recommendations for future research.

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CHAPTER 2

Literature Review

Recent developments in the application of Kraft pulping alkaline chemicals for lignocellulosic pretreatment: Potential beneficiation of green liquor dregs waste

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This chapter has been published in *Bioresource Technology* (306, 123225) with the title: Recent developments in the application of Kraft pulping alkaline chemicals for lignocellulosic pretreatment: Potential beneficiation of green liquor dregs waste.

The published review paper is presented in the following pages.

Highlights

- The Kraft pulping industry represents a major hub for alkaline solutions.
- Green liquor, although not a waste, has extensively been used for pretreatment.
- Green liquor dregs, a remarkable alkaline waste for lignocellulosic pretreatment.
- Development of innovative lignocellulosic biorefineries using wastes.

Bioresource Technology 306 (2020) 123225

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Review

Recent developments in the application of kraft pulping alkaline chemicals for lignocellulosic pretreatment: Potential beneficiation of green liquor dregs waste

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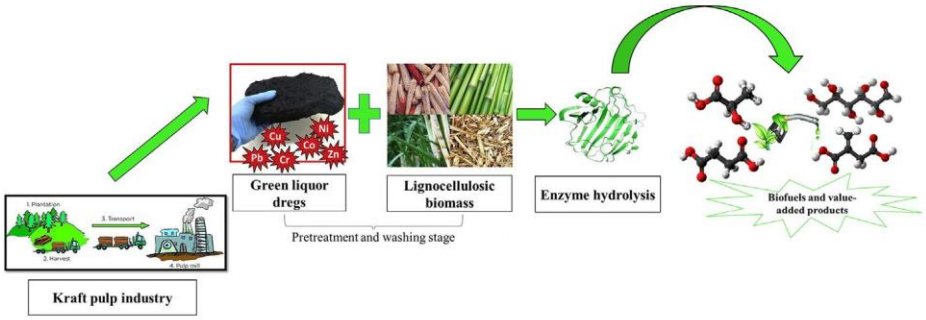
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Lignocellulosic pretreatment
Kraft pulping
Green liquor dregs
Biofuels
Value-added products

ABSTRACT

Lignocellulosic waste has offered a cost-effective and food security-wise substrate for the generation of biofuels and value-added products. However, its recalcitrant properties necessitate pretreatment. Of the various pretreatment methods, alkaline techniques have gained prominence as efficient catalysts. The kraft pulping industry represents a major hub for the generation of white, black and green liquor alkaline solutions during the paper making process. Despite its well-known significance in the kraft pulping process, green liquor (GL) has been widely applied for lignocellulosic pretreatment. Recently, green liquor dregs (GLD), an alkaline waste generated from the kraft pulping industry has piqued interest. Therefore, this review outlines the general flow of the kraft pulping process and the alkaline chemicals derived. In addition, the extensively studied GL for lignocellulosic pretreatment is discussed. Subsequently, the potential beneficiation of GLD for lignocellulosic pretreatment is presented. Furthermore, the challenges and prospects of lignocellulosic pretreatments are highlighted.

1. Introduction

Due to an increase in population size and industrialisation, fossil fuel reserves are drastically depleting. The extensive utilisation of these fossil fuel resources raises concern on its long-term supply in addition to severe environmental implications (Chohan et al., 2020). Consequently, this has sparked interest in the development of alternative energy carriers that remain renewable, sustainable and affordable for future requirements (Sarawan et al., 2019).

Lignocellulosic biomass (LCB) offers a promising feedstock that

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https://doi.org/10.1016/j.biortech.2020.123225
Received 24 January 2020; Received in revised form 16 March 2020; Accepted 17 March 2020
Available online 20 March 2020
0960-8524/

proves to be abundant, inexpensive, renewable and a food security-wise alternative for second generation biofuel production (Xu et al., 2019; Zhang et al., 2019). Examples of second generation feedstocks that have been assessed include sugarcane bagasse (Syawala et al., 2013), sorghum leaves (Sarawan et al., 2019), corn stover (Qing et al., 2016), wheat straw (Saha et al., 2011) and corn cobs (Sewsynker-Sukai et al., 2018), among several others. Bioconversion is considered an environmentally appealing approach for the valorisation of LCB that is derived from agricultural wastes (Sarsaiya et al., 2019). Recently, biorefinery systems have emerged as potential viable processes for the generation of biofuels and value-added products from LCB (Islam et al., 2019; Kim et al., 2019).

Even with the immense beneficial characteristics of LCB, its major structures (cellulose and hemicellulose) are cemented together by the complex and resistant lignin aromatic polymer, that hinders the enzymatic saccharification step (Chuetor et al., 2019). This in turn affects the metabolic activity of the fermentation microorganisms, since they are unable to metabolise complex sugars (Sarawan et al., 2019). However, these challenges may be overcome by pretreatment techniques that reduce cellulose crystallinity, increasing vulnerability of cellulose and hemicellulose to microorganisms and hydrolytic enzymes for the release of fermentable sugars (Liu et al., 2018; Sewsynker-Sukai et al., 2018).

Over the years, lignocellulosic pretreatment studies have explored several processes, which include hydrothermolysis, ball-milling, inorganic salt, organosolvents, microwave, acid, alkaline and thermal steam techniques, among others (Sewsynker-Sukai et al., 2018). Pretreatment criteria not only considers its ability to digest complex polysaccharides to its monomeric form but takes into account the feasibility, energy consumption and efficiency of these systems for industrial application (Qing et al., 2016). Therefore, pretreatment strategies aim to produce high fermentable sugar and biofuel yields with low concentrations of inhibitor compounds and short fermentation times. Once these criteria are met, downstream separation costs and energy consumption may be alleviated, thus enhancing industrial scale applicability (Sewsynker-Sukai and Gueguim Kana, 2018a,b).

Compared to other pretreatment methods, alkaline catalysts have proven to be an effective strategy due to its non-corrosive characteristics, decreased energy consumption and high lignin removal for effective sugar recovery (Sewsynker-Sukai et al., 2018). Despite the high sugar yields, alkaline pretreatments have excessive costs attached to it, due to the expensive chemicals required. This major drawback can be overcome by assessing and implementing the use of waste chemicals as a potential pretreatment agent.

Green liquor dregs (GLD) also known as dregs, are the undissolved particles that are alkaline chemical wastes generated from the kraft pulp and paper mill industry. In the recent time, GLD has piqued interest as a potential lignocellulosic pretreatment. This is mainly attributed to its remarkable alkaline characteristics, while it is deemed a kraft pulping chemical waste. GLD presents various limitations within the kraft pulping process, since it causes blockage of the filtration pipes and is therefore extracted and landfilled. Landfill deposition of GLD raises environmental concerns and drastically increases the process costs for the kraft pulping industry. To overcome these limitations, various avenues to either minimize the GLD production or manipulate its beneficial properties for valorisation towards the generation of biofuels and value-added products are highly sought after.

Interestingly, although green liquor (GL), a by-product that is actively involved in the kraft pulping industry has previously been extensively assessed for its pretreatment capabilities, its waste equivalent, GLD, to date has not been evaluated. Therefore, this review outlines the kraft pulping process and the alkaline chemicals that are derived. Additionally, previous GL lignocellulosic pretreatments are reviewed and its alkaline based mechanism is discussed. Subsequently, the background, current uses and potential beneficiation of GLD chemical waste for lignocellulosic pretreatment is presented. Furthermore, the current challenges and future outlook on lignocellulosic pretreatment systems are highlighted.

2. Lignocellulosic biomass

Agricultural food sources such as wheat, cassava and corn are used to produce first generation biofuels, but these do not comply with food sustainability (Xu et al., 2017). LCB offers a promising feedstock that proves to be abundant, inexpensive, renewable and a food security-wise alternative for second generation biofuel production and value-added products (Xu et al., 2019; Zhang et al., 2019). These substrates (LCB) are complex, heterogenous polymers composed of three fractions, namely; cellulose, hemicellulose and lignin that comprises of approximately 90% of its dry biomass (Kim et al., 2016; Muthuvelu et al., 2019). LCB consists of 38–50% cellulose, 23–32% hemicellulose and 15–25% lignin (McKendry, 2002). These polymers are interwoven together by hydrogen and covalent bonding, thus providing a structural scaffolding to the LCB (Rebello et al., 2020). Cellulose is the primary cell wall polysaccharide that contain inter- and intra-molecular hydrogen bonded chains of β-1,4-linked glucose units (Chang, 2007). Hemicellulose is regarded as a physical barrier that has a random and amorphous structure containing heteropolymers of different 5- and 6-carbon monosaccharide units of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose) and acetylated sugars (Bhatia et al., 2020). The hemicellulose networks link chains of cellulose into microfibrils and form cross links with lignin to provide mechanical strength to the biomass (Agbor et al., 2011; Scheller and Ulvskov, 2010). Conversely, lignin is a complex phenolic polymer consisting of hydroxylated and methoxylated phenylpropanoids with it corresponding monomeric units of *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) (Abdel-Hamid et al, 2013; Buranov and Mazza, 2008). The compressed structure plays a major role in the impermeable and recalcitrant nature of the barrier that protects cellulose and hemicellulose polymers from microbial and enzymatic deconstruction (Himmel et al., 2007; Mahmood et al., 2019). Its steric hindrance and non-productive enzyme adsorption prevent access of enzymes to the cellulose moieties (Min et al., 2014; Zhu et al., 2015). Lignin also provides structural support due to its rigidity and hydrophobicity (Buranov and Mazza, 2008). The quantity of lignin present within various biomass types will affect the enzymatic degradation of the polysaccharides. The strength and physical appearance of LCB is dependent on the ratios of these moieties and thus greatly influences its ability to be hydrolysed. Furthermore, the crystallinity index and degree of polymerization drastically affect the enzymatic digestibility of LCB (Xu et al., 2019).

The crystallinity index is an indication of the proportions of the highly ordered crystalline material in the substrate. High crystallinity hinders the accessibility of cellulase to cellulose moieties, resulting in a reduced substrate-enzyme digestibility of lignocellulose (Tu et al., 2017). The degree of polymerization of β-1, 4-glucans indicates a negative correlation with the hydrolysis of lignocellulosic materials. A reduction in the degree of polymerization enhances the biomass digestibility due to an increase in the number of reducing ends of cellulose chains (Cheng et al., 2018). The abovementioned factors play a crucial role in preventing microbial and chemical attack (Loow et al., 2015). Therefore, pretreatment is necessary to overcome these physical barriers by the disarray of lignin and breaking down of the LCB into its components before efficient enzymatic hydrolysis can occur (Kumar et al., 2020a).

In the present time, LCB is the most prominent resource for the production of biofuels and value-added products, due to its ability to yield numerous harvests from a single crop planting. Moreover, the use of crop waste is estimated to drastically decrease the annual costs related to establishing and managing the new harvests of energy crops since farmers do not have to continuously replenish the crops (Franks et al., 2006). LCB may be categorized into four major groups based on its source: (1) woody biomass, (2) cellulosic wastes (municipal solid

waste, pulp mill waste), (3) energy crops (switchgrass, miscanthus), and (4) agricultural residues (rice husk, wheat straw, corn stover, sugarcane bagasse, corn cob). Approximately 120 billion tons of lignocellulosic biomass is generated annually (Abraham et al., 2020), with corn, wheat, rice and sugarcane having maximum production.

3. Pretreatment strategies

Pretreatment of LCB is considered a crucial step in the production of fuel and other value-added chemicals (Kim, 2013; Amiri and Karimi, 2018). The rigid structural lignin components prevent cellulose and hemicellulose to be chemically or biologically broken down into its monomeric, more metabolizable sugars (Li et al., 2015a). Therefore, pretreatment applications ensure lignin removal, decreased cellulose crystallinity, increased porosity and surface area causing cellulose and hemicellulose to become accessible to enzymatic saccharification (Chuetor et al., 2017; Modenbach, 2013). The outcome of the pretreatment impacts subsequent steps in the process, namely, enzymatic hydrolysis, fermentation and downstream processing with regards to product recovery and purification.

Various pretreatment methods exist and include biological, physical and chemical as well as a combination of the different techniques (Yuan et al., 2018). Biological methods include the use of fungi, bacteria and enzymes that target lignin and hemicellulose decomposition (Maurya et al., 2015). Biological pretreatments show promise of decreased inhibitor compounds, energy efficiency and maintains mild operations that are eco-friendly (Sindhu et al., 2016). However, microbes require increased contact time with the biomass, since the rate of hydrolysis is slow (Sun and Cheng, 2002; Zabed et al., 2019). On the other hand, physical pretreatment is carried out to reduce the particle size of the biomass, which in turn increases surface area, decreases the degree of polymerization and crystallinity (Rajendran et al., 2018). These methods include milling, hackling, extrusion, sonication and pyrolysis amongst others. Physical methods have shown to be environmentally friendly and produce little to no toxic by-products. Studies have shown that mechanical treatment of feedstock has a significant effect on fractionation of cellulose and hemicellulose. Conversely, chemical pretreatments enable the decomposition of lignocellulosic biomass by initialising chemical reactions in aqueous solutions. Examples of chemical pretreatments include acid, alkaline, ionic liquid, inorganic salts and organosolvents with varying effects on the lignocellulosic structures as summarized in Table 1. Nevertheless, a combination of various pretreatments enhance the overall yield of fermentable sugars (Kumar et al., 2020a). For example, Lin et al. (2010) discovered that a combination of milling with alkaline pretreatments enhanced the enzymatic hydrolysis of corn stover by 1.67-fold when compared to wet milling alone. Likewise, Kumar et al. (2020b) assessed a combined physical, microbial and chemical pretreatment of sesame plant residue and achieved a 68% higher reducing sugar with the combined treatment compared to the microbial method alone.

Chemical pretreatments have been shown to enhance fermentable sugar recovery by approximately 80% when compared to pretreatment strategies that only garner 20% without chemical supplementation (Singhvi et al., 2014). The complex lignocellulosic structures are degraded and solubilised as a result of the chemical reactions in aqueous solutions and provide a platform for enzymes to easily access the saccharides for conversion to simple sugars (Kucharska et al., 2018).

For instance, organosolvent pretreatment requires organic solvents or their aqueous solutions for the release of cellulose residues for hydrolysis (Bhatia et al., 2020). Common organic solvents such as ethanol, acetone, glycol, glycerol and methanol are used in the presence of an acid, base or salt catalyst. The pretreatment studies by Kabir et al. (2015) and Alio et al. (2019) applied a solvent (ethanol) in the presence of an acid catalyst (H_2SO_4) and revealed maximum cellulose recovery of 48% and 82% respectively. Recently, Huang et al. (2020) developed a mild ethanol assisted alkaline peroxide pretreatment for enhancing the enzymatic hydrolysis efficiency of bamboo. These authors observed high delignification and glucan recovery of 80% and 83.3% respectively (Huang et al., 2020). Similarly, Wu et al. (2019) evaluated a novel crude glycerol pretreatment for selective saccharification of sugarcane bagasse via fast pyrolysis and noted a high delignification of 79.4%. Nevertheless, organic solvents may present itself as an inhibitor to the hydrolysis reactions or may engage in side-reactions, therefore, its removal prior to enzymatic hydrolysis is essential.

Ionic liquids (IL) are a class of organic solvents comprising of cations and anions existing at temperatures < 100 °C and insignificant vapour pressures that enhance cellulose and lignin solubilisation (Usmani et al., 2020). Examples of IL include: imidazole salts, cholinium acetate and cholinium amino acids. In the recent time, Sorn et al. (2019) examined the effect of a microwave-assisted ionic liquid/acidic pretreatment on rice straw and observed a glucan conversion of 55% (ionic liquid). Along with its enhanced cellulose conversion and sugar recovery, ionic solvents are considered environmentally friendly due to their low volatility and non-toxic nature (Alayoubi et al., 2020). However, industrial scale pretreatments encounter problems of recycling, inhibitor generation and incurrence of excessive costs (Kucharska et al., 2018; Kumar and Sharma, 2017). More recently, deep eutectic solvents (DES) that possess similar characteristics as ionic liquids are being investigated due to its cost-effectiveness, simpler process for synthesis, and higher biodegradability (Ling et al., 2020). Ling et al. (2020) investigated a novel levulinic acid (LA) based deep eutectic solvent pretreatment combined with hydrogen bonding acceptors (acetamide, betaine and choline chloride) using bamboo and observed the highest glucose yield of 79.07% (LA-Choline chloride method).

On the other hand, commonly used acid hydrolysis consists of the solubilisation of hemicellulose, which aid in the release of cellulose for saccharification (Sarawan et al., 2019; Kucharska et al., 2018; Kumar et al., 2020a). Acid hydrolysis is an attractive method since hemicellulose degradation may reach an efficiency of approximately 20–90%, depending on process variables (Hendriks and Zeeman, 2009; Łukajtis et al., 2018). Examples of strong acids such as H_2SO_4 and HCl have extensively been studied for their powerful pretreatment effect and flexibility regarding the choice of substrate that yield high fermentable sugar at mild temperatures (Sun and Cheng, 2002; Harmsen et al., 2010). However, temperature increases above 110 °C produce toxic inhibitor compounds, namely, furfural and 5-hydroxymethyl furfural, which halt enzymatic hydrolysis and microbial fermentation processes (Kucharska et al., 2018). Additionally, one of the major drawbacks of using concentrated acids are the corrosion of the reaction vessels and expenses attached to the chemicals needed (Kumar et al., 2020a). Alkaline pretreatments on the other hand, have risen as one of the most prominent chemical pretreatments due to its decreased energy consumption and high lignin removal for successful aromatic polymer degradation (Woiciechowski et al., 2020). Examples of some alkaline chemicals include sodium hydroxide, ammonia, calcium hydroxide as well as alkali salts such as sodium sulfide, sodium acetate and sodium carbonate, which act as viable catalysts.

3.1. Alkaline pretreatment mechanism of action, advantages and limitations

The alkaline pretreatment mechanism of action results in the cleavage of acetyl groups from xylan polymers, saponification of intermolecular ester bonds and alteration of the lignin structure that aids the removal of the lignin compounds (Kumari and Singh, 2018). Additionally, these chemicals alter the degree of polymerization, reduces crystallinity of cellulose while increasing surface area (Kim et al., 2016).

The cellulose moieties are embedded in a lignin and hemicellulose network with lignin offering physical protection against microbial or chemical cellulose degradation (Höfte and Voxeur, 2017). The presence of lignin is capable of binding to enzymes which form complexes that hinder the hydrolysis of carbohydrates (Yu, 2012). Alkaline

Table 1
Various common pretreatment strategies with its mechanism of action, advantages and disadvantages.

Pretreatment	Mechanism of action	Advantages	Disadvantages	References
Organosolvent	Cleavage of internal lignin bonds and 4-*O*-methylglucuronic bonds Hydrolysis of glycosidic bonds	Significant cellulose yield High purity product	Presents itself as an inhibitor and engages in side-reactions Volatile compounds cause risk of fires Harm to environment High cost Difficulty in removal	Bhatia et al. (2020); Alio et al. (2019); Wu et al. (2019); Huang et al. (2020); Sun and Cheng (2002)
Ionic liquids	Modifies intra- and intermolecular hydrogen bonds Cleaves β-*O*-4 linkage in lignin	High thermal stability and polarity Environmentally friendly Low volatility	Problematic recycling High inhibitor concentration Excessive costs	Usmani et al. (2020); Alayoubi et al. (2020); Ling et al. (2020); Sorn et al. (2019); Yoo et al. (2017); Kucharska et al. (2018); Kumar and Sharma (2017)
Acid	Solubilisation of hemicellulose	Flexibility with substrate choice High fermentable sugar yield at mild/low temperatures	Toxic inhibitor compounds Concentrated acids cause corrosion of the reaction vessels Expenses chemicals needed	Sarawan et al. (2019); Kumar et al. (2020a); Sun and Cheng (2002); Harmsen et al. (2010); Kucharska et al. (2018)
Alkaline	Lignin removal for aromatic polymer degradation Cleavage of acetyl groups from xylan polymers Alteration of the lignin structure	Low energy consumption Does not need specialized equipment Non-corrosive Low inhibitor concentrations	Excessive cost Irrecoverable salt production	Woiciechowski et al. (2020); Sewsynker-Sukai et al. (2018); Balat et al. (2008); Hendriks and Zeeman (2009)
Alkalic salt	Cleavage of ester and glycosidic bonds	Low cost Low inhibitor concentrations	Thermal energy is needed. Partial cellulose degradation	Sewsynker-Sukai and Gueguim Kana (2018a); Qing et al. (2016)
Inorganic salt	Disrupts the C-O-C and C–H bonds in cellulose	Show high catalytic activity	Results in partial degradation of lignocellulosic structure	Bhardwaj et al. (2020); Yu et al. (2011); Wei et al. (2011); Loow et al. (2015)
Milling	Reduces particle size Decreases the degree of polymerization and crystallinity Fractionation of cellulose and hemicellulose	Environmentally friendly Little to no toxic by-products	High energy	Zhang et al. (2019); Rajendran et al. (2018); Lin et al. (2010)
Biological	Utilises various enzymes or microorganisms that produce lignin degrading enzymes (lignin peroxidase, manganese-dependant peroxidases, which selectively break down lignin and hemicelluloses	No toxic by-products formed Low energy inputs and waste generation Mild pretreatment conditions Reduced costs Easy downstream processing	Slow pretreatment rate Loss of fermentable sugars to the microorganism, affecting overall yield Commercial application is limited Requires aseptic conditions for microbial growth Requires specific parameters Long incubation periods	Maurya et al. (2015); Sindhu et al. (2016); Abraham et al. (2020); Kumar et al. (2020a)
Microwave	Causes deviations in the dipole orientation of polar compounds within lignocellulosic structures	Low residence time High heating efficiency Low energy and time consumption	Generation of heat-induced inhibitors Sugar degradation Production of phenols	Pellera and Gidarakos, (2016); Feng et al. (2018); Abraham et al. (2020)
Hydrothermal	Acetic acids derived from acetyl groups and cleavage of uronic groups within lignocelluloses catalyse the degradation of various bonds of hemicellulose-lignin and polysaccharide fractions	No size reduction is needed No corrosion Low cost reactors No catalysts or chemicals required Low cost reactors	Formation of phenols and furan derivatives at high temperatures High energy and water requirements Sugar degradation	Maurya et al. (2015); Kumar et al. (2020a)

pretreatment chemicals diffuse by capillary action to the lumen and subsequent cell layers reaching the middle lamella and the secondary wall of the LCB. The desired polysaccharides are mainly located in the secondary wall regions while fewer polysaccharides are present in the middle lamella (Gu et al., 2012). For example, NaOH is dissociated into OH^- and Na^+ ions that attack the ester and ether linkage between lignin-hemicellulose complexes. Additionally, it has the ability to cleave the ester and carbon–carbon bonds in the lignin moieties (ferulic acid) (Kim et al., 2016). Other alkaline conditions result in the cleavage of phenolic and non-phenolic β-aryl ether linkages that directly aid in the depolymerization of lignin in lignocelluloses (Sjöström, 1983). The LCB in alkaline solutions provides moderate reaction conditions that minimize random hydrolysis and secondary peeling (Sjöström, 1993). Thus, resulting in higher retention of polysaccharides in the spent media.

Access by cellulase enzymes is thereby enhanced to ensure cellulose decomposition to produce glucose molecules (Jiang et al., 2016). Alkaline reagents are less caustic than acids such as H_2SO_4 and HCl and its pretreatment can operate under milder conditions and sometimes even at ambient temperature. Such methods eliminate the use of special reactor designs to cope with corrosion and severe reaction conditions and retrieval of reagents for pretreatment recycling is possible in some cases.

Due the immense benefits of alkaline pretreatments, it has been extensively studied on various lignocellulosic substrates (Table 2). Cheng et al. (2010) compared both lime and NaOH alkaline pretreatments on corn stover. These authors deduced that a 10% solid loading (SL), 0–10% lime concentration for 1–3 h at 95 °C observed delignification of up to 27% (Cheng et al., 2010). Similarly, Zheng et al. (2018) observed an increase in cellulose conversion rate from 38.1% to 65.8% when the NaOH concentration increased from 0.5% to 4% at a constant temperature of 121 °C on wheat straw. On the other hand, Xu et al. (2017) combined dilute aqueous ammonia and ultrasonic pretreatment on corn cobs and reported a maximum reducing sugar yield of 80.6%.

The recent study by Yan et al. (2020) investigated a hydrogen peroxide synergized dilute alkali (NaOH) pretreatment of grass waste and demonstrated high delignification (73.2%) and enzymolysis efficiency (83.5%). Similarly, Jiao et al. (2020) optimized an ultrasonic-assisted ammonium bicarbonate pretreatment on corn stalk and recorded a maximum saccharification rate of 82.61% under the optimal conditions. Conversely, Elalami et al. (2020) determined the effects of microwave, ultrasonic and alkaline (NaOH) pretreatment on olive pomace and recorded a maximum delignification of 68% using NaOH. The study by Jiang et al. (2020) reported a 44.9% glucose yield when giant reed was pretreated with NaOH. Likewise, Xia et al. (2020) established a combined liquid hot water with sodium carbonate-oxygen pretreatment to enhance enzymatic saccharification of reed and achieved a total sugar yield of 79.1%.

Alkaline pretreatment techniques do not require specialized equipment due to its non-corrosive characteristics. Nevertheless, alkaline pretreatments surface a major concern for industrial scale processes that is mainly attributed to the high cost, thus making it unfeasible (Wan et al., 2011). Moreover, high concentrations of inhibitor compounds are produced during harsh operating conditions and from substrates that contain high lignin content (Balat et al., 2008; Hendriks and Zeeman, 2009). The generation of inhibitor compounds negatively impacts on the cellulase enzymes and fermenting microbes (Harmsen et al., 2010; Gu et al., 2012). Pretreatment criteria that classifies the method's effectiveness not only considers its ability to digest complex polysaccharides to its monomeric form, but takes into account the cost feasibility, energy consumption and efficiency of these systems for industrial applications (Jagtap and Rao, 2018). The main aim of an efficient pretreatment method is to produce high sugar yields with low concentrations of inhibitor compounds (Sewsynker and Gueguim Kana, 2018b). Therefore, the development of a pretreatment technique that meet the above criteria and does not hinder large scale implementation is still highly sought after.

4. General flow of the chemical kraft pulping and paper making process

The kraft (sulfate) pulping process accounts for the largest portion of the global pulp production (Kinnarinen et al., 2016). This process (kraft pulping) engages in the removal of lignin from wood chips using a mixture of alkaline cooking chemicals, sodium hydroxide and disodium sulfide, known as white liquor (Golmaei et al., 2018a) (Table 3). Following the delignification process, a resultant weak black liquor containing degraded lignin, oxidized inorganic compounds (Na_2SO_4 and Na_2CO_3), organic materials and white liquor (Na_2S and NaOH), is pumped to the chemical recovery cycle (Bajpai, 2015). Its solids content is increased in evaporators to elevate its heat value before feeding it to the recovery boiler. The organic constituents of the concentrated black liquor are combusted and the sodium sulfate is reduced to sodium sulfide. As a result, an additional quantity of sodium sulfate is added to the black liquor to compensate for its loss, hence the term "kraft (sulfate) pulping process" (Bajpai, 2018). The heat from the combusted organic constituents of the black liquor produces an inorganic smelt enriched by Na_2S and Na_2CO_3 (known as green liquor or GL) in the recovery boiler (Cardoso et al., 2009). In the dissolving tank, the smelt is treated with a weak white liquor solution, forming the green liquor dregs (GLD) sludge in which the solid, undissolved particles are known as dregs (Sanchez, 2007; Tikka, 2008). The dissolved Na_2CO_3 content (GL) of the sludge is diverted back into the kraft pulping cycle and is converted to NaOH in the causticizing process (Golmaei et al., 2018a).

Since the GL should be free of suspended solids, the dregs are separated in the GL purification stage via sedimentation, centrifugation, cake filtration and cross-flow filtration (Golmaei et al., 2017; Kinnarinen et al., 2016). Finally, the inorganic waste fraction, GLD, is passed through a pre-coat lime mud filter which retrieves non-process elements (NPE) such as insoluble species and non-reactive metals found in wood (Mäkitalo et al., 2014). The final composition of GLD is

Table 2
Comparison of the different alkaline pretreatment methods used on various lignocellulosic waste.

Substrate	Pretreatment conditions	Pretreatment efficiency	Reference
Corn stover	10% (w/w) SL, 0–10% lime for 1–3 h at 95 °C	27%[a]	Cheng et al. (2010)
Corn stover	20% (w/w) SL, 0–4% NaOH for 1–3 h at 55 °C	23.1%[a]	Cheng et al. (2010)
Wheat straw	10% (w/v) SL, 4% NaOH for 1 h at 121 °C	65.8%[b]	Zheng et al. (2018)
Corn cob	15% (w/v) SL, 2% (w/w) NH_3 for 4 h at 70 °C under ultrasonic conditions of 90 W and 59 kHz	80.6%[d]	Xu et al. (2017)
Corn stover	10% (w/w) SL, 4% Na_2CO_3 for 20 min at 140 °C	0.268 g/g[c]	Kim et al. (2014)
Corn cobs	12.5% (w/v) SL, 0.5 M NaOH for 30 min at 121 °C	0.92[d]	Gao and Rehmann (2014)
Giant reed	10% (w/v) SL, 20% NaOH for 24 h at 24 °C	44.9%[c]	Jiang et al. (2020)
Grass waste	4% (w/v) SL, 1% NaOH and 2% H_2O_2 for 6 h at 55 °C, 150 rpm in a rotary shaker	73.2%[a]	Yan et al. (2020)
Olive pomace	2% (w/v) SL, 8% (w/w) NaOH for 24 h at 50 °C, 100 rpm in a rotary shaker	68%[a]	Elalami et al. (2020)
Corn stalk	8.3% (w/v) SL, 8% (w/v) $NaHCO_3$ for 11 min at 42 °C under ultrasonic conditions of 100 W and 20 kHz	82.61%[b]	Jiao et al. (2020)
Reed	10% (w/v) SL, 3.33% (w/v) Na_2CO_3 for 40 min at 150 °C under oxygen pressure of 0.6 MPa	79.1%[e]	Xia et al. (2020)

Note: [a] = Delignification, [b] = cellulose conversion rate, [c] = glucose yield, [d] = reducing sugar yield, [e] = total sugar yield, SL = Solid loading.

Table 3
Summary of the kraft pulping processes during the paper making process.

Kraft pulping chemical	Kraft pulping process	Key chemicals	Kraft pulping function	Reference
White liquor (WL)	Delignification	Mixture of sodium hydroxide and disodium sulfide	Removal of lignin from wood chips	Golmaei et al. (2018a)
Black liquor (BL)	Chemical recovery of solids by evaporation followed by boiling	Degraded lignin mixture	Degraded lignin is pumped to the chemical recovery cycle, where the solids are increased by evaporation to elevate its heat value before feeding it to the recovery boiler	Kinnarinen et al. (2016)
Green liquor (GL)	Causticizing process	Sodium carbonate and sodium sulfide	Heat from the combusted organic constituents of the BL produces a smelt (GL)	Cardoso et al. (2009)
Green liquor dregs (GLD)	GL is treated with a weak WL solution, forming the GLD	Calcium carbonate, sodium sulfide, magnesium hydroxide, sodium carbonate, metal sulfides, unburned carbon	No function, considered a waste that is landfilled	Mäkelä et al. (2016); Sanchez (2007); Tikka (2008)

strongly influenced by the combination of the NPE's as well as calcium carbonate, sodium sulfide, magnesium hydroxide, sodium carbonate, metal sulfides and unburned carbon during the kraft pulping process. The GLD is thereafter precipitated and sequentially removed from the kraft pulping chemical recovery circuit (Sartz et al., 2017). This waste (GLD) mainly consists of insoluble substances from wood and potential make-up chemicals, which do not play an active role in pulping and is considered a "dead load". In addition to being regarded as a dead load, the removal of GLD from the system is attributed to the high concentrations of calcite within alkaline precipitate that show signs of low hydraulic conductivity. This proves to be detrimental to the fibre line and chemical recovery, potentially causing operational problems within the kraft pulping mill. In an effort to avoid the adverse effects of GLD on the kraft pulping process, it is recovered and landfilled. According to Kinnarinen et al. (2016), the global annual production of GLD is approximately 4 to 11 kg per ton of pulp generated from kraft mills. In 2018, the total global kraft pulp production was estimated at 187.2 million tons (RISI, 2019), which translates to 0.74 to 2.10 million tons of GLD (since ~4 to 11 kg GLD is produced per ton of pulp). In South Africa alone, two of the largest mills have an estimated GLD production of ~100 000 tons of GLD/annum (Sebogodi et al., 2019). Beneficiation of GLD chemical wastes may improve the kraft pulping process while adding value to waste. Even though GLD has not been assessed for its pretreatment effect, the kraft pulping by-product GL, has been extensively studied and is detailed below.

4.1. GL and its implementation for lignocellulosic pretreatment

GL is a mixture of sodium carbonate and sodium sulfide generated as a by-product from the kraft pulping process (Jin et al., 2010). This by-product (GL) selectively removes the lignin moieties while leaving both hemicellulose and cellulose components in pretreatment slurry. The mechanism causes delignification, exposing the cellulose to enzymatic attack, resulting in high sugar recovery. Moreover, the considerable quantity of sodium sulfide (sulfidity up to 40%) is a valuable characteristic that aids in the delignification process (Gu et al., 2012). More specifically, GL affects the structure of aromatic fractions of the lignin in the LCB. A structural analysis of lignin in wheat straw reported that non-condensed guaiacyl, syringyl and *p*-hydroxyphenyl units undergo oxidation by GL pretreatment to produce three corresponding principal phenolic aldehydes, namely, vanillin, syringaldehyde and *p*-hydroxybenzaldehyde (Jiang et al., 2016). The cleavage of the α- and β-aryl ether bonds in the abovementioned units leads to the liberation, dissolution and structural changes of the lignin fraction. On the other hand, Gu et al. (2014) evaluated the pretreatment of LCB under GL conditions and deduced that HS^- and CO_3^{2-} species in the GL cleave the β-O-4 bond of the non-phenolic compounds. This results in disruption of the hydrogen bonding network, stable cellulose crystallinity and structural alternation of the residual lignin within the GL pretreated substrate while increasing porosity and surface area accessibility (Alvira et al., 2010). Thus, GL pretreatment aids in exposing the hydrolysing enzymes to the cellulosic and hemicellulosic fractions during enzymatic saccharification with the aim of generating fermentable sugars for subsequent fermentation stages.

Numerous studies have been performed on GL pretreatment for enhanced delignification and sugar release from various lignocellulosic wastes (Table 4). For instance, Gu et al. (2012) conducted a study on corn stover with GL (8% total titratable alkaline and 40% sulfidity) at 140 °C for 60 min. These optimized conditions yielded a 45% delignification and 70% polysaccharide conversion to fermentable sugars (28.5% glucan) (Gu et al., 2012). In a subsequent study, the same authors demonstrated a 39.4% delignification and 92.5% glucan conversion from rice straw under optimum GL conditions of 4% total titratable alkaline (TTA), 20% sulfidity at 140 °C for 60 min (Gu et al., 2013).

The study by Yu et al. (2013) coupled GL with ethylenediaminetetraacetic acid (EDTA) for the pretreatment of furfural residues and

Table 4
GL pretreatments previously studied on various lignocellulosic wastes.

Substrate	Pretreatment conditions	Pretreatment efficiency	Reference
Corn stover	8% TTA, 40% sulfidity, 16.67% SL, 140 °C for 60 min	28.5% ^{gc}, 45%^{d}	Gu et al. (2012)
Rice straw	4% TTA, 20% sulfidity, 16.67% SL, 140 °C for 60 min	92.5%^{gc}, 39.4%^{d}	Gu et al. (2013)
Whole rice waste	10% Na_2CO_3:Na_2SO_3 (1:1), 12% SL, 121 °C for 360 min	88%^{g}, 58.2%^{d}	Saratale et al. (2016)
Furfural residues	6 mL GL/g-DS, 0.6 g H_2O_2/g-DS, 1% EDTA, 3.3% SL, 80 °C for 180 min	90.4%^{g}, 56.2%^{d}	Yu et al. (2013)
Furfural residues	1 mL GL mixture/g-DS (50 ethanol:50 water), 0.4% AQ, 5% SL, 140 °C for 60 min	85.9%^{g}, 42.7%^{d}	Yu et al. (2014)
Sugarcane bagasse	1.5 mL GL/g-DS (50 ethanol:50 water), 1% AQ, 5% SL, 160 °C for 180 min	95.3%^{g}, 98.26%^{d}	Zhou et al. (2016)
Sugarcane bagasse	0.4 g Na_2SO_3/g-DS, 1.5 mL GL/g-DS, 5% SL, 140 °C for 180 min	96.8%^{g}, 76.2%^{d}	Zhou et al. (2017)
Sweet sorghum bagasse	18% TTA, 40% sulfidity (SGL), 7% SL, 160 °C for 110 min	89.6%^{gc}, 84.2%^{d}	Pham et al. (2018)
Wheat straw leaves	8% TTA, 20% sulfidity, 16.67% SL, 140 °C for 60 min	93.7%^{gc}, 28.9%^{d}	Jiang et al. (2016)
Loblolly pine chips	16% TTA, 25% sulfidity, 25% SL, 170 °C until H-factor = 800	43%^{gc}, 23.6%^{d}	Wu et al. (2012)
Mixed hardwood	16% TTA, 25% sulfidity, 25% SL, 160 °C until H-factor = 400	87%^{gc}, 33%^{d}	Jin et al. (2010)
Poplar	20% TTA, 25% sulfidity, 25% SL, 160 °C until H-factor = 400	89.9%^{gc}, 29.2%^{d}	Meng et al. (2014)

Footnote: TTA = total titratable alkali (Na_2CO_3 + Na_2S), sulfidity = Na_2S/TTA, SL = solid loading, GL = green liquor, g-DS = gram of dry substrate, EDTA = ethylenediaminetetraacetic acid, AQ = Anthraquinone, SGL = simulated green liquor, ^{g} = glucose, ^{gc} = glucan, ^{d} = delignification.

obtained a glucose yield and delignification of 90.4% and 56.2%, respectively. The same authors investigated a combined GL and anthraquinone (AQ) pretreatment method in a following study on furfural residues and recorded an 85.9% glucose yield and 42.7% delignification (Yu et al., 2014). The study by Zhou et al. (2016) also evaluated a combined GL and AQ pretreatment regime and achieved above 90% delignification and glucose yield from sugarcane bagasse. Similarly, Zhou et al. (2017) supplemented GL with sulfite and observed a high glucose yield (96.8%) and delignification (76.2%) under pretreatment conditions of 0.4 g/g-DS Na_2SO_3 and 1.5 mL/g-DS GL at 140 °C using sugarcane bagasse. The mechanism resulted in the removal of lignin where the sulfite portion engages in sulfonation, which solubilises the benzyl aryl ether, benzyl alcohol and benzyl alkyl ether linkages on the side chain of phenyl propane units (Zhou et al., 2017).

The recent study by Pham et al. (2018) investigated a simulated green liquor (SGL) for the pretreatment of sweet sorghum baggase and recorded 84.2% delignification and 89.6% glucan with optimum conditions of 18% TTA, 40% sulfidity and 7% SL at 160 °C for 110 min. The earlier reports by Jin et al. (2010) and Meng et al. (2014) investigated similar GL pretreatment conditions on mixed hardwood and poplar substrates, respectively and recorded high glucan (> 85%) and delignification (> 20%). Likewise, the studies by Saratale et al. (2016), Wu et al. (2012) and Jiang et al. (2016) reported high delignification and sugar recovery after GL pretreatment on whole rice waste, loblolly pine chips and wheat straw leaves, respectively (Table 4). The differences in the glucose/glucan recovery and delignification between the GL pretreatment studies in Table 4 may be ascribed to the treatment conditions and substrate types that consist of varying cellulose, hemicellulose and lignin content (Gu et al., 2012; Jiang et al., 2016; Zhou et al., 2017).

Recent studies have reported many advantages of conventional pretretreatment technologies such as acid, alkaline, organosolvents and inorganic salts resulting in enhanced cellulose recovery, high purity and flexible feedstock choices. Nevertheless, these benefits are challenged by the release of inhibitor compounds, toxicity, problematic disposal, corrosion of equipment and excessive costs. In comparison to the common pretreatment strategies, GL pretreatment presents major advantages of no toxic by-products such as furfural, acetic acid (from hemicellulose degradation) and metal ions that may affect the saccharification and fermentation stage. It is capable of selectively removing lignin from LCB while keeping majority of the hemicellulose and cellulose moieties in the pretreatment slurry. Furthermore, it is void of corrosive properties and has been proposed to reduce production costs by merging the biofuel and pulp milling plants. It represents an attractive pretreatment approach that can be channelled towards the production of biofuels and other valuable biochemicals via microbial fermentation.

Despite the effectiveness of GL as a lignocellulosic pretreatment agent, it consists of compounds that discharge reduced toxic sulfur compounds such as hydrogen sulfide, dimethyl sulfide, methyl mercaptan and dimethyl disulfide into the air during the pulping process resulting in atmospheric pollution (Yu et al., 2013). A major contributor of hydrogen sulfide is as a result of the reaction between sodium sulfide and carbon dioxide. Methyl mercaptan and dimethyl sulfide are formed in reactions with lignin moieties within lignocellulosic feedstocks, while dimethyl disulfide is produced through the oxidation of mercaptan groups derived from the lignin. In order to reduce the toxic sulfur components in the atmosphere, certain process modifications can be set in place. For example, black liquor oxidation systems result in sulfides being oxidised into less reactive thiosulfates that minimise the odorous emissions. Similarly, passing non-condensable odorous gases through lime kiln can eliminate pollutants through thermal oxidation. Additionally, maintaining sufficient oxygen, residence time and turbulence can significantly decrease the reduced sulfur components from the source (USEPA, 1995). Furthermore, the dissolved Na_2CO_3 fraction of green liquor sludge is required to be re-routed back into the kraft pulping cycle to be converted to NaOH by the causticizing process (Golmaei et al., 2018a). Therefore, GL is essential for the continuation of the kraft pulping cycle and this places its use for lignocellulosic pretreatment under major scrutiny, since it is a valuable by-product that is required for the paper making process. Unlike the by-product GL, its waste counterpart GLD, has not yet been realised for its chemical pretreatment capability. Recently, green liquor dregs (GLD), an alkaline chemical waste generated from the kraft pulping industry, has emerged as a potential pretreatment regime.

4.2. Background and uses of GLD

Although landfill deposition of pulp and paper mill residues such as GLD has gradually reduced in the last few years, it is costly and suitable applications for these wastes (GLD) remain limited (Mäkelä et al., 2016, 2014). Regulations have been set in place with the aim to conserve the environment, which has not only raised the cost with regards to maintenance of landfilling but has hindered the acquisition of new disposal sites. GLD contains heavy metals such as Cd, Cu and Zn and landfilling enables leaching of these elements into the soil and water in close proximity of the dumpsites. The exposure of elevated concentrations of the heavy metal to the environment are considered hazardous to ecosystems (Manskinen et al., 2011).

This is attributed to its non-degrading ability which exist in the environment for long periods of time after being released. Heavy metals have shown to interact with cell components such as DNA and nuclear proteins leading to DNA damage and conformational changes causing carcinogenesis and apoptosis in humans (Tchounwou et al., 2012). Therefore, separation of these elements is currently being investigated (Mäkelä et al., 2016; Golmaei et al., 2018a,b). For instance, chelating

(complexing) agents have the ability to extract heavy metals by enhancing its mobilisation from the solid phase. While other extractants such as strong acids and redox agents need to decompose the solid matrix to release the metals, chelating agents favour complexation of the heavy metals (Manskinen et al., 2011). There are two mechanisms in which these agents can proceed towards removing heavy metals from solids. The first being a fast, thermodynamic mechanism that causes the weak bonds between cationic metals and solids to be broken down by the chelator and forms a complex with the metal atom (Dwyer, 2012; Zhang et al., 2010). The second mechanism engages in indirect mobilisation of the metals bound to the oxides and organic matters through a slow chelating agent-promoted dissolution, which disrupts the solid structure partially (Garrabrants and Kosson, 2000; Zhang et al., 2010). Various chelating agents include ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), anionic polyacrylamide (APAM) and ethyleneglycol tetraacetic acid (EGTA) amongst others. One of the most commonly utilised chelating agents for divalent metal extraction is EDTA due to its strong chelating ability, but its low biodegradability causes some environmental implications for field applications (Giannis et al., 2010). Conversely, its relatively low price makes it economically feasible for large scale use (Leštan et al., 2008). A study by Golmaei et al. (2018b) showed that EDTA was effective in extracting hazardous metals such as Cd, Pb, Co, Cu, Mn, Ni and Zn with traces of Cr and Ca from the green liquor dregs. On the other hand, sequential leaching fractionation is as a result of the individual components of a sample becoming soluble and mobile when in contact with extraction solvents (Lorentzen and Kingston, 1996). Nurmesniemi et al. (2005) showed that heavy metals had enhanced leachability when in contact with the easily reduced fraction ($HONH_3Cl$) and oxidizable fraction (H_2O_2 and CH_3COONH_4). A more recent study by Mäkelä et al. (2016) revealed that cyclone processing has become a potential method to reduce heavy metal content in green liquor dregs by separating its particles according to size. The treatment was successful in separating the dried dregs from the finer particles containing the metals to the reject fraction, enabling suitable application for the green liquor dregs (Mäkelä et al., 2016). Similarly, a hydrocyclone device can be utilized for continuous phase separations in a solid–liquid suspension by a centrifugal force, where the solid particles are separated into finer overflow and coarser underflow fractions based on their size, density, shape and magnetic field (Bai et al., 2009; Ghadirian et al., 2015). A study by Golmaei et al. (2018a) utilised this concept for the separation of heavy metals from the solid dregs fraction. It was observed that hazardous metals Cd, Ni, Pb and Zn along with other trace metals were mostly found in the finer overflow fractions. Also, high concentrations of Cd and Ni in green liquor dregs do not meet maximum allowed concentrations requirement of the European commissions, however, the outcome of the study by Golmaei et al. (2018a) showed that the concentrations of these two hazardous elements may be reduced to a level lower than the maximum threshold.

In pursuit of a way to overcome these difficulties, researchers are continuously seeking various avenues to either minimize the GLD production or manipulate its beneficial properties for valorisation towards the generation of value-added products. Some recent applications of GLD are depicted in Table 5. The repurposing concept results in the co-disposal of GLD and substances that are detrimental to the environment. For instance, Jia et al. (2013) and Mäkitalo et al. (2014) reported the use of GLD as an alkaline barrier layer that stabilises mine tailings by minimizing the water percolation and oxygen transport, thus preventing acid rock drainage. Additionally, the highly alkaline compounds and salts present within the GLD are being used as fertilizers and soil amendments to prevent the acidification in soils (Mäkelä et al., 2012). Likewise, Sebogodi et al. (2019) studied GLD application for the treatment of acid mine drainage and noted that its acidity at low doses of GLD was significantly reduced, offering a competitive advantage over commercial $CaCO_3$. Recently, Simão et al. (2018) and dos Santos et al. (2019) investigated the use of GLD as a substitute in building materials and ceramic products due to its high calcium carbonate content and low hydraulic conductivity. Thus, there is a renewed interest on the use of GLD waste as a result of its beneficial chemical characteristics.

4.3. GLD and its potential pretreatment mechanism

This paper proposes the use of green liquor dregs (GLD), a kraft pulp and paper mill waste material as a promising pretreatment strategy based on the alkaline pretreatment principle. Although green liquor (GL) is well established as an efficient lignocellulosic pretreatment agent derived from the kraft pulping process (Wu et al., 2012; Gu et al., 2012, 2013), its waste counterpart, GLD, has not yet been evaluated for pretreatment processes. Like GL, due to a similar chemical composition, GLD waste offers numerous pretreatment benefits of no fermentation inhibitors and high delignification under milder pretreatment conditions (short contact time, low temperature and pressure). Interestingly, the presence of the alkaline species ($CaCO_3$, Na_2S and Na_2CO_3) in the GLD, similar to GL, contributes to its strong alkalinity (pH $>$ 10) and is viewed as an advantageous characteristic in pretreatment systems (Golmaei et al., 2018a). Additionally, these chemicals are considered a "dead load" in the pulping process and therefore provide a means of "green" disposal for the paper and pulping industry. Furthermore, it provides a replacement for expensive alkaline pretreatments since it is a waste generated from a thriving kraft pulping industry. The development of effective GLD pretreatments will result in the production of high value fuels and bioproducts.

GLD (pH $>$ 10) can be characterised as an alkaline chemical proposed to mimic effective catalysts such as NaOH (pH 13). Strong alkaline agents cleave the ester bonds between the lignin and hemicellulose polymers by hydrolysis, causing structural damage (Kim, 2013; Saratale and Oh, 2015). GLD has surfaced as a completely disposable waste and is composed of $CaCO_3$, Na_2CO_3 and Na_2S, influencing its strong alkalinity and excellent buffering capacity (Mäkelä et al., 2016). The presence of these individual species has been noted to confer advantageous characteristics within pretreatment systems (Cheng et al., 2010; Gu et al., 2013). The chemical mechanistic effects of GLD consist of combined alkalic salts (Na_2CO_3 and Na_2S) that target various sites in cellulose, hemicellulose and lignin. The Na_2CO_3 component causes cleavage of the ester and glycosidic bonds in the cell wall matrix, therefore resulting in the alteration of lignin structure, cellulose swelling, and the partial decrystallization of cellulose (Cheng et al., 2010). Moreover, the strong nucleophilic attack of the HS^- species of the Na_2S promotes the cleavage of phenolic β-aryl ether bonds of lignin, while removing lignin moieties with limited attack on carbohydrates (Gu et al., 2013). The combined alkalic salt effect of GLD proposes a more efficient and enhanced pretreatment method compared to the commonly used NaOH that only targets specific bonds such as the hydrolysis of the ester bonds between the ferulic acid and hemicellulose causing lignin degradation and lignocellulose particle swelling (Modenbach, 2013). In addition to the proposed pretreatment abilities of GLD, the strategy highlights many benefits attached to an effective pretreatment system, including feasibility, energy usage and efficiency.

Various studies have assessed the potential of lignocellulosic biorefinery technologies and observed that the pretreatment step accounts for up to 40% (equivalent to more than one third) of the total processing cost for the production of biofuels and value-added products (Kucharska et al., 2018). Therefore, finding an alternative to expensive chemicals used in lignocellulosic biomass pretreatment is of utmost importance. The use of GLD lowers lignocellulosic pretreatment operational costs, since GLD is a waste chemical that is discarded by the kraft paper and pulping process. Moreover, its strong alkalinity (pH $>$ 10) requires reaction conditions that are generally milder (lower temperature, pressure and residence time) with little to no problematic enzyme or fermentation inhibitor compounds generated during the chemical pretreatment stage (Zhao et al., 2019). The lower

Table 5
Characteristics and applications of GLD.

Characteristics	Elements/Compounds	Applications of GLD	Reference
Highly alkaline	pH > 10, Na_2CO_3, Mg $(OH)_2$, Na_2S	Neutralising acidic pulp mill wastewater Neutralising acidic soils	Nurmesniemi et al. (2007); Cabral et al. (2008)
High buffering capacity	$CaCO_3$ CaO, $Ca(OH)_2$, calcite	Liming potential to replace commercial agricultural limestone Neutralisation for oxidized mining waste GLD can substitute primary calcareous resources in building materials e.g. soil–cement bricks. Alternative raw material on ceramic products	Johnson and Hallberg (2005); Ragnvaldsson et al. (2014); Sebogodi et al. (2019); Simão et al. (2018); dos Santos et al. (2019)
Low hydraulic conductivity	Calcite	Alkaline sealing barrier that minimizes water percolation and oxygen transport for prevention of acid rock drainage in the mining industry and landfill sites	Jia et al. (2013); Mäkitalo et al. (2014); Farage et al. (2019)
Non-process elements (NPEs)	Ba, Cl, Cr, Cu, Fe, Mn, Ni, P, K, Zn	Low concentrations can be used at forest fertilizers	Manskinen et al. (2011)
Macronutrients	Na, K, Ca, Mg	Fertilizers and soil amendments	Mäkelä et al. (2012)
Micronutrients	Cu, Fe, Mn, Zn	Fertilizers and soil amendments	Mäkelä et al. (2012)

temperature, pressure and residence time minimises the dehydration of uronic acids and pentoses within lignocellulosic feedstocks into furfural and 5-Hydroxymethylfurfural (Jönsson and Martín, 2016). Furthermore, alkaline chemicals are less caustic as opposed to acidic agents, thus, eliminating the need for expensive materials and advanced reactor designs to cope with extensive reaction conditions and corrosion (Kim et al., 2016). The abovementioned characteristics of GLD leads to an effective biomass pretreatment technology that decreases the capital cost and net energy consumption while enhancing the operational function of the bioprocessing facility.

However, like conventional alkaline pretreatments such as NaOH, GLD generates aqueous black liquor (BL) after pretreatment. BL is a toxic and harzardous waste hydrolysate that contains cellulosic and hemicellulosic oligomers, soluble salt ions, alkali and alkali-soluble lignin and cross-linked macromolecules containing aromatic groups such as phenols and catechols. Taking into account the high pH (> 11), chemical oxygen demand (COD), biological oxygen demand (BOD) and presence of suspended solids, correct management of this waste is of high priority due to severe environmental implications (Li et al., 2015b; Pola et al., 2019), thus a sustainable approach for the treatment of BL is required. In recent years, BL has served as a cheap and renewable source of lignin due to its numerous applications and high value as a biopolymer. Several methods such as adsorption, precipitation with an acid, combustion, ultrafiltration and electrocoagulation have been developed to purify lignin from BL (Li et al., 2015b). Once extracted, lignin can be modified as a dispersant for gypsum paste (Matsushita and Yasuda, 2005), a plasticizer for cement (Kamoun et al., 2003), a heavy metal chelator (Sena-Martins et al., 2008) or an additive in coatings and paintings (Sena-Martins et al., 2008). After the separation of lignin, the non-lignin fraction containing hemicellulose, a naturally synthesized biopolymer, shows to be a promising sustainable raw material with a wide range of valuable properties, such as biodegradability and bioactivity (Luo et al., 2019; Mendes et al., 2017). In addition to the abovementioned residues, carboxylic acids may also be directly purified from BL. It has also been noted that some constituents of BL may be involved in pretreatment technologies and its recycling concept plays a major role in decreasing its potency, accordingly (Cha et al., 2016). Additionally, organic compounds in BL have been observed as effective solvating agents of lignin within corn stover for enhanced enzymatic hydrolysis (Xu et al., 2012). A recent study by Goshadrou et al. (2019) employed a BL pretreatment strategy that substantially improved the enzymatic hydrolysis of cogongrass from 24.8% to 90.8% and thereafter it progressively decreased to a minimum value of 66.4% due to sequential BL recycling. Nevertheless, previous studies have observed that the addition of a non-ionic surfactant maintains the high conversion rates of fermentable sugars (Wang et al., 2016; Goshadrou et al., 2019). Therefore, the challenges observed with the proposed GLD pretreatment could be considered as opportunities to further produce high value products whilst reducing operating issues and environmental concerns.

5. Challenges and future prospects

5.1. Current lignocellulosic pretreatments

Various pretreatment methods have been analysed as effective catalysts for the successful breakdown of recalcitrant moieties from lignocellulosic waste. Chemical pretreatments have shown favourable results with regards to fermentable sugar recovery of approximately 80% as opposed to pretreatments employed without chemicals. Nevertheless, these significant yields have been masked by the major drawbacks that hinder its implementation on a commercial scale. Acid hydrolysis produces toxic inhibitor compounds, cause corrosion to the reaction vessels and is expensive. Alkaline chemicals have demonstrated various advantages over acid treatment, since it results in high sugar yields with minimal damage to reactors. Nevertheless, the high cost limits alkaline-based methods. Ionic solvents and organosolvents also encounter increases in inhibitor concentration, are not easily recyclable and incur excessive costs.

Apart from the aforementioned chemicals, green liquor (GL), an alkaline mixture of sodium carbonate and sodium sulfide, is a by-product that is derived from the kraft pulp mill and has previously been used for pretreatment processes. Despite the potential of GL as an attractive pretreatment alternative, it is diverted back into the kraft pulping cycle for the conversion of sodium carbonate to NaOH in the causticizing process. The GL recyclability is a crucial step that is necessary in the kraft pulping process during paper making, which raises concerns on its use as a lignocellulosic pretreatment strategy. Therefore, the development of chemical waste pretreatment systems is highly sought after.

5.2. Green liquor dregs waste for lignocellulosic pretreatment

Green liquor dregs (GLD), a kraft pulp and paper mill alkaline waste has sparked interest as an effective lignocellulosic pretreatment technique. The solid GLD has presented problems for the fibre line of the kraft pulping process and is landfilled, which adds to the process costs and raises environmental concerns. Therefore, the application of GLD in alternative processes such as lignocellulosic pretreatment in an effort to mitigate the aforementioned issues while adding value to the waste should be explored. The GLD waste consists of various alkaline species (Na_2S and Na_2CO_3) that can be channelled towards alkaline pretreatment as well as excellent buffering capabilities due to the presence of $CaCO_3$. Additionally, each compound within the GLD may target specific sites of the lignocellulosic biomass, thus increasing its pretreatment effect. There is a lack of knowledge on GLD as a potential alkaline

treatment with its lignocellulosic pretreatment efficiency yet to be discovered.

Another challenge for the biorefinery process is the extreme quantities of water that are required for both pretreatment and conditioning of lignocellulosic wastes. Water is known to be the greenest solvent in pretreatment technologies and strongly impacts the efficiency of thermochemical conversion and delignification mechanisms of the biomass. However, most countries around the world experience variable rainfall, therefore, water availability is of dire concern. The extensive use of water during the biorefinery process poses a major hindrance to the pretreatment of recalcitrant waste material. A possible solution to this problem is the application of wastewater from industrial production lines. The kraft paper and pulping industry produces an alkaline paper wastewater that requires disposal at the end of the production process. This disposal outlet can be diverted to the pretreatment of lignocellulosic waste that serves both as a water source and an alkaline enhancement to the proposed GLD treatment strategy. Using wastewater during pretreatment technologies not only decreases production cost and relieves the water crisis, but also results in the beneficiation of problematic industrial effluent to valuable commodities. The optimization of these pretreatment parameters will lead to the development of an economically viable and efficient system, which enables increased sugar yields for microbial fermentation processes towards biofuels and value-added products.

6. Conclusion

Efficient pretreatment techniques are imperative for the breakdown of recalcitrant lignocellulosic substrates. The kraft pulping industry has long since been looked towards for alkaline chemicals that may be harnessed for lignocellulosic pretreatment. However, the feasibility of these alkaline chemicals for lignocellulosic pretreatment still faces major scrutiny. Conversely, green liquor dregs (GLD), an alkaline waste generated from the kraft industry has recently attracted attention as a potential pretreatment catalyst. This review discusses the recent developments in the application of kraft pulping alkaline chemicals for lignocellulosic pretreatment and the potential beneficiation of GLD. Additionally, the challenges and prospects of lignocellulosic pretreatments are highlighted.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge the National Research Foundation (NRF) of South Africa (Grant numbers 115465 and 122080) for providing funding towards this research. Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily attributed to the NRF.

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CHAPTER 3

Extended Literature review

This chapter elaborates on aspects which are not covered in the published literature review in Chapter 2. It explores integrated biorefinery systems, covering outlooks such as microbial lactic acid (LA) fermentation processes, the potential of low cost minimally supplemented media formulation, process optimization and artificial intelligence modelling, kinetic studies, and scale-up strategies.

Advancements in Lactic Acid Production: Harnessing minimally supplemented dairy wastewater media for sustainable bioprocessing, optimization and modelling

Abstract

The escalating demand for lactic acid (LA) necessitates the reassessment of conventional production methods, owing to the significant cost and resource burden, particularly due to its nutrient-rich media formulations and requirement for freshwater. The paradigm shift towards a minimally supplemented medium formulation that incorporates wastewater in place of freshwater challenges the costly traditional De Man, Rogosa and Sharpe medium, by strategically selecting cost-effective raw materials and tailoring its compositions for enhanced LA production. The dairy industry provides a major supply of dairy waste, particularly dairy wastewater (DWW) that can be diverted from effluent disposal to the bioprocessing of LA. This review delves into the potential incorporation of economical enhancement agents (nitrogen source, carbon source and surfactant) in a DWW medium and the exploration of different physicochemical parameters (buffer agents, pH effects, MnO nanoparticles, and micronutrients) for LA production. It also navigates through various bioprocess types, process optimization strategies, kinetic modelling, and scale-up optimization with emphasis on resource efficiency and economic feasibility. Furthermore, the current challenges and future perspectives are highlighted.

Keywords: Lactic acid, Minimally supplemented media, Dairy wastewater, Optimization, Kinetic modelling, Scale-up, Value-added products

3.1. Introduction

The ever-growing global population is expected to rise to 9.6 billion by 2050 according to the International Energy Outlook (2023). This exponential population growth has led to a surge in the overall universal demand of fossil fuel-derived energy and commodity chemicals, while placing tremendous strain on industrial markets (Song et al., 2019). Recently, there has been significant research surrounding the implementation of a carbon-neutral bioeconomy and the commercialization of lignocellulosic derived-microbial fermentation processes for the production of value-added bioproducts (Diaz et al., 2018). These bioproducts include biofuels (bioethanol, biodiesel, biohydrogen), biochemicals (lactic acid, itaconic acid, succinic acid) and biopolymers (polyhydroxyalkanoates, polylactic acid) among others (Diaz et al., 2018). One such bioproduct of interest is lactic acid (LA), since it is a robust organic acid that has high commercial value, due to its wide variety of applications in food, cosmetics, pharmaceutical and other commodity chemicals (Nwamba et al., 2021). Moreover, due to the sparked interest in bioplastics such as polylactic acid (PLA), it is estimated that the LA market will drastically increase to facilitate its production (Tian et al., 2018). The production of LA can be achieved either by the chemical synthesis or microbial fermentative routes. The latter accounts for approximately 90% of the LA production worldwide and gives rise to either D-LA, L-LA or a mixture, based on the choice of the LA producing microorganism, known as LABs and specific microbial strains (Ahmad et al., 2020). LABs include bacteria belonging to genus *Lactococcus, Lactobacillus, Bifidobacterium, Streptococcus* and *Leuconostoc*, amongst others. In particular, *Lactobacillus* strains are known to be the most economically important for the production of LA, attributable to its high acid tolerance, enhanced yield and productivity as well as amenability to genetic engineering (Kylä-Nikkilä et al., 2000). Moreover, species selections are also based on its enantiomeric configurations, desirability in industrial applications and nutritional requirements (Abedi and Hashemi, 2020;

Macedo et al., 2020). The latter characteristic can influence the LABs carbon metabolism and drives it either towards the homofermentative or heterofermentative pathway (Figure 3.1). The homofermentative pathway converts monosaccharides almost solely to LA, with a theoretical yield of 1 g/g (Martinez et al., 2013). On the other hand, heterofermentative metabolism gives rise to split pathways with different products such as LA, acetic acid, ethanol and carbon dioxide, with a theoretical LA yield of 0.5 g/g (Abdel-Rahman et al., 2011). In terms of economical relevance, homofermentative LABs are desired as a result of high yields (almost 100%), high optical purity (>99%) and productivity (Abedi and Hashemi, 2020). LA pathways are also streamlined according to their respiratory needs. LABs are known to be facultative anaerobes, whereby they can grow in the presence or absence of oxygen (Sano et al., 2020). Generally, under anaerobic conditions, LABs tend to generate energy through glycolysis by converting pyruvate to lactate (Sano et al., 2020). Conversely, in the presence of oxygen, the minimal electron transport chain is activated, thus promoting high energy metabolism for increased biomass growth and replication (Smetanková et al., 2012). Some microbial strains can represent a mixed respiro-fermentative metabolism, in which these microorganisms can switch metabolic routes upon availability of oxygen (Sewsynker-Sukai and Gueguim Kana, 2018). For this reason, knowledge of the mechanistic pathways and influence of oxygen requirements will notably impact LA process design for industrial scale-up.

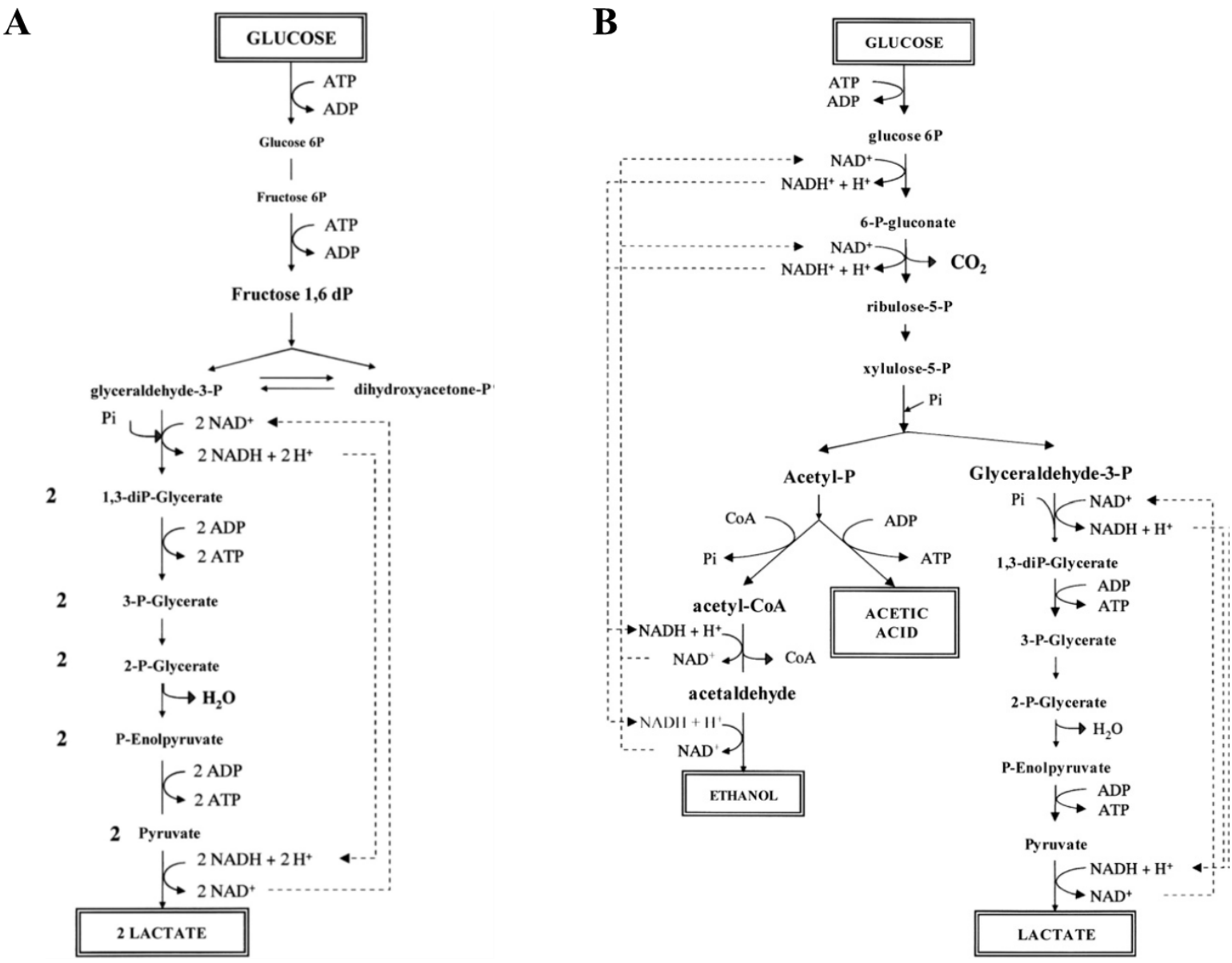

Figure 3.1. Schematic diagram of the (A) homofermentative and (B) heterofermentative pathways of glucose fermentation in LA bacteria (Adapted from Martinez et al., 2013; modified).

Lignocellulosic LA production is still in the infancy stage within the global markets, due to its low economic viability. Lignocellulosic substrates particularly derived from sources such as agricultural biomass (corn, sorghum, sugarcane, rice, and wheat), are waste components produced in large quantities and does not hinder food security, therefore presenting a

promising feedstock for biorefinery systems (Karimipour-Fard et al., 2024). The wastes generated include corn cobs, sugarcane leaves and bagasse, sorghum chaff, rice straw, and wheat straw, amongst others. It constitutes a complex matrix and rigid structure comprising cellulose (38–50%), hemicellulose (23–32%), and lignin (15–25%) (McKendry, 2002). More specifically, lignin is a complex aromatic polymer that acts as a protective shield around the cellulose and hemicellulose moieties, and its intertwining nature creates a matrix that is resistant to enzymatic and microbial degradation (Mahmood et al., 2019). Nevertheless, lignocellulosic pretreatment is a critical step in the processing of lignocellulosic biomass (LCB), aimed at disrupting its intricate structure. It facilitates enhanced enzymatic accessibility, essential for maximizing sugar release during subsequent saccharification towards LA fermentation stages (Chakraborty et al., 2024). In essence, the journey from lignocellulosic substrates through pretreatment to LA production underscores the intricate interplay of biological and chemical principles, offering a pathway toward more sustainable and economically viable bioprocessing while achieving complete valorization of waste-based substrates. Therefore, intervention of novel methodologies and cost-effective strategies is crucial to overcome the technological challenges and enhance the economic feasibility of lignocellulosic LA production, aligning with the principles of the circular bioeconomy. Implementing innovative approaches, optimizing bioprocessing steps, and integrating sustainable practices can pave the way for its widespread adoption and commercial success in the global market.

This chapter explores the implications of dairy waste effluents on the environment, recognizing the ecological concerns associated with its discharge after expensive treatment. Subsequently, the chapter delves into the potential of dairy wastes as valuable substrates for biofuel and bioproduct generation, highlighting the diversity of high-value compounds that can be derived. Thereafter, specific emphasis is placed on the potential of dairy wastewater

(DWW) in LA production, in addition to its composition and inherent characteristics for microbial fermentation. Expanding on this, the chapter explores strategies for the minimal supplementation of DWW with essential components (nitrogen source, carbon source and surfactant) and the exploration of different physicochemical parameters (buffer agents, pH effects, and micronutrients) necessary for optimizing LA production. The narrative then shifts towards the crucial aspects of process optimization and the integration of Artificial Intelligence (AI) modelling that offers a cutting-edge approach to optimize and predict outcomes in the complex bioprocessing landscape. Furthermore, it taps into kinetic modelling, with evaluation into the dynamics governing microbial cell growth and LA production. The review culminates in addressing the strategies associated with scaling up the bioprocess from laboratory to bioreactor levels, outlining different mixing efficiencies and the potential of utilizing dairy waste in large-scale applications. Lastly, the challenges and future perspectives toward enhancing lignocellulosic LA production are presented.

3.2. Generation of dairy waste in the dairy processing industry

In the last few decades, the rapid growth of global populations has led to a rapid pace of industrialization in sectors such as agriculture, food production, animal farming, and processing (Usmani et al., 2022). In particular, the dairy industry has seen significant development of innovative technologies aimed at increasing the productivity of milk and milk-based products to serve the tremendous upsurge of the global populace. As reported by the food and agriculture organization (FAO) of the United Nations, the global milk production stood at ~930 million tons in 2022 (FAO, 2023). This was comparable with a total global milk production of 906 million tons accounting for a 2.58% increase and 928 million tons (0.22% increase) in 2020 and 2021, respectively (FAO, 2021; FAO, 2022). While catering to the needs of the population, the dairy industry processing plants release large

quantities of polluting agents into the environment due to substantial use of water and emission of effluents. In fact, at every stage of the dairy production and processing, significant amounts of waste are generated from the dairy farming, processing, transportation, and packaging of products (Usmani et al., 2022). This waste mainly comprises of processing waste, spoiled goods, and mishandled products and materials (Mahboubi et al., 2017). It has been estimated that the annual dairy waste residues of ~4–11 million tons are generated globally in the form of solid waste and effluents while 0.2-10 L of wastewater is produced per litre of processed milk (Usmani et al., 2022).

3.2.1. Implications of dairy wastewater effluent release

While the dairy industry generates substantial volumes of waste, the release of these wastes into the environment poses significant challenges. One such implication is the breakdown of the milk proteins in the waste raw milk containing various organic and inorganic functional groups of nitrogen including NO^{-2}, NO^{-3}, NH^{+4} (14–830 mg/L of total nitrogen concentration) which may be converted to nitrites, thus contaminating groundwater, causing methemoglobinemia (Kavitha et al., 2019). Furthermore, DWW is typically characterized by high chemical oxygen demand (COD=1-10 g/L) and biological oxygen demand (BOD=0.3-5.9 g/L) (Kothari et al., 2017; Yonar et al., 2018). The high organic load leads to a rapid depletion of dissolved oxygen in aqueous systems when the effluent is directly discharged, thus inadvertently harming the aquatic ecosystems and consequently impacting the overall environment (Usmani et al., 2022). In addition, the high BOD and COD in the presence of lactose within the dairy effluent can encourage the growth of specific microorganisms that produce organic acids and alcohols, further exacerbating water quality issues (Slavov, 2017). More so, the oily and greasy fat residues may form a film on the surface of water bodies, thus preventing the oxygen transfer and adversely impacting the aquatic flora and fauna (Kavitha

et al., 2019). Overall, the consumption of dairy products by humans necessitates stringent hygiene standards in dairy processing facilities, leading to the use of surfactants and detergents that ultimately end up in the generation of waste effluents and solid wastes. For this reason, DWW requires adequate management for environmental and human safety prior to release, based on the wastewater characterization, treatability studies, planning of proper units and processes for effluent treatment. The pollution regulatory board has assigned discharge standards for industrial effluent treatment and hence, it must meet the effluent discharge standard norms before releasing the treated effluent on to land or any surface water body (Sivaprakasam and Balaji, 2021). This waste effluent is broadly classified as biological and chemical components, consisting of biodegradable organic materials and non-biodegradable solids. Therefore, the general routes for treatment of dairy waste include physicochemical and biological methods. The biological strategies consist of anaerobic and aerobic treatments such as activated sludge processing, up-flow sludge anaerobic blanket (USAB), sequential batch reactors (SBR), moving bed biological reactor (MBBR), wetlands and co-composting amongst others (Kwapinska et al., 2020; Ahmad et al., 2019; Zhou et al., 2015). In addition, physicochemical methods such as flocculation, flotation, adsorption, membrane filtration, coagulation, and nanofiltration are also initiated (Awasthi et al., 2022). More importantly, handling and treatment of this waste residue is a significant challenge that makes up almost 60% of total treatment cost in the processing unit (Kwapinska et al., 2020). New technologies are required to bypass the cost and energy intensive processes to enable more flexible, stable, clean, and energy-saving procedures for DWW treatment (Roufou et al., 2021a, Roufou et al., 2021b).

3.2.2. Potential of dairy waste in bioprocessing

One striking feature of dairy waste is the rich organic nature, which makes it a favourable medium for the sustenance of microbial communities. This characteristic makes it an ideal medium to facilitate the production of a diverse range of value-added products through microbial bioprocessing (Lappa et al., 2019) (Figure 3.2). The application of dairy waste effluents in microbial media provides an alternate disposal means to the dairy industry by completely by-passing its effluent treatment process, while simultaneously supplying bioprocessing units with sufficient water, without the implications of consuming freshwater resources.

Systematic valorization and biorefining of the extensive industrial dairy waste through various microbial bioprocesses brings to light a new perspective on the effective dairy waste management and economic benefit for the vast and continually expanding global dairy market. In that respect, manipulating the beneficial properties of dairy waste effluents as a medium for the generation of value-added products has become a potential pathway for utilization (Table 3.1). For instance, the production of biofuels such as bioethanol and biobutanol from dairy whey are prospective biorefinery approaches due to its stable and eco-friendly nature. In particular, Nooshkam et al. (2018), reported ethanol yields ranging from 30g/L to 35 g/L through the hydrolysis and subsequent anaerobic fermentation of lactose in whey powder. The study employed various yeast species, including *Kluveromyces marxianus* DSMZ-7239, immobilized *Saccharomyces cerevisiae* and *Candida inconspicua* to achieve these yields (Nooshkam et al., 2018). Moreover, dairy waste is rich in proteins and moisture, creating favourable conditions for the growth of bacterial communities which can produce biosurfactants by using the waste as the carbon source. Vera et al. (2018) applied *Lactococcus lactis* CECT-4434 on 15% whey, resulting in 8.9 mg/L production of

biosurfactant with a surface tension reduction of ~18.1 mN/m. Another important area of innovation lies within the production of antioxidant and anticancer bioactive peptides from dairy whey, utilizing the proteolytic activity of microorganisms, starter cultures and enzymatic hydrolysis facilitated by digestive enzymes (Mohan et al., 2015). In an attempt to produce antihypertensive peptides that boost angiotensin-converting enzyme activity during gastrointestinal digestion, Alvarado et al. (2019) used whey protein hydrolysate as the carbon source, resulting in a 10% increase in enzyme activity. Dairy effluent containing sugar lactose and dissolved lipids have also been harnessed as feed substrates for the production of biopolymers such as polyhydroxyalkonates (PHA) and polyhydroxybutyrates (PHB). Recently, Liu et al. (2021) employed a recombinant *E. coli* strain in untreated acid whey, achieving a process yield of 4 g/L of PHB during growth phase.

Over the years, there has been a consistent demand for organic compounds as additives in industrial processes involving food, beverages, cosmetics, and pharmaceuticals. These platform chemicals are employed for the preservation, stabilization, emulsification, acidification, and enhancement of flavours in consumable products (Usmani et al., 2022). Dairy waste in various forms is a potential medium for organic acids production such as acetic, citric, succinic, propionic and LA (De Jesus et al., 2015). A recent study by Longanesi et al. (2018) indicated that using *Actinobacillus succinogenes* ATCC 55,618 in deproteinated cheese whey was able to produce 0.72 g/L of succinic acid per hour. Another study by Jiang et al. (2015) illustrated the potential of immobilized *Propionibacterium acidipropionici* on whey lactose to generate propionic acid (135 ± 6.5 g/L), an important chemical for the preservation of vitamin B12 in the production process. Pandey et al. (2019) also states that treating cheese whey with *Lactobacillus acidophilus* to produce biohydrogen may also result in byproducts like acetic, lactic, pyruvic, formic, propionic, and butyric acid. More

specifically, LA production has been reported using whey permeates by the application of *Lactobacillus casei* to produce a maximum concentration of 33.7 g/L (Ricciardi et al., 2019).

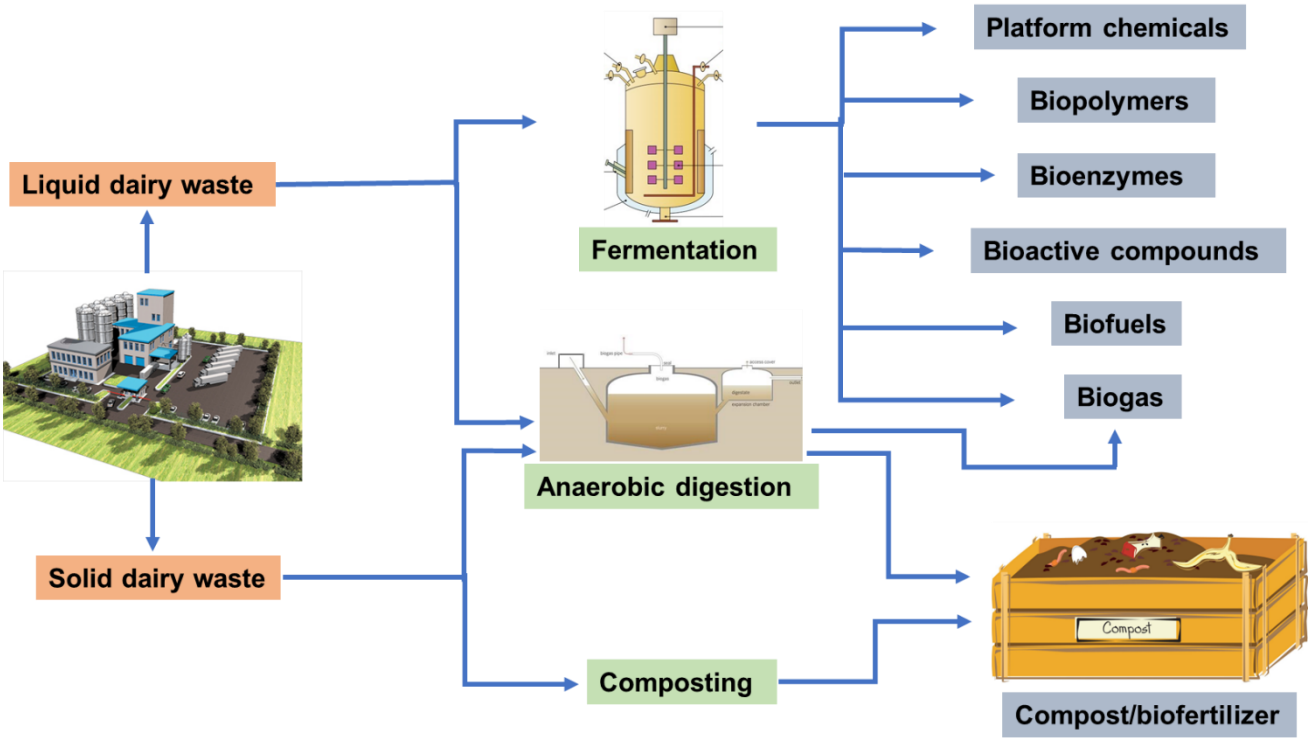

Figure 3.2. Overview of bioproducts generation using dairy waste.

Table 3.1. Microbial fermentation for biofuels and commodity chemical production using dairy waste

Dairy waste	Bioproduct	Reference
Dairy whey powder	Bioethanol	Nooshkam et al. (2018)
Dairy whey	Biosurfactants	Vera et al. (2018)
Dairy whey	Antioxidant and anticancer bioactives	Mohan et al. (2015)
Whey protein hydrolysate	Antihypertensive peptides	Alvarado et al. (2019)
Raw acid whey	polyhydroxybutyrates	Liu et al. (2021)
Deproteinated cheese whey	Succinic acid	Longanesi et al. (2018)
Cheese whey	Biohydrogen	Pandey et al. (2019)
Whey permeates	Lactic acid	Ricciardi et al. (2019)
Whey lactose	Propionic acid	Jiang et al. (2015)
Crude cheese whey and wastewater	Biohydrogen	Mete et al. (2024)
Dairy wastewater	Bioethanol	Dhandayuthapani, et al. (2022)
Dairy waste paneer whey	Lactic acid	Verma et al. (2023)
Dairy sludge	L-glutamate	Ghazanfari et al. (2023)

3.3. Lactic acid production

3.3.1. Potential of dairy waste for lactic acid production

Although solid waste generation such as cheese whey, curd and whey permeate amongst others are well established as favourable substrate for bioconversion to value-added products, the wastewater effluent derived after filtration is seen as an excellent freshwater substitute and nutrient enhancement platform. This review proposes the use of DWW (DWW) effluent as a promising baseline medium for the production of valuable bioproducts, in particular, LA. Interestingly, DWW consists of residual nutritional components such as carbohydrates, soluble organic compounds, nitrogen and protein that assist in stimulating the growth of several LABs (Kaur, 2021). DWW contains elements such calcium, essential for intracellular signalling and regulation of multiple cellular processes of LAB species such as cell division and development, stress response and motility, amongst others (Domínguez et al., 2015; King et al., 2020). It acts as a cofactor for certain enzymatic pathways involved in LA production. Potassium is another important nutrient as it is involved in maintaining cell osmotic balance and pH regulation, which are critical for the survival and growth of these bacteria during fermentation. The DWW consists of manganese (Mn^{2+}) and magnesium ions (Mg^{2+}) that serve as cofactors of enzymatic reactions involved in the overall metabolic function of LABs. These ions also synergistically improve the binding affinity of the enzyme-substrate complex during carbohydrate metabolism (Yu et al., 2008, Lew et al., 2013). Moreover, both Mg^{2+} and Mn^{2+} are integral in the biosynthesis of lipoteichoic acid and peptidoglycan within microbial cell walls (Lew et al, 2013). In addition, phosphorus is a key component of nucleic acids, adenosine triphosphate (ATP), and other cellular molecules that necessitates bacterial cell growth and LA production. Nitrogen is critical for the growth of LABs since it provides the building blocks in the synthesis of key biomolecules such as amino acids, proteins, nucleotides, and coenzymes involved in metabolism (Ye et al., 2018). Moreover, organic carbon, such as sugars or other carbohydrates, are the primary substrates for LA fermentation in which LABs use it as a source of energy.

3.3.2. Approaches in the supplementation of dairy wastewater for lactic acid production

The successful production of LA is reliant on various essential nutrients, trace elements, and suitable carbon sources to maintain a balanced medium. This is due to LABs being auxotrophic, meaning it requires an external source of complex nutrients such as carbon, phosphorus, nitrogen, vitamins, and minerals for growth, since they lack many biosynthetic capabilities (Chen et al., 2020a). In the case of LA production, the widely used De Man, Rogosa and Sharpe (MRS) media is a nutrient-rich source that is routinely used for the cultivation of various LABs. Nevertheless, due to the specific nutritional requirements of *Lactobacilli*, the cost associated with the intricate supplementation of MRS media has limited its utilization to laboratory-scale cultivation and fermentative operations. Therefore, strategically selecting raw ingredients and formulating optimum fermentation media that are both effective and economically feasible is considered one of the most crucial stages in the advancement of LA processes towards large-scale production. This has led to the innovation of supplementing the existing DWW effluent from the dairy industry with inexpensive alternatives. This novel media formulation will enable a sustainable biorefinery production facility that will not only deal with the burden of waste disposal in the dairy industry and freshwater consumption in LA generation but will also extract the benefits of various nutrient supplements to enhance its commercial value to the fullest potential (Awasthi et al., 2019).

As mentioned above, biological processes such as LA fermentation thrive with an adequate nitrogen source, due to its fundamental role in the biosynthesis of key constituents such as amino acids, proteins, and nicotinamide adenine dinucleotide (NAD) that are essential for cell functioning (Martinez-Burgos et al., 2021). The standard MRS media contains three different nitrogen sources in the form of peptone, meat extract and yeast extract with the latter being the main nitrogen contributor but also the most expensive, accounting for approximately 30%

of the production costs (Tang et al., 2013). Consequently, researchers are in search of nitrogen sources that meet the requirements for LA fermentation with cheaper costs attached. Previously, studies have shown use of cheaper alternatives to yeast extract such as protein hydrolysate, soybean meal and corn steep liquor (CSL), amongst others (Mis Solval et al., 2019; Jiang et al., 2019; Li et al., 2021). More specifically, CSL is an emerging nitrogen source in LA production due to its rich composition of amino acids, polypeptides, inorganic salts, and considerable amounts of vitamin B-complexes, as indicated by Hofer et al. (2018). These essential nutrients are functional aspects in satisfying the growth of LABs, including *Lactobacillus s*pecies. As a byproduct of the corn wet milling industry, CSL offers several advantages, including cost-effectiveness approximating one fifth of the cost of conventionally used yeast extract, secured availability, and its ability to support robust bacterial growth and high LA yields (Tan et al., 2016). Therefore, CSL can be used for the partial or total substitution of yeast extract within the fermentation medium to assist in the overall cost reduction, sustainability and efficiency while maintaining all essential nutrients required for microbial cell growth and product formation.

As focus shifts to the source of energy for microbial fermentation, carbon sources such as pure glucose, which has conventionally been used in fermentation media, is no longer a sustainable or viable option in the progression of green bioeconomy. LCBs are regarded as renewable residues that hold promise as an attractive carbon source for the fermentative synthesis of valuable industrial commodities such as LA. In this context, corn cob waste (CCW) stands out a potential raw material for extracting fermentable sugars, owing to its widespread global availability, rich carbohydrate content and low lignin content (Potumarthi et al., 2012). Nonetheless, the commercialization of LA production from lignocellulosic sources has faced challenges in light of expensive and resource-intensive processes. These difficulties often result in low product yields and costly downstream procedures (Kumar and

Sharma, 2017). To address these shortcomings, there is a growing need for approaches that reduce costs, energy consumption, and resource utilization while concurrently achieving high sugar yields. In line with this, alkaline Kraft pulping and paper waste, green liquor dregs (GLD) and paper wastewater (PWW), offers a promising solution as an innovative waste-based pretreatment method for use in the lignocellulosic biorefinery.

Another component of interest within commercial MRS media is the presence of Tween 80, a non-ionic surfactant that contains a mono-unsaturated fatty acid (oleate). The presence of Tween 80 was shown to have a multi-fold effect within lignocellulosic microbial fermentation processes (Taoka et al., 2011; Zhang et al., 2018). For instance, within a microbial suspension, it allows for enhanced permeability and reduced surface tension of the cell membranes resulting in improved nutritional uptake of nutrients surrounding the cell body (Taoka et al., 2011). Additionally, Tween 80 has been employed as an emulsifying agent where immiscible compounds or the distribution of nutrients is critical (Taoka et al., 2011). It can improve the solubility and bioavailability of complex hydrophobic nutrients such as fats and oils in the fermentation medium. Moreover, it aids in dispersion and stabilization of the insoluble compounds in the aqueous medium, ensuring better contact with the microbial cells. Furthermore, it enhances enzymatic hydrolysis of LCB by promoting strong interactions between the substrate-enzyme complex, increasing stability, and reducing unproductive enzyme binding (Zhang et al., 2018). In some cases, Tween 80 has also been proposed to enhance the stress tolerance of LABs by protecting the cells from environmental stresses such as osmotic stress as well as stabilizing cell membranes and improving its integrity.

Extending on the essential requirements of a LA fermentation medium, the effects of additional supplementations such as buffers and micronutrients necessitates further research

for enhancing LA production. Buffer agents are of paramount importance in the LA synthesis processes. Its primary role is to ensure pH stability, thus providing a conducive environment for LABs to thrive. LABs are highly sensitive to fluctuations in pH and its microbial growth and metabolic activity is reliant on an optimal pH range, typically below pH 4.5 to 6.5 (Anagnostopoulou et al., 2022). Accordingly, careful selection of an appropriate buffer system is essential to facilitate optimal LA production. Buffering agents encompass a variety of compounds, including K_2HPO_4, $NaHCO_3$, $Na_2HPO_4 \cdot 12H_2O$, $Na_3C_6H_5O_7$, Na_3PO_4 and $CaCO_3$ amongst others. Therefore, it is essential to validate the compatibility and effectiveness of the selected buffers through laboratory-scale experiments prior to scaling up to industrial applications. Specifically, $CaCO_3$ is a widely utilized neutralizing agent in LA mediums. It essentially aids in preventing excessive acidification, a condition that could prove detrimental to the viability of the LAB (Yanga et al., 2015). $CaCO_3$ reacts with LA to form calcium lactate salt and carbon dioxide gas, thereby initiating a shift towards a neutral pH environment (Wang et al., 2014). In its initial form, $CaCO_3$ exhibits alkaline properties, creating an environment less conducive for the growth of contaminating microorganisms that may contend with LABs during fermentation.

In addition to buffer agents, the supplementation of micronutrients is a significant factor in achieving optimal bacterial growth, increasing biomass production, enhancing overall fermentation performance, and maximizing yield generation. In this case, $MgSO_4$ ($Mg^{2+)}$ and $MnSO_4$ (Mn^{2+}) act as cofactors to enhance the catalytic activity of enzymes in biosynthetic pathways. It also works to synergistically improve the enzyme-substrate binding affinity in carbohydrate metabolism (Yu et al., 2008, Lew et al., 2013). More so, Mg^{2+} ions bolster the stress tolerance of LABs in challenging circumstances characterized by high osmolarity, low pH and elevated fermentation temperatures (Archibald and Fridovich, 1981; Watanabe et al., 2012). Conversely, Mn^{2+} ions maintain the redox balance of reducing equivalents such as

NADH (nicotinamide adenine dinucleotide) and NADPH (nicotinamide adenine dinucleotide phosphate) by ensuring availability of the necessary reducing power for LA synthesis. The Mn^{2+} ions also act as an antioxidant defence system against reactive oxygen species (ROS) that are sometimes produced during LA fermentation (Archibald and Fridovich, 1981; Watanabe et al., 2012). Manganese-containing enzymes, such as superoxide dismutase effectively scavenge and neutralize ROS, safeguarding LABs from oxidative stress and preserving its cellular components. The remarkable capabilities of these micronutrient capabilities have prompted the development of nanoparticles, offering several potential advantages over its chemical counterpart. Notably, Manganese oxide (MnO) nanoparticles exhibit unique physicochemical properties at a nanoscale with its high surface-to-volume ratio and distinctive surface properties that enable efficient adsorption and activation of reactant molecules, thereby enhancing LA production (Sanusi et al., 2020). It has garnered much traction for its recovery and reusability potential, thus contributing to cost-effectiveness and sustainability of the production process. It is worth highlighting that the utilization of MnO nanoparticles in LA production remains an active area of research since the application of micronutrient nanoparticles opens up new possibilities for precise and efficient delivery of essential elements to LABs. This promotes specific target delivery, improved nutrient absorption, and overall effectiveness in LA bioprocessing (Powell et al., 2010; Abdelsalam et al., 2016).

3.3.3. Bioprocess types for lactic acid production

Another important consideration for lignocellulosic LA production is the choice of bioprocess system, namely, (1) separate hydrolysis and fermentation (SHF), (2) simultaneous saccharification and fermentation (SSF) and (3) prehydrolysis with SSF (PSSF) (Carrillo-Nieves et al., 2017). The SHF process involves separate enzymatic hydrolysis and

fermentation steps at different optimal temperatures, leading to extended processing times, operational costs, and risks of contamination. In contrast, SSF combines hydrolysis and fermentation in a single vessel with a median temperature, eliminating lengthy process time and the exposure to cellulase inhibition. Interestingly, PSSF bridges the gap by incorporating a short prehydrolysis step (6-24 hr) before fermentation, optimizing both the enzymatic saccharification and fermentation (Sewsynker-Sukai and Gueguim Kana, 2018). Nonetheless, the process exhibits slightly longer processing time and higher costs than the SSF process. While SSF demonstrates advantages for large-scale applications, both the SSF and PPSF processes require comparative assessment in terms of economics, productivity, and yield output. All three bioprocesses (SHF, SSF and PSSF) have been explored extensively in our book chapter (Sewsynker-Sukai et al., 2023a; Sewsynker-Sukai et al., 2023b) attached as appendix B (*Section 10.3*) and appendix C (*Section 13.6*).

3.4. Bioprocess optimization and modelling

3.4.1. Process optimization

In the past, researchers often took part in the arbitrary selection of process variables or raw material combinations for achieving certain product characteristics as its lignocellulosic bioprocess optimization strategies. However, this practice is often without consideration for relevant experimental designs and in such situations, it is difficult to compare the data since it precludes ideas concerning interactions between process variables, sensitivity of specific variable combinations and effects of the process variables on the product quality features in question. In addition, the relationships between process variables and product responses necessitate the evaluation of a large number of process variable combinations. To achieve reliable bioprocess optimization with a single-dimensional search, the one variable at a time (OVAT) approach, whereby changing one variable while fixing the other variables at a

certain level is necessary. Nevertheless, this methodology can be laborious and time consuming especially when it includes a large number of variables (Ratnam et al., 2005). The primary goal of optimization is to provide a precise map of the path that has the highest probability towards successful product formation (Ruguo, 1999; Crapiste, 2000).

To develop and optimize lignocellulosic bioprocesses, studies have found interest in the use statistical analysis tools such as Response Surface Methodology (RSM) that use experimental designs and exploratory data to predict process outputs in relation to the input parameters presented in multivariable systems (Kucharska et al., 2018). In the context of lignocellulosic bioprocessing, RSM plays a critical role in optimizing various parameters to enhance the production of biofuels, enzymes, platform chemicals, or other valuable products from LCB. RSM models have important applications in the design, analysis and optimization of existing products and unit operations, its use decreasing thus the volume of experiments, reagents, time, financial input, energy, among others (Montgomery, 2009). In accordance with its demand, several experimental designs have been applied in lignocellulosic bioprocesses in order to achieve the most appropriate combination of factors that will render the best characteristic of a product and/or process response. Some of these designs include full factorial, central composite, mixture and box-Behnken designs amongst others. Moreover, based on the purpose of design and availability of data generated, it is important to note that in order to achieve a final objective, it is sometimes necessary to use a sequence of two or more designs (Granato and de Araujo Calado, 2014). The full factorial design is a systematic approach where all possible combinations of the levels of each factor are evaluated. It provides a complete illustration of how each factor independently and interactively affects the output response (Granato and de Araujo Calado, 2014). However, the full factorial design can be resource-intensive if the number of factors and levels are high, resulting in a large number of experiments and may not be feasible for complex or high-dimensional issues. Furthermore,

the Central Composite Design (CCD) extends the full factorial design by adding centre points that are augmented with a group of corner and star points for the estimation of curvature, allowing for fitting a second-order polynomial model, covering both linear and non-linear responses (Annor et al., 2009). This model permits for the identification of critical process parameters and their optimal levels. This particular design efficiently models quadratic responses and interaction effects, reduces the number of experimental runs compared to a full factorial design and provides insights into curvature of the response surface. Regardless of these advances, the CCD requires a significant number of experimental runs, especially as the number of factors increases. In addition, mixture designs are used when the factors are proportions or percentages that sum to a constant (Granato and de Araujo Calado, 2014). It addresses cases where the total composition remains constant (sum of proportions=100%) and efficiently studies how changing proportions of components affect the response. More, specifically, the Box-Behnken Design is a response surface design that focuses on fitting a second-order polynomial model without including estimation of pure quadratic effects at extreme factor levels. Important for efficiency and cost effectiveness, this design requires fewer experimental runs compared to the abovementioned designs. Furthermore, it is suitable for moderate to high-dimensional problems and effectively models quadratic responses. As it stands, each design has its own strengths and is suitable for specific situations based on the nature of the factors, the desired response surface exploration, and the available resources. The choice of the design is crucial for an effective and efficient application of RSM in process optimization.

3.4.2. Artificial Intelligence modelling

The advent of the fourth industrial revolution (4IR) aligned with global sustainability objectives has guided research toward the utilization of artificial intelligence (AI) systems. This technology has piqued the interest of researchers, particularly within the realm of

bioenergy, biofuel and bioproduct research. The profound impact of AI in this specific domain has spurred discussions on numerous AI strategies aimed at enhancing existing systems and addressing challenges in lignocellulosic bioprocessing units. Machine learning algorithms can analyse vast datasets to identify patterns, facilitating optimization of process parameters. This adaptive approach enables continuous improvement and enhanced scalability. With this in mind, AI presents a plethora of sophisticated mechanisms in lignocellulosic bioprocessing technologies, promoting effective resource utilization, reduce laboratory experimentation, environmental consciousness, and efficient bioproduct generation from natural resources (Cheng et al., 2023). Integration of AI tools allow for real-time monitoring and control, enabling dynamic adjustments to optimize performance during process development and scale-up with minimal experimental iterations. Moreover, the incorporation of AI enables swift assessment of risks or potential disruptive events throughout the supply chain, contributing to more effective risk mitigation (Soori et al., 2023). Much like other sectors, the field of bioprocessing is also harnessing AI to support its long-term objectives. AI offers a diverse range of optimization tools, such as the Evolutionary Algorithms (Amenaghawon et al., 2024), Genetic Algorithm (GA) (Izquierdo et al., 2024), and artificial neural network (ANN) modelling (Mansour et al., 2024). These optimization tools can also leverage support for economic forecasting and the large-scale commercialization of bioproduct conversion technologies.

More so, ANN's facilitate virtual experiments that alleviate exorbitant costs and intensive labour. It simulates the cognitive responses of the human brain, enabling the mathematical modelling of intricate non-linear systems without prior knowledge of kinetics, metabolic fluxes or the bioprocessing medium ((Zhu and Liu, 2022). These data-driven tools leverage correlations between the process inputs and corresponding outputs, training machine learning algorithms for precise yield prediction (Soori et al., 2023). In turn, this leads to maximized

product generation and minimized inhibitory factors (Sebayang et al., 2017). In addition, ANNs incorporate feature selection techniques to identify and prioritize the most relevant input features from the dataset, enhancing the network's efficiency, accuracy, and interpretability. In choosing the most influential features, it optimizes the model's complexity by excluding redundant inputs and mitigates the risk of overfitting, leading to faster training and evaluation (Chen et al., 2020b). To achieve these goals, one of the methods employed for this purpose is sensitivity analysis. Sensitivity analysis stands out as a feature selection method that point identifies essential input parameters for predicting the output variable and quantifies how the modifications in these input values affects the model's responses (Chen et al., 2020b). It improves model predictive performance and generalization of the model. As a result, these intelligent models expedite screening evaluations of the fermentable sugar release from pretreated LCB, offering foresight into potential industrial scale applications.

While ANN is a robust tool for predicting responses, making complex decisions and recognizing patterns, there has been a growing interest in exploring the potential of advanced Generative Artificial Intelligence (GAI) models like ChatGPT to unravel complex perceptions in the domain of bioprocess development. By harnessing the capabilities of deep learning algorithms, the GAI models possess the ability to process, analyse and synthesize intricate patterns from vast and multifaceted scientific datasets, generating articulations similar to human-like language (Ray, 2023). Unlike human interpretation that relies on contextual knowledge, GAI models offer unbiased analysis based on statistical associations within the presented dataset (Ray, 2023).

Nevertheless, these models lack expertise and comprehension specific to the domain, potentially restricting its capacity to offer nuanced interpretations intrinsic to the specialized field. To overcome this challenge, an integrated strategy that synergistically combines the extensive computational and data processing capabilities of GAIs with human interaction can

be exercised to ensure precise and contextually informed decisions. This collaborative approach assists in identifying possible discrepancies and cognitive biases that could arise in both human and model interpretations, ultimately propelling research advancements and fostering the generation of factual, scientific knowledge.

3.4.3. Kinetic modelling

As it stands, lignocellulosic biorefineries are complex systems with multi-scale phenomena, from molecular-level reactions to macro-scale transport processes. Kinetic models offer valuable insight into the rational design of microbial metabolic processes and exploring the phenomena in intricate biological systems pertaining to reaction rates, mechanisms and intermediate steps. This knowledge expediates cell factory engineering and defines the parameters for bioprocess control, particularly when considering industrial-scale applications (Oliveira et al., 2016). Bioprocess modelling encompasses the mathematical representation and simulation of biological, physical, and chemical aspects inherent to the bioconversion of LCB into valuable products. This aims to meticulously select and optimize the process parameters that influence microbial growth and product formation (Fedailaine et al., 2015).

The kinetics models provide insights using rate equations to describe how the concentrations of reactants change over time and are based on empirical observations derived from experimental outcomes. Thus, predicting the optimal conditions and behavioural conditions over time within a fermentation process assists in (1) enhancing product yield and productivity, (2) efficiently managing resource utilization, (3) reducing undesired byproduct formation, (4) optimizing cost and time efficiency, and (5) transitioning from lab-scale reactions to industrial-scale reactions, whilst maintaining high product purity (Kucharska et al., 2018). Modelling the kinetics of microbial growth necessitates accounting for the regulatory effects and dynamics of the biological system at a metabolite level (Costa et al.,

2016). These approaches typically rely on a set of mathematical expressions that quantitatively describe the systematic occurrences in light of reaction rate constants, activation energies, and adsorption coefficients. Well-designed experiments and data analysis techniques such as regression, optimization strategies are employed to forecast the response to various input conditions, accurately. Various kinetic models, including but not limited to the Monod model, logistic model and Haldane model, modified Gompertz model, and modified logistic model, are commonly used to characterize the specific growth rates of microorganisms and product synthesis. These models conceptualize microorganisms as independent entities that interact with their environment, and their growth rates are influenced by the biomass and substrate concentrations (Dong et al., 2015; Panikov, 1999). Overall, kinetic modelling for bioprocess development has been explored extensively in appendix B (*Section 10.4*) (Sewsynker-Sukai et al., 2023a).

3.5. Bioprocess scale-up

When transitioning a bioprocess from flask to reactor scale, considerations regarding kinetics and mixing efficiency in the bioreactor are required. Mixing efficiency is a fundamental feature in ensuring homogeneous distribution of nutrients, gases, and microorganisms throughout the fermentation medium. It directly influences the mass transfer rates, heat transfer and reaction kinetics within the system. Therefore, optimal mixing is essential for maintaining consistent physicochemical conditions and preventing localized concentration gradients that may hinder microbial growth, product formation or lead to undesirable by-products (Xia et al., 2015). By assessing the impact of mixing regime parameters such as constant power input per unit volume (P/V) and constant impeller tip speed (V_{tip}) on the bioprocess, it becomes possible to achieve successful scale-up with improved productivity, yield and product quality. Constant P/V refers to the amount of power required to operate the bioreactor relative to the volume of the input liquid media. It serves as a quantitative measure

of the energy efficiency and understanding the reactors energy requirements should be considered as a comprehensive analysis method to enable improved industrial-scale bioreactor design, overall bioprocess performance and productivity. In the same vein, constant V_{tip} is another critical parameter that refers to the velocity of the impeller blade tip during agitation in the bioreactor. The impeller tip speed affects various aspects of the fermentation process, including mixing efficiency, mass and heat transfer, substrate utilization and shear stress on the cells. A reduced power-input and in agitation speed leads to a notable decline in the rate of mass, heat and gas transfer that can impact negatively on the process performance (Deniz et al., 2015). Conversely, high impeller tip speeds (>3.0 $m.s^{-1}$) generate significant energy input, increased power consumption and shear stress to microbes (Marques et al., 2010). Deviation from the preferred tip speed range can negatively impact cell growth, product formation, or overall process performance. As the scale of the bioprocess increases, the constant P/V and constant V_{tip} parameters are modified accordingly without compromising the mixing efficiency, temperature regulation and microbial cell integrity throughout the bioreactor.

3.6. Challenges and future prospectives

The increasing demands of LA coupled with economic and environmental development have stimulated the need for its renewable and sustainable production. The routinely used commercial MRS media provides an optimal environment for the growth and metabolic activity of LABs with several factors such as its complex nutrient composition, pH regulation, microbial selection and versatility, contributing to its suitability for LA production. While MRS media is commonly used for laboratory-scale studies and optimizing LA production conditions, its nutrient rich formulation accounts for approximately 30% of the production costs, impairing the economics of the system (Tang et al., 2013). In order to negate these excessive costs, the meticulous selection of cheap yet effective raw materials are

desired for technoeconomic viability. Moreover, media formulations tailored for specific LAB strains and LA production processes are often preferred. As it stands, biological systems require carbon and nitrogen sources to control biosynthetic pathways for LA production. Additionally, surfactant-assisted systems may enhance nutrient uptake, improve enzymatic saccharification processes and promote homogeneity during fermentation (Taoka et al., 2011; Zhang et al., 2018). In this context, buffer agents such as K_2HPO_4, $NaHCO_3$, $Na_2HPO_4.12H_2O$ and $CaCO_3$ amongst others, primarily ensure pH stability, thus providing a conducive environment for LABs to thrive and produce the product of interest. Furthermore, nanoparticle-assisted fermentation exhibits high surface-to-volume ratio and distinctive surface properties that enable efficient adsorption and activation of reactant molecules, thereby enhancing LA production (Sanusi et al., 2020). More importantly, the freshwater footprint of bioprocessing units represents an ongoing issue that affects sustainability, environmental development and feasibility. Therefore, the use of industrial wastewater streams such as DWW instead of fresh water into the media formulation processes may reduce expenses and resources, while converting waste to wealth. To this end, prospects lie in harnessing a blend of low-cost raw materials within a DWW medium based on the abovementioned nutritional and enhancements requirements. Beyond LA production, the resultant effluent and biomass discharge from the process may display residual nutrient compositions that hold potential for repurposing as valuable resources. As a result, the nutrient rich effluent could potentially serve as animal feed and biofertilizer for local subsistence farmers, contributing to sustainable waste utilization and integrated biorefineries whereby continuous streams of commodity chemicals are produced while recycling all other waste outputs with little to no discharge, achieving a circular bioeconomy.

With the synergistic interactions of a minimally supplemented DWW medium in mind, bioprocess optimization provides a comprehensive understanding of process efficiency,

productivity and yield comparison, process economics and potential scale-up considerations that are vital for tailoring strategies to meet specific production goals. However, there is a paucity of studies on the optimization of key input parameters that form part of a feasible media formulation using various fermentation process types (SSF and PSSF). Another aspect to consider with a fourth industrial revolution upon us is the integration of Generative Artificial Intelligence tools such as Artificial Neural Networks (ANN) and ChatGPT for advanced process optimization that allows for real-time monitoring and control as well as enabling dynamic adjustments for scale up performance. In particular, ANNs can model intricate relationships between multiple variables, facilitating the identification of optimal conditions for enzymatic hydrolysis, fermentation, and downstream processing. On the other hand, ChatGPT integrates its natural language processing capabilities with textual data, research findings and contextual information. This integration enables a more comprehensive approach to process optimization, considering both numerical data and qualitative insights. These tools poise lignocellulosic biorefineries with transformative advancements that contribute to enhanced process understanding, adaptive control, collaborative decision-making and optimization, positioning bioprocessing units at the forefront of sustainable and efficient biobased production.

Overall, there is a dearth of knowledge on the mathematical kinetic modelling of *Lactobacillus* species cell growth and LA formation. *Lactobacillus* species engages in a high energy metabolism in the presence of oxygen, which is directed towards biomass growth and replication (Smetanková et al., 2012). On the other hand, under anaerobic conditions, the microbial metabolism favours LA formation since the energy produced through glycolysis may not be sufficient for abundant cell growth (Smetanková et al., 2012). This information is imperative for industrial scale-up of commodity chemicals such as LA since maintaining anaerobiosis incurs excessive cost. For this reason, it is essential to have an in-depth

understanding of the bioprocess kinetics with the aim of enhancing the feasibility and productivity of LA production. Therefore, insight on the kinetics of microbial cell growth and LA formation of the optimized process parameters under varying oxygen environments (microaerophilic and anaerobic) are needed. These aspects bring to the forefront valuable advancements on the bioprocess design for potentially improved yields and economic return on a commercial scale.

Subsequent to optimization and kinetic studies, scaling up involves transitioning from laboratory-scale processes to larger production units. Ensuring seamless integration of unit operations such as pretreatment, enzymatic hydrolysis and fermentation, while maintaining efficiency, increasing productivity and minimizing energy consumption, is a complex challenge. This is due to the intricate nature of lignocellulosic biorefinery processes that exhibit diverse and dynamic interactions involved in biomass conversion to fermentation end products. Thus, achieving comparable conditions while maintaining product quality at larger scales requires the consideration of hydrodynamic complexity, shear sensitivity, mass and heat transfer efficiency and bioreactor geometry. For this reason, optimization of scale-up criteria such as constant V_{tip} and constant P/V are imperative for preserving biological activity, uniform reactor environment and scale up reproducibility. These considerations are fundamental to the success of large-scale biorefineries and the economic viability of biobased production processes. Advancements in these methodologies offer the potential to enhance process efficiency, reduce costs and optimize resource utilization. Overall, it will pave the way for sustainable and economically viable lignocellulosic biorefineries in future endeavours while establishing interconnections to the food-energy-water (FEW) nexus.

3.7. Conclusion

This review explores the dynamic landscape of lactic acid (LA) production with a specific focus on the innovative use of dairy waste, specifically dairy wastewater as a promising fermentation medium. It also presents the potential of incorporating cost-effective enhancement agents (nitrogen source, carbon source and surfactant) and varying physicochemical parameters (buffer agents, pH effects, and micronutrient) to enhance the LA production. It covers aspects including bioprocess types, optimization strategies, modelling kinetics as well as the application of artificial intelligence to improve lignocellulosic bioprocessing. Moreover, mixing criteria such as constant V_{tip} and constant P/V for scale-up optimization was briefly discussed in terms of the yield output and productivity. Furthermore, key challenges and future perspectives in the LA bioprocess development trajectory was outlined.

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CHAPTER 4

Development of Kraft waste-based pretreatment strategies for enhanced sugar recovery from lignocellulosic waste

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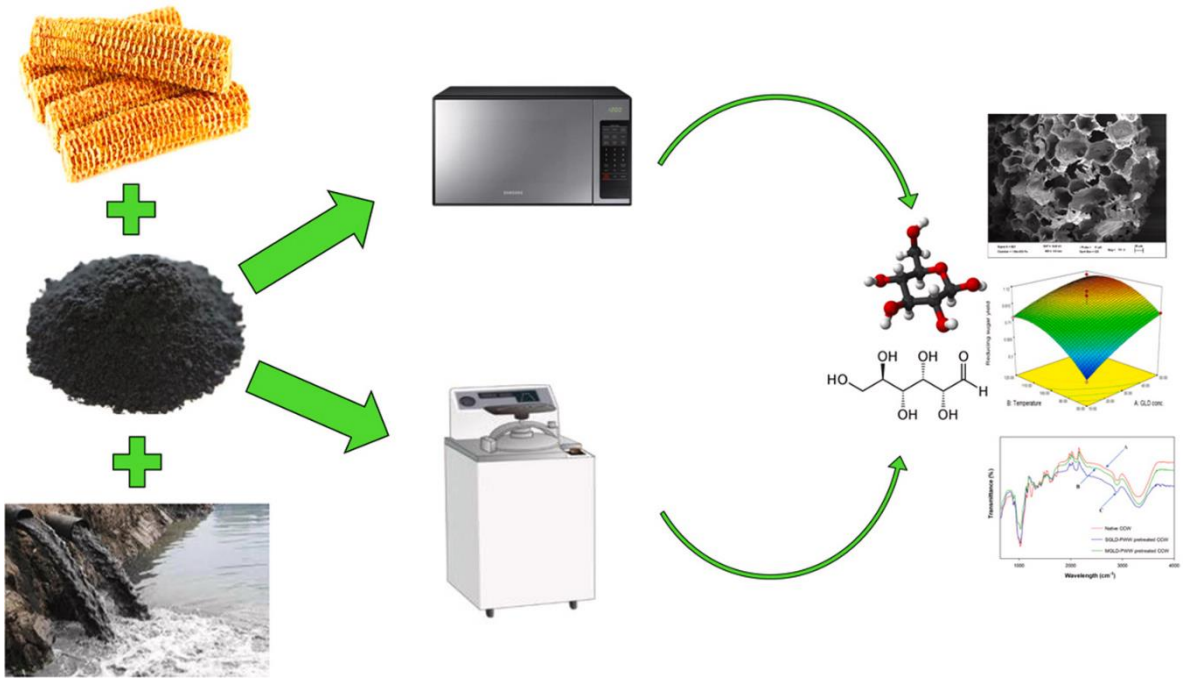
This chapter has been published in *Industrial Crops & Products* (174, 114222) with the title: Development of Kraft waste-based pretreatment strategies for enhanced sugar recovery from lignocellulosic waste.

The published paper and supplementary material are presented in the following pages.

`Highlights

- First report on combined green liquor dregs and paper wastewater pretreatments.
- Optimization revealed high reducing sugar (1.53 g/g) and glucose yield (0.85 g/g).
- The developed pretreatment methods eliminate the use of chemicals and fresh water.
- This study demonstrates the beneficiation of Kraft wastes for pretreatment.

Graphical Abstract



Industrial Crops & Products 174 (2021) 114222

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Development of Kraft waste-based pretreatment strategies for enhanced sugar recovery from lignocellulosic waste

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ARTICLE INFO

Keywords:
Green liquor dregs
Paper wastewater
Microwave irradiation
Steam
Complete waste-based pretreatment

ABSTRACT

The Kraft industry generates large quantities of alkali wastes in the form of green liquor dregs (GLD) and paper wastewater (PWW). These wastes are potential catalysts for lignocellulosic pretreatment and propose a suitable alternative to conventional, expensive, chemical-based methods. This study optimized two novel complete Kraft waste-based pretreatments. The developed methods included a steam-assisted combined GLD and PWW (SGLD-PWW) and a microwave-assisted combined GLD and PWW (MGLD-PWW) for enhanced sugar recovery from corn cobs. The SGLD-PWW and MGLD-PWW models gave coefficient of determination values > 0.87. Pretreatment optimization revealed a higher reducing sugar (1.53 ± 0.36 g/g) and glucose yield (0.85 ± 0.16 g/g) for the SGLD-PWW pretreatment compared to 1.04 ± 0.01 g/g (reducing sugar yield) and 0.51 ± 0.06 g/g (glucose yield) for the MGLD-PWW strategy. This study demonstrated that using Kraft pulping industry residues for the pretreatment of lignocellulosic feedstock significantly enhanced the sugar recovery, thus representing the "waste to wealth" concept.

1. Introduction

Lignocellulosic biomass (LCB) is a carbon neutral, non-food-based, renewable source that is considered an ideal substrate for the production of valuable bio-based products (Xu et al., 2017). The global production of LCB is approximately 120 billion tons per annum, equating to 2.2×10^{21} Joules of energy (Guo et al., 2015). The general composition of lignocellulosic wastes consists of cellulose (38–55 wt%), hemicellulose (23–32 wt%) and lignin (15–25 wt%) with traces of inorganic salts and extractive portions (resins, tannins, fatty acids) (McKendry, 2002). Over the years, various potential lignocellulosic substrate types have been evaluated as a feedstock for the production of biofuels and platform commodity chemicals. These substrates include rice straw (Kumar et al., 2019), sugarcane bagasse (Zhang et al., 2020), giant reed (Jiang et al., 2020), corn stover (Amenaghawon et al., 2014) and corn cobs (Xu et al., 2017), amongst others. Corn has been tagged as one of the major cultivated crops with an estimated global annual production of over 1 billion metric tons (USDA, 2020). Apart from the harvested corn kernels, the corn plant is also made up of non-food-based portions such as the leaves, husks, stalks and cobs that account for approximately 50% of the global corn production and is either discarded or burnt in fields (Sewsynker-Sukai and Gueguim Kana, 2018). Among the existing corn waste, corn cobs represent one such feedstock that is generated with a high global output of approximately 500 million metric tons per annum (David et al., 2020).

Although lignocellulosic wastes such as corn cobs represent attractive precursors for sugar recovery, its highly crystalline and rigid structure presents numerous hurdles to the microbial and enzymatic degradation of polysaccharides. This is due to the resistant lignin that is tightly bound to the cellulose and hemicellulose layer and functions to provide a cohesive and impermeable cell wall structure, thus hindering enzymatic hydrolysis (Sewsynker-Sukai et al., 2020). Pretreatment is imperative to disintegrate cross-linked lignin fractions of lignocellulosic waste in order to make the cellulose microfibrils and hemicellulose matrices more amenable to enzymatic processes (Kim et al., 2015). Numerous pretreatment strategies have been developed and these include acid (Amenaghawon et al., 2014), alkaline (Jiang et al., 2020), ionic liquids (Sorn et al., 2019), ultrasound (Ramadoss and Muthukumar, 2016), microwave and steam-assisted inorganic salt (Moodley and Gueguim Kana, 2017). Alkaline pretreatment technologies have been coined the most effective, due to its high depolymerization of lignin, less intensive process conditions and low polluting nature (Moodley and

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https://doi.org/10.1016/j.indcrop.2021.114222
Received 17 July 2021; Received in revised form 27 September 2021; Accepted 24 October 2021
Available online 8 November 2021

Gueguim Kana, 2017). Several alkaline pretreatment techniques have been investigated to date, however, their industrial applicability is hampered by the high operational costs, enormous consumption of expensive chemicals and increased energy demands. Consequently, recent pretreatment studies are inclined towards cost-effective, environmentally friendly and energy saving approaches, with the aim of maintaining the fundamental characteristics of an effective lignocellulosic bioconversion process.

Various industrial chemical by-products from the Kraft pulping industry such as green liquor (GL) (Pham et al., 2018) and black liquor (BL) (Goshadrou, 2019) have gained wide interest as pretreatment alternatives to the conventional alkaline chemical technologies. Despite the efficiency of GL and BL, these pretreatment strategies are not characterized as waste-based methods, since they are usually diverted back into Kraft industrial plants for use in the paper making process (Sewsynker-Sukai et al., 2020).

With tremendous emphasis placed on waste beneficiation, green liquor dregs (GLD), a waste residue generated by the Kraft paper and pulp mill industry, has become prominent over commonly reported GL and BL based pretreatments (Sewsynker-Sukai et al., 2020). The removal of GLD from the Kraft paper and pulp mill cycle is imperative for its smooth functioning, since the high calcite concentrations within the GLD is destructive to the fiber lines and chemical recovery. To avoid the negative impact of GLD, the Kraft pulping industry currently landfills it as its major means of disposal. Nevertheless, this has presented numerous problems with regards to acquisition and maintenance costs as well as environmental regulations. Therefore, various applications for GLD are currently being explored and implemented to encourage the "waste to wealth" concept. One striking characteristic of GLD is its composition that consists of alkaline compounds such as $CaCO_3$, Na_2S and Na_2CO_3, which provide a highly alkaline (pH $>$ 10) and stable medium for pretreatment of lignocellulosic biomass (Golmaei et al., 2018; David et al., 2020). Alkaline pretreatments such as GLD offers milder pretreatment conditions, a possibility of little to no inhibitor compounds and reduced costs with regards to the purchase of chemicals, since it is a waste residue, thus matching some criteria of a suitable pretreatment technology (David et al., 2020). However, even with the reduced costs and low energy consumption that is associated with the development of a GLD pretreatment strategy, the high-water usage still remains a critical issue for lignocellulosic biorefinery processes. Water is a universal solvent that engages in the thermochemical conversion and delignification of LCB. However, the scarcity of a natural resource such as water, raises questions around its utilization in lignocellulosic pretreatment plants. An emerging concept of recycling wastewater generated from industrial production circuits may lead to an effective technical flow in lignocellulosic pretreatment systems. Kraft paper and pulping has become one of the most prevalent chemical processes globally. Along with its high production and demand, this industry consumes huge quantities of fresh water and contributes approximately 40% of the worlds industrial wastewater (Toczylowska-Maminska, 2017). The paper wastewater discharge contains an array of contaminants including chlorinated organic compounds, resins, nitrogen compounds, dissolved lignin and complexing agents such as EDTA (Ashrafi et al., 2015). In addition to these components, the high suspended solid content and brown hue causes water bodies to become esthetically unappealing (Haq and Raj, 2020). Therefore, it is necessary to treat the vast quantities of the paper wastewater (PWW) released into the environment to a required compliance level as set by various governmental authorities (Ashrafi et al., 2015). Numerous wastewater treatment processes have demonstrated efficiency in reducing pollutants and removing turbidity, however, these technologies are energy exhaustive, expensive and contribute to greenhouse gas emissions (Ashrafi et al., 2015). As a result, Kraft industries are in pursuit of a more sustainable concept in comparison to the conventional wastewater treatments. An alternative solution to this acute problem, is the diversion of the untreated and discarded portion of the paper wastewater effluent to replace fresh water that is usually required for lignocellulosic pretreatment. With this approach in mind, profits generated from the lignocellulosic biorefinery processes can be used to cover the final wastewater treatment process, which may be cheaper, carried out under milder conditions and is less invasive. Therefore, substituting the PWW generated from the Kraft pulping industry as a water source in the lignocellulosic pretreatment process would reduce freshwater consumption, relieve industrial plants of high effluent treatment costs and serve as an alkaline enhancement to the proposed GLD treatment strategy. The present study aims to develop a pretreatment strategy that holistically utilizes waste residues such as GLD and PWW from the Kraft pulp and paper industry. This technology reduces both the chemical costs and water usage, with the potential to release comparable sugar yields to otherwise defined chemical pretreatments that are usually expensive and hinder industrial scale feasibility.

Despite the excellent pretreatment properties of GLD and the proposed use of PWW, the energy demand also plays a key role in the total cost within a lignocellulosic pretreatment plant. Therefore, to increase efficiency of sugar recovery, reduce energy inputs and shorten process time, a combination of pretreatment methods is being developed. Steam-assisted heating is a conventional method that is centered on the use of water at high temperatures and pressures (Toscan et al., 2019) that induces changes in the chemical properties of water and catalyzes the hydrolysis of covalent bonds within the lignocellulosic structure (Amenaghawon et al., 2014). This processing strategy involves short residence time and does not require expensive corrosive-resistant materials (Kumari and Singh., 2018). These characteristics propose the basis of commissioning larger reactor vessels that may result in fewer pretreatment cycles, thus reducing the cost and energy, while improving the productivity. Furthermore, the high-pressure system maintains water in a liquid state even at the elevated temperatures to prevent dehydration of the reaction medium (Kumari and Singh., 2018). However, the elevated physical conditions may: (1) elicit high water demands, and (2) result in sugar degradation and the formation of enzymatic and fermentation inhibitory compounds (Maurya et al., 2015). On the other hand, microwave irradiation has recently gained attention for its ability to induce the breakdown of lignocellulosic constituents (Sorn et al., 2019). The microwave mechanism works through molecular collisions caused by dielectric polarization on chemical covalent bonding within lignocellulosic feedstocks (Sorn et al., 2019). Benefits of this approach include short processing times, high uniformity, engages in direct heating and reduced energy requirements, since the generation of heat is instantaneous (Kumar et al., 2019). Nevertheless, the sole use of this pretreatment method at elevated temperatures ($>$ 200 °C) degrade sugars and induce the formation of inhibitor compounds such as phenols and furfurals. Additionally, the scalability of microwave-assisted heating for large-scale implementation remains a primary challenge, since new reactor concepts, microwave-material interactions, kinetic modeling and simulations are yet to be explored (Arpia et al., 2021). Limited studies have been performed on the comparison of steam- and microwave-assisted pretreatment for enhanced sugar recovery from lignocellulosic biomass (Lai and Idris, 2016; Moodley and Gueguim Kana, 2017). Therefore, optimizing steam and microwave-assisted waste-based pretreatments and analyzing the resultant outcome aims to fill the knowledge gaps and improve yields in view of commercial production. Considering the emphasis placed on waste reduction and recycling, cost, energy consumption, feasibility and efficiency of pretreatment strategies in lignocellulosic biorefineries, the present study optimized: (a) a steam-assisted combined GLD and PWW (SGLD-PWW) and (b) a microwave-assisted combined GLD and PWW (MGLD-PWW) pretreatment using corn cobs for improved sugar recovery. Furthermore, structural changes in the untreated and optimized SGLD-PWW and MGLD-PPW pretreated corn cobs were systematically characterized using scanning electron microscopy (SEM) and Fourier Transform Infrared (FTIR) analysis.

2. Materials and methods

2.1. Materials

The pretreatment experiments were conducted using corn cob waste (CCW) substrate obtained from the Ukulinga research farm (Pietermaritzburg, South Africa) (29° 67′ E, 30° 40′ S). The CCW was oven dried at 60 °C for 24 h and milled to a particle size of less than 1–2 mm (Hammermill, South Africa). The powdered substrate was stored in an airtight container at room temperature. A local paper Kraft pulp and paper mill industry supplied both the green liquor dregs (pH 10–12) and paper wastewater (pH ~ 8) (Mondi, Richards Bay, South Africa) with their chemical compositions as shown in Table S10 and S11, respectively. The commercial cellulase-based enzyme blend, Cellic CTec 2 was generously provided by Novozyme (Novozymes A/S, Denmark). All chemicals employed in this study were purchased from Merck, South Africa.

2.2. Pretreatment of corn cobs

2.2.1. Preliminary screening

A preliminary screening was carried out to determine the combined effect of the GLD and PWW on the enzymatic saccharification of corn cobs using microwave- or steam-assisted heating. The microwave-assisted combined green liquor dregs and paper wastewater (MGLD-PWW) model inputs consisting of the GLD concentration (10–50%, w/v), power intensity (100–900 W) and pretreatment time (2–10 min) was based on the ranges by Moodley and Gueguim Kana (2017) and David et al. (2020). Alternatively, for the steam-assisted combined green liquor dregs and paper wastewater (SGLD-PWW) model, the GLD concentration (10–50%, w/v), temperature (80–120 °C) and pretreatment time (5–15 min) was selected from previous studies by Shao et al. (2020) and David et al. (2020). Median input values from the aforementioned ranges were implemented for the preliminary assessment and denoted as follows: (1) preliminary microwave-assisted combined GLD and PWW ($MGLD\text{-}PWW_{preliminary}$) and (2) preliminary steam-assisted combined GLD and PWW ($SGLD\text{-}PWW_{preliminary}$) pretreatments. The pretreatment experiments were performed in 1.8 L beakers with a total working volume of 150 mL and a standard solid loading of 10% (w/v). The beakers consisted of 30% (w/v) GLD submerged in paper wastewater. The pretreatments were subjected to two different heating systems namely, microwave and steam (autoclave). For the microwave-assisted pretreatment, the process was carried out in a microwave oven (Samsung, ME9114S1) at 500 W for 6 min. The steam-assisted pretreatment was performed in a laboratory autoclave at 100 °C for 10 min. Control pretreatment experiments were performed on CCW, and these included: (1) 30% (w/v) GLD in deionized water heated by microwave (MGLD-W) or steam (SGLD-W), (2) deionized water alone heated by microwave (MW) or steam (SW) and (3) paper wastewater alone heated by microwave (MPWW) or steam (SPWW). The resulting pretreated CCW was thereafter filtered using a domestic sieve (< 1 mm) and washed multiple times with deionized water, until the water appeared transparent and free of any residual GLD and PWW. The washed, pretreated CCW substrate was oven dried at 70 °C overnight prior to the enzymatic hydrolysis stage. The reducing sugar and glucose that were retrieved after enzymatic hydrolysis was used as an index of pretreatment efficiency.

2.2.2. Response surface methodology (RSM) modeling of the developed pretreatments

The response surface methodology (RSM) (Box-Behnken design) (Design Expert 7.0, Stat Ease Inc, USA) was used to develop the experimental design for optimization of the microwave-assisted combined GLD and PWW (MGLD-PWW) and steam-assisted combined GLD and PWW (SGLD-PWW) pretreatment on the CCW. The design generated a total of seventeen experimental runs for each model. The pretreatment input parameters for the MGLD-PWW included GLD concentration (10–50%, w/v), power intensity (100–900 W) and heating time (2–10 min). For the SGLD-PWW pretreatment, input parameters that were investigated included GLD concentration (10–50%, w/v), temperature (80–120 °C) and heating time (5–15 min). The CCW substrate solid loading was maintained at 10% (w/v) for all pretreatment experimental runs. The milled CCW was submerged in 150 mL of PWW containing varied concentrations of GLD, mixed thoroughly and were thereafter subjected to different power intensities and heating times in a microwave oven for the MGLD-PWW experiments (Table 1). The same approach was adopted for the SGLD-PWW experiments with the exception of autoclave heating at different temperatures and times as specified within the experimental design (Table 2). Following the pretreatment process, the solid residue was filtered, washed and dried as previously stated above prior to enzymatic hydrolysis.

2.2.3. Optimization and validation of the MGLD-PWW and SGLD-PWW models

The experimental outputs consisted of reducing sugar ($_{rs}$) and glucose ($_{g}$) yields and thus, two process response surface models were assessed for each pretreatment. The MGLD-PWW model was designated as $MGLD\text{-}PWW_{rs}$ and $MGLD\text{-}PWW_{g}$ while the SGLD-PWW model consisted of $SGLD\text{-}PWW_{rs}$ and $SGLD\text{-}PWW_{g}$. The experimental data was used to fit the polynomial model equations in order to study the interactive effect of the independent input variables for improved sugar recovery. Subsequently, the optimum conditions for the MGLD-PWW (48.70% GLD, 800 W, 9 min) and SGLD-PWW (49.89% GLD, 118 °C, 5 min) models were determined using the developed RSM models. These optimized conditions were used to validate each model.

2.3. Enzymatic hydrolysis

The Cellic CTec 2 enzyme activity of 160 FPU/mL was determined according to the Laboratory Analytical Procedure (LAP) of the National Renewable Energy Laboratory (NREL, 2008). Enzymatic hydrolysis was carried out in 100 mL Erlenmeyer flasks, where the MGLD-PWW or SGLD-PWW pretreated CCW was immersed in 10 mL sodium citrate buffer (pH 4.8, 0.05 mol/L) with a constant solid loading of 10% (w/v). Cellic CTec2 enzyme (10 FPU/g substrate) was added to the reaction mixture. The flasks were incubated at 50 °C and 120 rpm for 72 h. Following the saccharification stage, the samples were centrifuged at 9000 rpm for 5 min and the supernatant was analyzed for reducing sugar and glucose.

Table 1
MGLD-PWW pretreatment of CCW using the Box-Behnken design.

Run	Inputs			Outputs	
	GLD concentration (%)	Power intensity (W)	Pretreatment time (min)	Reducing sugar (g/g)	Glucose (g/g)
1	50	500	10	0.85	0.41
2	30	900	2	0.36	0.21
3	30	100	2	0.40	0.20
4	30	500	6	0.69	0.27
5	50	500	2	0.32	0.20
6	10	900	6	0.39	0.19
7	50	100	6	0.54	0.14
8	30	500	6	0.68	0.26
9	10	100	6	0.35	0.16
10	30	500	6	0.72	0.28
11	10	500	2	0.29	0.17
12	30	500	6	0.61	0.19
13	50	900	6	0.96	0.35
14	30	100	10	0.27	0.17
15	30	900	10	0.80	0.32
16	10	500	10	0.55	0.20
17	30	500	6	0.46	0.23

g/g = g reducing sugaror g glucose/ g pretreated dry weight corn cobs.

Table 2
SGLD-PWW pretreatment of CCW using the Box-Behnken design.

Run	Inputs			Outputs	
	GLD concentration (%)	Heating temperature (°C)	Pretreatment time (min)	Reducing sugar (g/g)	Glucose (g/g)
1	50	100	15	1.15	0.42
2	30	120	5	0.88	0.53
3	30	80	5	0.45	0.14
4	30	100	10	0.90	0.34
5	50	100	5	0.99	0.55
6	10	120	10	0.75	0.50
7	50	80	10	0.81	0.27
8	30	100	10	1.07	0.52
9	10	80	10	0.30	0.15
10	30	100	10	0.86	0.34
11	10	100	5	0.66	0.25
12	30	100	10	1.01	0.43
13	50	120	10	1.08	0.56
14	30	80	15	0.83	0.30
15	30	120	15	1.05	0.63
16	10	100	15	0.81	0.33
17	30	100	10	0.86	0.35

g/g = g reducing sugar or g glucose/g pretreated dry weight corn cobs.

2.4. Analytical techniques

2.4.1. Sugar analysis

The reducing sugar and glucose yields obtained after enzymatic hydrolysis were quantified using the 3.5-dintrosalicylic acid method (Miller, 1959) and Megazyme glucose kits (Megazyme, Wicklow, Ireland), respectively. All pretreatment experiments for both the MGLD-PWW and SGLD-PWW models were performed in duplicate.

2.4.2. Compositional analysis

The chemical compositional analysis of the untreated (native) and optimized (SGLD-PWW or MGLD-PWW) pretreated substrate was determined according to the Van Soest method (Van Soest and McQueen, 1973). The neutral detergent fiber (NDF) analysis was conducted by boiling the sample in a neutral detergent fiber solution (pH 7.0). The NDF consisted of cellulose, hemicellulose and lignin. Acid detergent fiber (ADF) was carried by boiling the sample in an acid detergent fiber solution to remove the soluble portion with the resultant components being cellulose and lignin. The ADF components (cellulose and lignin) were treated with 72% H_2SO_4 to yield acid detergent lignin (ADL) and contained lignin.

2.4.3. Scanning electron microscopy (SEM)

The morphological features of both the native (untreated) and optimally pretreated MGLD-PWW and SGLD-PWW CCW samples were assessed using a scanning electron microscope (ZEISS EVO LS 15). Prior to viewing, the CCW samples were fixed onto aluminum specimen stubs, sputtered in a conductive gold coating (Q150R ϵS) and images were captured at 750× magnification for the native and pretreated samples.

2.4.4. Fourier transform infrared (FTIR) analysis

The functional group shifts of the native and optimally pretreated MGLD-PWW or SGLD-PWW CCW samples were detected by Fourier Transform Infrared (FTIR) spectroscopy using an Agilent Cary 630 spectrometer (Santa Clara, CA, USA). The CCW samples were ground and pressed to produce diameter pellets. The resultant FTIR spectra were scanned in transmittance mode within the wavenumber range of 650 and 4000 cm^{-1}.

3. Result and discussion

3.1. Preliminary screening

According to the preliminary screening pretreatments (Table S1) carried out, high reducing sugar (1.27 g/g) and glucose yield (0.45 g/g) were obtained for the MGLD-$PWW_{preliminary}$ pretreatment. Similarly, the SGLD-$PWW_{preliminary}$ pretreatment released 1.33 g/g and 0.47 g/g of reducing sugar and glucose, respectively. The high sugar outputs were comparatively evaluated against the reducing sugar (0.27 g/g) and glucose (0.17) yields of the native CCW (42.35% cellulose, 39.18% hemicellulose and 12.13% lignin). Alongside the abovementioned screening, a set of control experiments were performed using the same pretreatment parameters, with the exception of using deionized (pure) water instead of PWW. The control experiments using pure water revealed slightly higher reducing sugar (rs) and glucose (g) yields for the SGLD-W (rs = 1.44 g/g and g = 0.40 g/g) and MGLD-W (rs = 1.42 g/g and g = 0.40 g/g). This translates to a slightly higher reducing sugar (up to 7.64% and 10.56%) when compared to the SGLD-PWW and MGLD-PWW pretreatments respectively using PPW. Nevertheless, the application of Kraft wastes such as PWW in the latter strategy compensates for the expense attached to pure water consumption in the system, thus enhancing the technoeconomic output. Additionally, the control experiments using PWW and pure water alone (without GLD) resulted in considerably lower sugar yields as follows: SPWW alone (rs = 0.49 and g = 0.21 g/g), MPWW alone (rs = 0.49 and g = 0.19 g/g), SW alone (rs = 0.35 and g = 0.17 g/g) and MW alone (rs = 0.35 and g = 0.13 g/g). The abovementioned control experiments were performed prior to the optimization to evaluate the combined pretreatment ability of GLD and PWW as well as to establish the median input ranges for the SGLD-PWW and MGLD-PWW models.

3.2. MGLD-PWW and SGLD-PWW RSM model development

Reducing sugar and glucose yields from the MGLD-PWW and SGLD-PWW pretreatment experiments shown in Tables 1 and 2 were used to generate the polynomial model equations. The quadratic model equation illustrates the interaction between the significant parameters and the fermentable sugar response (Table S2). Analysis of Variance (ANOVA) (Table S3-6) was used to assess the model fitness for both the MGLD-PWW and SGLD-PWW models. The MGLD-PWW model significance is denoted by low p-values (0.021 and 0.013) and high F-values (5.12 and 6.11) for the reducing sugar and glucose yields, respectively. Similarly, the SGLD-PWW pretreated model presented high F-values (12.17 and 5.59) and low p-values (0.002 and 0.017) for the reducing sugar and glucose yields, respectively (Table 3). Generally, high F-values indicate that there is a low chance for noise to occur, while p-values less than 0.05 represent model and parameter significance (Kumar et al., 2019). From the overall assessment, pretreatment time (0.012) and power intensity (0.007) were considered the most significant input variables for the MGLD-PWW_{rs} and MGLD-PWW_g models, respectively. These parameters play an important role in the efficient heating, since it leads to increased reaction rates. As heat penetrates throughout the lignocellulosic biomass, thermal heat zones are formed, which cause molecules to vibrate (Farag et al., 2012). The accelerated motion of the molecules leads to the disruption of inter- and intramolecular hydrogen bonds within lignocellulosic biomass. Additionally, the pretreatment time impacts the amount of contact that the GLD has with the substrate for effective disabling of the lignocellulosic structure. On the other hand, the SGLD-PWW_{rs} and SGLD-PWW_g models found the GLD concentration (0.0004) and temperature (0.0004) to be most significant, respectively. The temperature contributes to the rate at which evaporation occurs, therefore determining the measure of water available within the pretreatment reaction. The sufficient water content enables uniform heat flow throughout the solid medium, which in turn increases the temperature for faster reactions rates. With this in mind, the high

Table 3
Analysis of variance (ANOVA) of the developed polynomial models for the optimized MGLD-PWW and SGLD-PWW pretreatments.

Model	Parameter significance													Model significance		
	p-value													F-value	p-value	R^2
	A	B	B*	C	AB	AB*	AC	BC	B*C	A^2	B^2	B^{*2}	C^2			
MGLD-PWW_{rs}	0.013	0.023	–	0.012	0.139	–	0.274	0.045	–	0.801	0.334	–	0.085	5.12	0.021	0.87
MGLD-PWW_g	0.010	0.007	–	0.016	0.045	–	0.048	0.101	–	0.633	0.142	–	0.726	6.11	0.013	0.89
SGLD-PWW_{rs}	0.0004	–	0.0006	0.007	–	0.307	0.967	–	0.246	0.236	–	0.007	0.732	12.17	0.002	0.94
SGLD-PWW_g	0.034	–	0.0004	0.359	–	0.707	0.196	–	0.704	0.601	–	0.900	0.771	5.59	0.017	0.88

MGLD-PWW_{rs} = microwave-assisted combined GLD and PWW-reducing sugar, MGLD-PWW_g = microwave-assisted combined GLD and PWW-glucose, SGLD-PWW_{rs} = Steam-assisted combined GLD and PWW-reducing sugar, SGLD-PWW_g = Steam-assisted combined GLD and PWW-glucose, A = GLD concentration, B = Power intensity, C = Pretreatment time, B* = Temperature, F-value = Fisher-Snedecor distribution value, p-value = probability value, R^2 = coefficient of determination.

temperatures also aid in disabling the strong matrix surrounding the hydrogen bonding, van der Waals forces and dipole attraction (Ramadoss and Muthukumar, 2016). Furthermore, the uptake of the GLD waste chemical into the substrate enables the degradation of the ester and ether linkages between the lignocellulosic matrices, thus exposing cellulose to the action of enzymes (Toscan et al., 2019). The model fitness was analyzed using the coefficient of determination (R^2) value as a statistical index. An R^2 value between 0.7 and 1 indicates model fitness. High R^2 values of 0.87, 0.89, 0.94 and 0.88 were observed for the MGLD-PWW_{rs}, MGLD-PWW_g, SGLD-PWW_{rs} and SGLD-PWW_g models, respectively. This accounts for 87% (MGLD-PWW_{rs}), 89% (MGLD-PWW_g), 94% (SGLD-PWW_{rs}) and 88% (SGLD-PWW_g) of the variability in the observed data response. These statistical approaches imply that the models displayed a good correlation between the variables and the fermentable sugar outputs.

3.3. *Effect of the pretreatment parameters on the reducing sugar and glucose yields*

The pretreatment parameters along with the resultant reducing sugar and glucose yields were presented in Table 1. The MGLD-PWW model observed a minimum reducing sugar yield of 0.27 g/g for pretreatment run 14 (30% GLD, 100 W, 10 min) and a maximum sugar yield of 0.96 g/g for run 13 (50% GLD, 900 W, 6 min). Additionally, the lowest glucose yield (0.14 g/g) was observed for run 7 (50% GLD, 100 W, 6 min) while the highest glucose yield (0.41 g/g) was observed for run 1 (50% GLD, 500 W, 10 min). The variation in these outputs showed that the sugar recovery was highly dependent on the model input parameters of power intensity and pretreatment time.

The interactive effects of process input variables on the responses for the MGLD-PWW model (Fig. 1A–D) is illustrated using 3D response graphs. Fig. 1A and B shows the reducing sugar and glucose yields, respectively, as a function of the power intensity and GLD concentration, while keeping the pretreatment time constant at its median value. The reducing sugar yields increased proportionally from 0.40 to 0.91 g/g with a simultaneous increase in GLD concentration (10–50%) and power intensity (100–900 W) (Fig. 1A). Similarly, a simultaneous increment in the GLD concentration and power intensity from 10% to 50% and 100–900 W, respectively led to an increase in the glucose yield from 0.16 to 0.35 g/g (Fig. 1B). The high reducing sugar and glucose yields observed under the elevated GLD concentrations may be ascribed to the ability of the strong alkaline characteristics to efficiently permeate the rigid corn cob structures and cause physical damage. GLD comprises of alkalic salts, Na_2CO_3 and Na_2S, that target specific bonds within the lignocellulose fractions. For instance, the Na_2CO_3 component attacks the ester and glycosidic bonds in the outer wall matrix, while the Na_2S encourages the strong nucleophilic attack on the phenolic β-aryl ether bonds of lignin (Cheng et al., 2010; Qing et al., 2016). Cleavage of these bonds cause hemicellulose and lignin disbanding, partial decrystallization of cellulose and modifications in the lignin fractions (Cheng et al., 2010). Additionally, structural adaptations include swelling of biomass fibers and enhanced pore formation which encourages the penetration of hydrolytic enzymes for improved sugar recovery (Kim et al., 2015). A previous study by Sewsynker-Sukai and Gueguim Kana (2018) recorded similar observations for the reducing sugar yields (0.16–0.69 g/g) when the Na_2CO_3 and microwave power intensity was varied from 0% to 15% and 0–800 W, respectively. Additionally, a study by Qing et al. (2016) reported a similar trend where the reducing sugar and glucose yields reached the highest value at 78.46% and 55.13%, respectively, when the Na_2PO_4 and Na_2S concentration were increased from 1% to 4% and 1 to 10%, respectively.

The pairwise effect of pretreatment time and GLD concentration on the reducing sugar and glucose yield when power intensity is kept at its center point, is presented in Fig. 1C and D, respectively. An increase in the pretreatment time from 2 to 10 min in combination with the GLD concentration from 10% to 50%, led to an increase in the reducing sugar yield from 0.30 to 0.85 g/g (Fig. 1C) and glucose yield from 0.20 to 0.38 g/g (Fig. 1D). These results suggest that the longer pretreatment time along with the GLD saturated reaction solutions enhances breakdown of the lignin-rich structures (Qing et al., 2016). This may be due to the longer time available for the alkaline GLD pretreatment to permeate the substrate for degradation towards polymeric sugar conversion. Furthermore, an increase in temperature as a result of longer pretreatment times improve both mass transfer and rates of reaction (Ramadoss and Muthukumar, 2016). The elevated temperature increases the diffusion rate of the pretreatment chemical, in this case GLD, within the substrate. Moreover, advances in temperature, increases the pressure of the saturated chemical (GLD) solution, which facilitates the GLD penetration into the cavities of the substrate and in turn causes the disintegration of the lignocellulosic structure (Ramadoss and Muthukumar, 2016). Similar trends were depicted on rice straw and sugarcane leaf waste that was subjected to $FeCl_3$ (Kumar et al., 2019) and $ZnCl_2$-NaOH (Moodley and Gueguim Kana, 2017) pretreatments, respectively.

Table 2 represents the SGLD-PWW pretreatment input parameters with the experimental reducing sugar and glucose yields that ranged between 0.30 and 1.15 g/g and 0.14–0.63 g/g, respectively. A low GLD concentration of 10%, pretreatment time of 10 min at 80 °C resulted in a minimum reducing sugar yield of 0.30 g/g (run 9) while a GLD concentration of 50%, pretreatment time of 15 min at 100 °C led to a maximum sugar yield of 1.15 g/g (run 1). Furthermore, the lowest glucose yield (0.14 g/g, run 3) was recovered under a 30% GLD concentration, at a temperature of 80 °C for 5 min. Alternatively, the highest glucose yield (0.63 g/g, run 15) was obtained using 30% GLD, at a temperature of 120 °C for 15 min

Fig. 1E–H is a graphical representation of the process inputs and experimental outputs for the SGLD-PWW model. Fig. 1E and F depicts the interactions of the GLD concentration and temperature on the reducing sugar and glucose yields, respectively, when the pretreatment time is kept at its midpoint value. An increasing trend in both the GLD concentration (10–50%) and temperature (80–111.92 °C) resulted in an increase in the reducing sugar yield from 0.33 to 1.10 g/g (Fig. 1E). Furthermore, maximum glucose yield (0.11–0.60 g/g) was recovered when a simultaneous increase in the GLD concentration from 10% to 50% and temperature from 80° to 120°C was observed (Fig. 1F). The

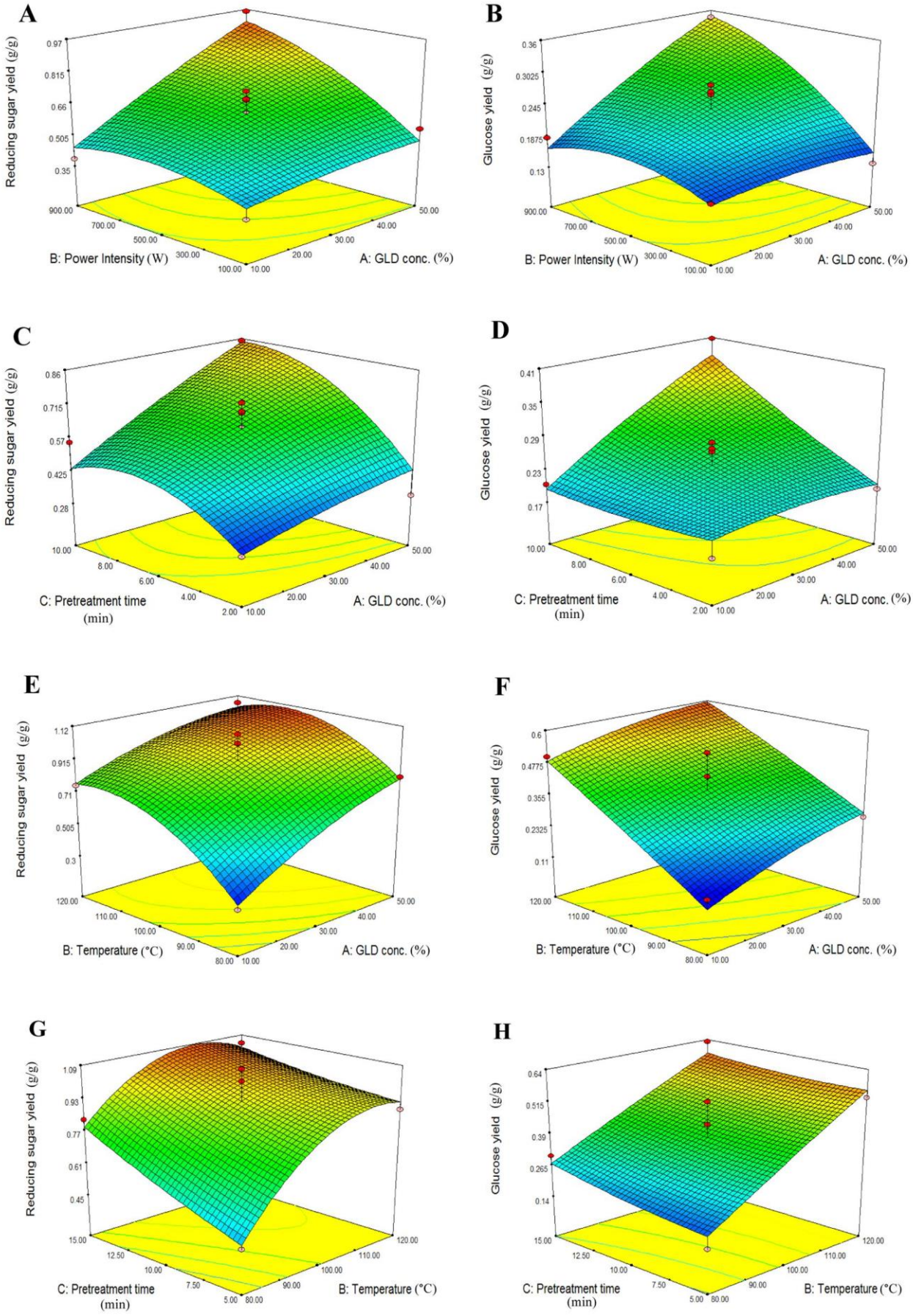

Fig. 1. 3D Response surface plots representing the effect of the various input parameters on the fermentable sugar recovery from corn cobs: (A) GLD concentration (%) and power intensity (W) (MGLD-PWW$_{rs}$), (B) GLD concentration (%) and power intensity (W) (MGLD-PWW$_{g}$), (C) GLD concentration (%) and time (min) (MGLD-PWW$_{rs}$), (D) GLD concentration (%) and time (min) (MGLD-PWW$_{g}$), (E) GLD concentration (%) and temperature (°C) (SGLD-PWW$_{rs}$), (F) GLD concentration (%) and temperature (°C) (SGLD-PWW$_{g}$), (G) temperature (°C) and time (min) (SGLD-PWW$_{rs}$), and (H) temperature (°C) and time (min) (SGLD-PWW$_{g}$).

proportional increments in both the GLD concentration and temperature led to high sugar yields. The elevated temperatures allow for solubilization of the CCW substrate, hence causing the substrate to become more susceptible to chemical (in this case GLD) permeation (Zhang et al., 2020). The highly alkaline GLD (pH > 10) cleaves the intermolecular ester bond between the lignin and hemicellulose components, thus disintegrating the lignocellulosic structure and making it vulnerable to enzymatic saccharification (Kim, 2013). These results were in line with a study by Kim et al. (2014) which exhibited an optimum glucose yield of 266.9 g/kg from corn stover when the Na_2CO_3 concentration increased from 2% to 4.1% and the temperature increased from 120° to 142.6°C. Conversely, when the GLD concentration is maintained at 50% and the time is increased from 111.92° to 120°C, a decrease in the reducing sugar yield was observed from 1.10 to 1.05 g/g, as shown in Fig. 1E. High temperatures facilitate the evaporation of water, which adversely affects the pretreatment efficiency. The substrate tends to hold the water tightly within its pores, decreasing the water potential (Modenbach, 2013). This affects the viscosity of the reaction solution, which in turn hinders the diffusion of GLD into the CCW.

The combined effects of temperature and pretreatment time on the reducing sugar and glucose yields are represented in Fig. 1G and H, respectively, when the GLD concentration is kept at its median. Fig. 1G revealed that reducing sugar yield increased from 0.47 to 1.09 g/g when the pretreatment time and temperature increased proportionally from 5 to 15 min and 80–107.73 °C, respectively. An increase in both the temperature and pretreatment times enhance mass transfer and rates of reaction. During steam-assisted pretreatment, high temperatures induce a change in the chemical properties of water by ionization to form H_3O^+ and OH^- ions (Toscan et al., 2019). As a result, the reaction medium becomes more acidic, which implies an increase in the concentration of H^+ ions for rapid reaction rates. The presence of H^+ ions act as in-situ-formed catalysts for the hydrolysis of ester and ether linkages between the lignocellulosic components for accessibility to the cellulose moiety (Zhang et al., 2020). Moreover, solubilization of the substrate at high temperatures and longer pretreatment times, enhanced the uptake of the GLD into the solid structure for improved sugar recovery. However, a further rise in the temperature (107.73–120 °C), when the pretreatment time was at its maximum brought about a slight decline in the reducing sugar yield from 1.09 to 1.03 g/g. The relationship between the pretreatment temperature and time has a notable impact on the physical-chemical characteristics of the LCB. The higher temperatures and contact time during pretreatment strongly influence the fractionation of the substrate, however, severe operational conditions result in thermal degradation of sugars (Ruiz et al., 2013). Fig. 1H depicts the glucose yield as a function of temperature and pretreatment time when the GLD concentration is kept constant at 30%. A proportional change in the temperature (80–120 °C) and pretreatment time (5–15 min) caused the glucose yield to significantly increase from 0.19 to 0.58 g/g. Nevertheless, varying the pretreatment time between 5 and 15 min, while keeping the temperature at its maximum (120 °C), revealed a relatively similar glucose yield ranging between 0.56 and 0.58 g/g. This suggests that the pretreatment time had little effect on the glucose yield whereas temperature showed significant positive change. Engaging in longer pretreatment times results in the evaporation of water from the pretreatment system, which in turn impedes mass transfer and sufficient penetration of the GLD chemical into the substrate (David et al., 2020). Conversely, higher temperatures generally favor lignocellulosic degradation towards more efficient enzymatic saccharification (David et al., 2020).

3.4. Validation of the optimized pretreatment models

The validity of the developed models were determined with the aim of maximizing the reducing sugar and glucose yields (Table S7). According to the MGLD-PWW model prediction, the reducing sugar and glucose yields obtained under optimum conditions of 48.70% GLD, 800 W and 9 min were recorded as 1.05 g/g and 0.43 g/g, respectively. A comparable reducing sugar (1.04 g/g±0.01 g/g) and glucose yield (0.51 ± 0.06 g/g) was reported for the experimental validation carried out in duplicate. Conversely, the SGLD-PWW model (49.89% GLD, 118 °C, 5 min) predicted a reducing sugar yield of 1.02 g/g along with a glucose yield of 0.63 g/g. Upon experimental validation, the SGLD-PWW model generated a reducing sugar and glucose yield of 1.53 ± 0.36 g/g and 0.85 ± 0.16 g/g, respectively. The overall results revealed that the optimized SGLD-PWW pretreatment produced a 32% and 40% higher reducing sugar and glucose yield, respectively, compared to the MGLD-PWW pretreatment. This result may be attributed to the mechanistic effect of steam-assisted heating that creates an acidic reaction environment in which solvents can permeate the components of the lignocellulosic biomass, hydrate cellulose and degrade the ester and ether linkages for structural collapse (Toscan et al., 2019). On the other hand, the lower fermentable sugar yields during MGLD-PWW pretreatment may be due to cellulose degradation in the hexoses and oligosaccharides by thermal processing (Ruiz et al., 2013). As a result, a lower fermentable sugar release is observed when the pretreated CCW is subjected to enzymatic hydrolysis, since the damaged cellulose cannot be successfully converted to monosaccharides. Interestingly, both the MGLD-PWW and SGLD-PWW developed pretreatments displayed reducing sugar yields above 1 g/g. The reducing sugar yields observed above 1 g/g can be accounted for from a scientific standpoint. For instance, Wyman et al. (2005) indicated that reducing sugar yields > 1 g/g can be explained by a reaction that occurs during cellulose and hemicellulose hydrolysis, where each sugar unit reacts with a water molecule, resulting in a mass gain of 11.1% and 13.6%, respectively. Additionally, Fan et al. (2016) revealed that hydrothermal pretreatment influences the average molecular weight of cellulose (Fan et al., 2016). Overestimation in the reducing sugar may be as a result of a reaction between aldehyde molecules (lower molecular weights than glucose) and the DNS reagent (Rivers et al., 1984). Furthermore, during extreme pretreatment conditions (high temperatures and pressures), reducing sugars derived from lignocellulosic biomass may be decomposed to furans that contains a common free carbonyl group, which interestingly is also present in fermentable sugars. The DNS method results in the reduction of 5-dinitrosalicylic acid to the corresponding 3-amino-5-nitrosalicylic acid, which in turn oxidizes its carbonyl center of both furans and fermentable sugars to carboxylic acid (Deshavath et al., 2020). This reaction, regardless of the carbonyl group source will drive a color change from yellow to brick red (Deshavath et al., 2020). Despite the discrepancies presented when reducing sugar yield is used as an expression unit, it provides comparability and consistency with the majority of lignocellulosic pretreatment studies that are available within the scientific domain. Nevertheless, in addition to the DNS method for reducing sugar, an enzymatic assay was used in the present study to quantify the glucose yield, thus providing a true representation of the fermentable sugars that are available.

3.5. Influence of the optimized pretreatments on the structural morphology of corn cobs

3.5.1. Compositional analysis

During pretreatment, the glucose-rich cellulose that is tightly embedded in the matrix of lignin and hemicellulose polymers may be rendered accessible to enzymatic attack (Kumar et al., 2019). The analysis revealed the cellulose, hemicellulose and lignin content of the native (untreated), MGLD-PWW and SGLD-PWW optimized CCW samples (Table S8). For the native (untreated) CCW sample, the composition of the cellulose, hemicellulose and lignin was 42.35%, 39.18% and 12.13%, respectively. The MGLD-PWW optimized sample revealed a 53.30% cellulose, 26.59% hemicellulose and 16.13% lignin content. Similarly, the composition of the SGLD-PWW optimized sample was 53.14% cellulose, 25.12% hemicellulose and 19.49% lignin. The

cellulose content for the MGLD-PWW pretreatment improved by 25.86%. Microwave irradiation manipulates the polarity and dielectric properties of molecules in order to achieve polarization of the chemical bonds. The polarized molecules collide with each other causing friction. As a result, the oscillating movement of the polar particles are accelerated causing rapid increases in the temperature, thus breaking the hydrogen bonds within the lignocellulose components and releases the cellulose fractions (Eskicioglu et al., 2007). Likewise, the SGLD-PWW pretreatment depicted a 25.48% cellulose increment. During steam-assisted heating the involvement of high pressures maintain the water in a liquid state even at the elevated temperatures to prevent dehydration of the reaction medium (Kumari and Singh., 2018). This aids in adequate GLD transfer through the medium, increasing GLD-CCW contact. The permeation of the alkaline GLD causes the covalent bonds between the lignin-hemicellulose complex to undergo hydrolysis, which aids in the liberation of cellulose (Kim, 2013). These results indicate that both the combined microwave or steam mechanism coupled with the GLD and PPW was effective in releasing the bound cellulose fraction of the lignocellulosic biomass. Furthermore, similar hemicellulose solubilization was obtained for the MGLD-PWW (38.13%) and SGLD-PWW (35.89%) pretreated CCW samples. In the case of the MGLD-PWW pretreatment, the significant decline in the hemicellulose fraction may be attributed to the direct heating interactions with the CCW. Its effect causes the disintegration of the heteropolysaccharide building blocks and its conversion to oligosaccharides (Lai and Idris, 2016). Under the SGLD-PWW pretreatment conditions, hydrolysis of acetyl groups within the heterogenous polysaccharides allows for the partial solubilization of hemicellulose (Toscan et al., 2019). The results of the compositional analysis in the present study using alkaline waste residues (GLD and PWW) revealed that the changes in cellulose and hemicellulose was in accordance with previous alkaline pretreatment studies (Lai and idris, 2016; Xu et al., 2017; Moodley and Gueguim Kana, 2017). On the other hand, the lignin fraction increased by 32.98% and 60.68% for the MGLD-PWW and SGLD-PWW pretreatments, respectively, which did not follow the general trend found in previous studies. Pretreatment techniques intend on reducing the lignin component within the lignocellulosic feedstock. However, the present study adopted the Van Soest method of lignin determination which may present drawbacks with regards to overestimation of lignin within the substrate (Van Soest, 1963). Samples containing protein within the acid insoluble lignin fraction is required to be sufficiently removed or accounted for in the gravimetric analysis since protein contamination may result in a higher recorded lignin value (Van Soest, 1963; Sluiter et al., 2008).

Moreover, studies have indicated that freely available monosaccharides may undergo degradation and incorporates itself into the lignin structure to produce carbon-rich aromatic complexes via polycondensation (Sannigrahi et al., 2011; Hu et al., 2012). These structures known as pseudo-lignin occurs as a result of the elevated temperature conditions during thermal pretreatments and has shown to be one of the main contributing factors for the higher lignin quantities observed. For example, a previous study by Meng et al. (2015) noted a similar observation whereby an increase in lignin from ~ 22% in the native populus substrate to ~ 33% in the hot water pretreated sample at 160 °C for 60 min was observed. Xiao et al. (2014) developed a hot water pretreatment using bamboo residues and also noted an increase in the lignin content from 25.9% to 41.1%, potentially indicative of pseudo lignin formation as observed in the present study.

Similarly, lignocellulosic biomass subjected to aqueous conditions above the melting point of lignin lead to the coalescence of lignin fractions, thus aiding in the loss of highly ordered structures (Donohoe et al., 2008). Xu et al. (2010) carried out a pretreatment strategy using $Ca(OH)_2$ as the catalyst, and suggested that lignin forms complexes with carbohydrates and the calcium ions, resulting in spherical structures known as lignin droplets. In conjunction, a study by Yan et al. (2015) was also carried out by comparing $Ca(OH)_2$ and NaOH pretreatments. The study observed that lignin droplets were present on the biomass after $Ca(OH)_2$ pretreatment due to calcium ion absorption, while the NaOH pretreated substrate was void of these complexes (Yan et al., 2015). According to the composition of GLD (Table S10) in the present study, calcium is present in the form of $CaCO_3$ and $Ca(OH)_2$. Therefore, the developed MGLD-PWW and SGLD-PWW pretreatments may have resulted in a similar phenomenon, where lignin droplets formed due to the presence of calcium ions within the pretreatment catalyst. The formation of these complexes is not only capable of reducing the recalcitrance within the lignocellulosic biomass but retains carbohydrates due to the calcium ions crosslinked with lignin and carbohydrates (Xu et al., 2010). Subsequently. the carbon rich lignin complexes produced within the cell wall tends to migrate to the cell surface after thermal expansion (Donohoe et al., 2008). This leads to more exposed carbohydrates and higher pore volume, thereby improving the efficiency of enzymatic hydrolysis for enhanced sugar yields (Xu et al., 2010). Despite the higher lignin content observed for the developed pretreatments in the present study, the increased glucose recovery observed after the treatment process demonstrates effective degradation to the lignocellulosic structures. Additionally, SEM and FTIR analysis were performed to corroborate the abovementioned results by demonstrating the structural damage to the CCW.

3.5.2. *Scanning electron microscopy (SEM) analysis*

The surface morphology of the native (untreated), MGLD-PWW and SGLD-PWW pretreated corn cob waste was visualized using scanning electron microscopy (SEM) (Fig. 2). The surface structure of the native (untreated) CCW appeared to be smooth, rigid and compacted. This may be attributed to the dense lignin network surrounding the cellulose and hemicellulose moieties (Phitsuwan et al., 2016). Alternatively, noticeable changes were induced by the MGLD-PWW and SGLD-PWW pretreatments to the CCW surface. The optimized MGLD-PWW and SGLD-PWW pretreatments demonstrated a compromised cellular integrity with extreme cavitation, detached fibers and expanded surface area. These structural aberrations inflicted by the optimized pretreatment strategies may be due to the disbanding of the lignin fraction and solubilization of hemicellulose from the CCW residues. The collapse of the rigid polymer structure causes cellulose to become accessible to enzymatic attack for significant bioconversion to fermentable sugars. These observations were in accordance with pretreatment studies using microwave-assisted NaOH on cassava stem (Kamalini et al., 2018), steam-assisted NaOH on napier grass (Phitsuwan et al., 2016) and steam-assisted GLD pretreatment on corn cobs (David et al., 2020).

3.5.3. *Fourier transform infrared (FTIR) spectral analysis*

The FTIR spectral analysis identifies functional groups in the native (untreated) and pretreated lignocellulosic biomass and compares the changes with regards to chemical bonding in the carbohydrate and lignin moieties. These chemical modifications are illustrated by using various banding patterns for the native, MGLD-PWW and SGLD-PWW pretreated CCW (Fig. 3). The band intensities of the SGLD-PWW pretreated biomass, followed by the MGLD-PWW pretreatments were significantly higher than that of the native CCW samples with reference to the cellulose, hemicellulose and lignin. The C-O-C stretching at the β-glycosidic linkages between amorphous cellulose and hemicellulose sugar units is represented by the peak at 896 cm^{-1} (Ramadoss and Muthukumar, 2016). The pronounced banding at 1028 cm^{-1} may be associated with the C-O-H stretching of the primary and secondary alcohols of cellulose (Pang et al., 2012). The adsorptive peak formation at 1116 cm^{-1} and 1315 cm^{-1} are associated with skeletal vibrations of the C-O-C ring and C-H bending within the cellulosic moieties, respectively (Ramadoss and Muthukumar, 2016). These results portray the structural changes that occurred within the cellulose fractions as a result of the CCW pretreatment. The occurrence of C=O elongation of the aromatic ring in lignin, xylan and ester groups, and the formation of the -OCH_3 group in syringic acid of lignin are represented by 1246 cm^{-1} and

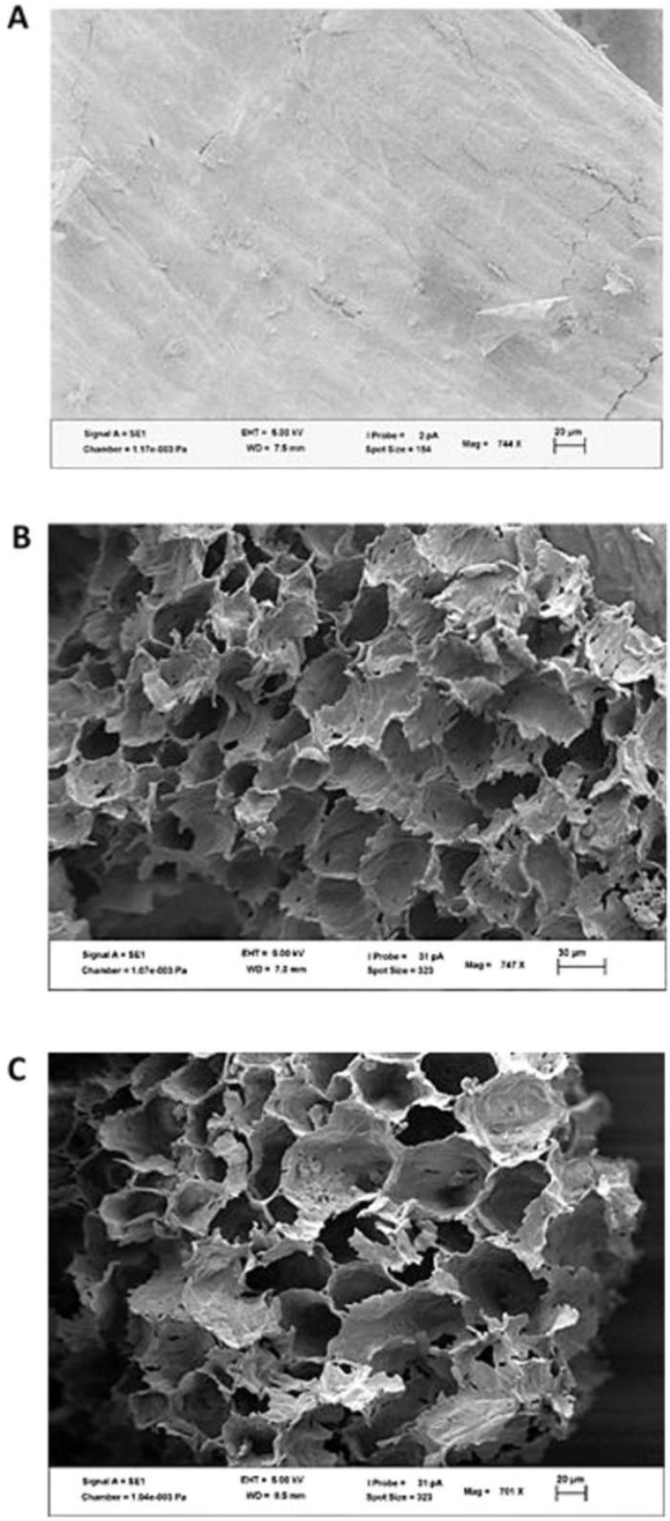

Fig. 2. Scanning electron microscopy images of (A) native, (B) optimized MGLD-PWW pretreated and (C) optimized SGLD-PWW pretreated CCW.

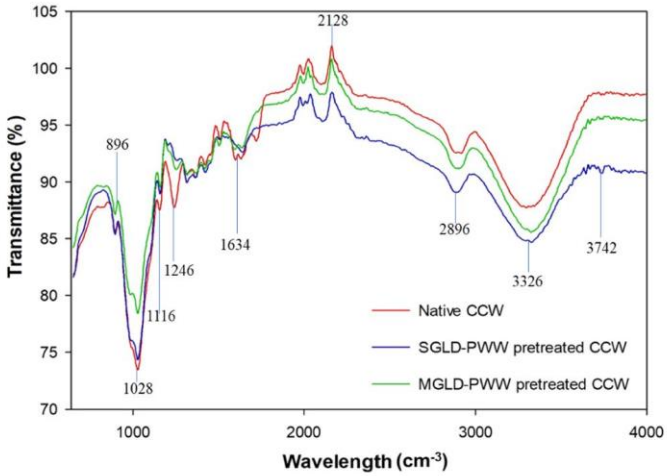

Fig. 3. Fourier transform infrared spectrum of the native, optimized MGLD-PWW and optimized SGLD-PWW pretreated CCW samples.

1421 cm^{-1}, respectively (Kamalini et al., 2018). The C=C stretching of the aromatic skeletal ring within lignin and bending mode of absorbed water is related to peaks at 1505 cm^{-1} and 1634 cm^{-1}, respectively (Pang et al., 2012). The characteristic bands at 2896 cm^{-1} corresponds to the C-H extension of the methyl and methylene groups while the peak at 3326 cm^{-1} is linked to the O-H stretching in aliphatic and phenolic groups (Ramadoss and Muthukumar, 2016). These banding patterns have increased significantly after SGLD-PWW and MGLD-PWW pretreatment when compared to the native CCW. The banding pattern at 3742 cm^{-1} for the SGLD-PWW and MGLD-PWW pretreated samples indicate the manifestation of an asymmetrical O-H group widening (Ramadoss and Muthukumar, 2015). As a result, the peak is less pronounced in the spectra of the pretreated CCW residues and thus suggests that the molecular of the cellulose structures was altered due to the rupturing of this linkage after pretreatment. Pretreatments approaches that incorporate alkaline-type chemicals target the ester and glycosidic bonds within the cell wall, causing hemicellulose and lignin disbanding, partial decrystallization of cellulose and modifications in the lignin fractions (Cheng et al., 2010). The aforementioned trends observed for the MGLD-PWW and SGLD-PWW optimized pretreatments coincide with previous alkaline pretreatment studies on lignocellulosic residues (Sewsynker-Sukai and Gueguim Kana, 2018; Kamalini et al., 2018, David et al., 2020).

3.6. *Evaluation of the optimized MGLD-PWW and SGLD-PWW pretreatments with reported studies on various lignocellulosic residues*

A comparative assessment of the optimum sugar yields from the waste-based MGLD-PWW and SGLD-PWW optimally pretreated CCW with recent reports on alkaline pretreatment regimes was performed (Table S9). The present SGLD-PWW pretreatment study displayed a more effective pretreatment strategy in terms of higher reducing sugar and glucose yields as opposed to various chemical pretreatment studies using different substrates. For instance, the SGLD-PWW pretreated corn cobs under optimized conditions revealed a 50.59% higher glucose recovery in comparison to our previous study that used pure water (as opposed to PWW in the present study) with 15.86% GLD, 3.97% solid loading at a temperature of 121 °C for 50.33 min (David et al., 2020). Additionally, the present study not only displayed a higher sugar yield but also used a significantly shorter pretreatment time (5 min) and a slightly lower temperature (118 °C) than our previous study using GLD (David et al.,2020). Furthermore, previous studies have reported lower glucose yields during steam-assisted alkaline pretreatments on napier grass (Phitsuwan et al., 2016) and corn stover (Shao et al., 2020). The noticeably higher fermentable sugar yields obtained in the present study when evaluated against reported studies of alkaline pretreated lignocellulosic biomass may be accounted for by the dual impact of Na_2CO_3 and Na_2S present within GLD. These alkalic salts are viable pretreatment catalysts for the degradation of specific bonds within the lignocellulosic structure. The Na_2CO_3 promotes cleavage of the ester and glycosidic bonds within cell wall, while the strong nucleophilic species (HS^-) present in Na_2S attack the phenolic β-aryl ether bonds of lignin (Cheng et al., 2010; Qing et al., 2016). The application of individual alkalic salt pretreatments are considered to be as effective as strong alkaline

chemicals such as NaOH, however, using a combination of alkalic salts (as is in the case of GLD) has the potential to exceed pretreatment ability. Furthermore, the $CaCO_3$ in the GLD provides a buffering effect to prevent fluctuations in pH during chemical reactions (Golmaei et al., 2018). Along with its effective delignification ability and buffering characteristics, GLD offers milder pretreatment conditions.

Interestingly, Moodley and Gueguim Kana (2017) recorded a 19.38% higher reducing sugar yield using a microwave-assisted sequential salt-alkali ($ZnCl_2$-NaOH) pretreatment on sorghum leaves compared to the present MGLD-PWW strategy. However, despite the higher reducing sugar yield obtained by Moodley and Gueguim Kana (2017), the implementation of sequential pretreatment regimes is detrimental to the environment by exposing two different chemicals in addition to the negative impact on the cost, time and energy, which in turn affects the efficiency and feasibility of the pretreatment system. Lignocellulosic pretreatment costs account for approximately 40% of the biorefinery process costs (Kucharska et al., 2018). The large fraction of expenses associated with pretreatment processes has become the driving force towards finding a method that reduces costs and energy, while producing high sugar yields. The Kraft paper and pulp industry generates high quantities of alkaline waste such as GLD and PWW that are either landfilled or necessitate costly and energy exhaustive treatment processes to meet the environmental regulations and standards. With the abovementioned drawbacks in mind, the present developed pretreatment strategies could assist the Kraft industry by: (1) utilizing their waste products, thus avoiding high landfill costs and negative environmental implications, and (2) generating an additional revenue stream by partnering with biotechnology conglomerates to produce value-added compounds that can compensate for the costs associated with end process wastewater treatments.

4. Conclusion

Two novel Kraft waste-based pretreatments using microwave- (MGLD-PWW) or steam heating (SGLD-PWW) were optimized on corn cobs in this study. The optimized SGLD-PWW pretreatment resulted in a 1.7-fold higher glucose yield with a 44% lower pretreatment time compared to the MGLD-PWW method. The developed waste-based pretreatments have shown significant potential to tackle global challenges that are centered around water, food and energy by: (1) utilizing lignocellulosic wastes that do not impact food security, (2) applying Kraft wastes that eliminate chemical and water usage during pretreatment processes and (3) generating high sugar that may be harnessed for the microbial production of biofuels and other value-added compounds.

CRediT authorship contribution statement

Anthea Naomi David: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition. **Y. Sewsynker-Sukai:** Conceptualization, Methodology, Software, Validation, Writing – review & editing, Project administration, Resources, Supervision, Funding acquisition. **E.B. Gueguim Kana:** Methodology, Software, Validation, Data curation, Writing – review & editing, Resources, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work is based on research supported in part by the National Research Foundation (NRF) of South Africa (Grant numbers 122080 and 129556). Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the NRF.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2021.114222.

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Chapter 4: Supplementary Material

Table S1. Preliminary screening of the microwave-and steam-assisted combined GLD and paper wastewater pretreatment of CCW

Run	Input				Output	
	GLD concentration (%)	Power intensity (Watts)	Temperature (°C)	Pretreatment time (min)	Reducing sugar (g/g)	Glucose (g/g)
MGLD-PWW$_{preliminary}$	30	500	-	6	1.27	0.45
MGLD-W	30	500	-	6	1.42	0.40
MPWW	-	500	-	6	0.49	0.19
MW	-	500	-	6	0.35	0.13
SGLD-PWW$_{preliminary}$	30	-	100	10	1.33	0.47
SGLD-W	30	-	100	10	1.44	0.40
SPWW	-	-	100	10	0.49	0.21
SW	-	-	100	10	0.35	0.17

Footnote: g/g = g reducing sugar or glucose/ g pretreated dry weight corn cobs, MGLD-PWW$_{preliminary}$= preliminary microwave-assisted combined green liquor dregs and paper wastewater, MGLD-W= GLD in deionized water heated by microwave, MPWW= paper wastewater alone heated by microwave, MW= deionized water alone heated by microwave, SGLD-PWW$_{preliminary}$= preliminary steam-assisted combined green liquor dregs and paper wastewater, SGLD-W= GLD in deionized water heated by steam, SPWW= paper wastewater alone heated by steam, SW= deionized water alone heated by steam.

Table S2. Polynomial model equations representing the relationship between the input parameters and the sugar yields for the MGLD-PWW and SGLD-PWW pretreatments

Model	Equation	Equation number
$MGLD\text{-}PWW_{rs}$	$0.63 + 0.14A + 0.12B + 0.14C + 0.14BC$	1
$MGLD\text{-}PWW_{g}$	$0.25 + 0.046A + 0.050B + 0.042C + 0.046AB + 0.045AC$	2
$SGLD\text{-}PWW_{rs}$	$0.94 + 0.19A + 0.17B^{*} + 0.11C - 0.15B^{*2}$	3
$SGLD\text{-}PWW_{g}$	$0.40 + 0.071A + 0.17B^{*}$	4

Footnote: $MGLD\text{-}PWW_{rs}$=microwave-assisted combined GLD and PWW-reducing sugar, $MGLD\text{-}PWW_{g}$=microwave-assisted combined GLD and PWW-glucose, $SGLD\text{-}PWW_{rs}$=Steam-assisted combined GLD and PWW-reducing sugar, $SGLD\text{-}PWW_{g}$=Steam-assisted combined GLD and PWW-glucose, A=GLD concentration, B=Power intensity, C=Pretreatment time, B*=Temperature.

Table S3. Analysis of variance (ANOVA) of the optimized microwave-assisted combined GLD and paper wastewater pretreatment model for reducing sugar yields

Source	Sum of Squares	Degrees of freedom*(df)*	Mean Square	F-value	p-value (probability > F)	
Model	0.62	9	6.9×10^{-2}	5.12	2.13×10^{-2}	Significant
A-GLD conc.	0.15	1	0.15	10.85	1.32×10^{-2}	
B-Power intensity	0.11	1	0.11	8.36	2.33×10^{-2}	
C-Pretreatment time	0.15	1	0.15	11.24	1.22×10^{-2}	
AB	3.80×10^{-2}	1	3.80×10^{-2}	2.78	0.14	
AC	1.90×10^{-3}	1	1.90×10^{-3}	1.41	0.27	
BC	0.08	1	0.08	5.92	4.52×10^{-2}	
A^2	9.28×10^{-4}	1	9.28×10^{-4}	6.90×10^{-2}	0.80	
B^2	1.50×10^{-2}	1	1.50×10^{-2}	1.08	0.33	
C^2	5.40×10^{-2}	1	5.40×10^{-2}	4.02	8.51×10^{-2}	
Residual error	9.5×10^{-2}	7	1.40×10^{-2}			
Lack of fit	5.3×10^{-2}	3	1.80×10^{-2}	1.68	0.31	Not significant
Pure Error	0.04	4	0.01			
Cor Total	0.72	16				

Footnote: A= GLD (%), B= Power intensity (Watts), C= pretreatment time (min).

Table S4. Analysis of variance (ANOVA) of the optimized microwave-assisted combined GLD and paper wastewater pretreatment model for glucose yields

Source	Sum of Squares	Degrees of freedom*(df)*	Mean Square	F-value	p-value (probability > F)	
Model	7.70×10^{-2}	9	8.61×10^{-3}	6.11	1.31×10^{-2}	Significant
A-GLD conc.	1.70×10^{-2}	1	1.70×10^{-2}	12.26	0.01	
B-Power intensity	0.02	1	0.02	14.44	6.70×10^{-3}	
C-Pretreatment time	1.40×10^{-2}	1	1.40×10^{-2}	9.88	1.63×10^{-2}	
AB	8.41×10^{-3}	1	8.41×10^{-3}	5.96	4.46×10^{-2}	
AC	8.06×10^{-3}	1	8.06×10^{-3}	5.72	4.81×10^{-2}	
BC	5.01×10^{-3}	1	5.01×10^{-3}	3.55	0.10	
A^2	3.52×10^{-4}	1	3.52×10^{-4}	0.25	0.63	
B^2	3.86×10^{-3}	1	3.86×10^{-3}	2.74	0.14	
C^2	1.88×10^{-4}	1	1.88×10^{-4}	0.13	0.73	
Residual error	9.87×10^{-3}	7	1.41×10^{-3}			
Lack of fit	4.75×10^{-3}	3	1.58×10^{-3}	1.24	0.41	Not significant
Pure Error	5.12×10^{-3}	4	1.28×10^{-3}			
Cor Total	8.70×10^{-2}	16				

Footnote: A= GLD (%), B= Power intensity (Watts), C= pretreatment time (min).

Table S5. Analysis of variance (ANOVA) of the optimized steam-assisted combined GLD and paper wastewater pretreatment model for reducing sugar yields

Source	Sum of Squares	Degrees of freedom*(df)*	Mean Square	F-value	p-value (probability > F)	
Model	0.74	9	0.08	12.17	1.70×10^{-3}	Significant
A-GLD conc.	0.28	1	0.28	41.26	4.00×10^{-4}	
B*-Temperature	0.23	1	0.23	34.56	6.00×10^{-4}	
C-Pretreatment time	0.09	1	0.09	13.96	7.30×10^{-3}	
AB*	8.20×10^{-3}	1	8.20×10^{-3}	1.22	0.31	
AC	1.28×10^{-5}	1	1.28×10^{-5}	1.90×10^{-3}	0.97	
B*C	0.01	1	0.01	1.61	0.25	
A^2	0.01	1	0.11	1.68	0.24	
B^{*2}	0.10	1	0.10	14.66	6.50×10^{-3}	
C^2	8.60×10^{-4}	1	8.60×10^{-4}	0.13	0.73	
Residual error	4.70×10^{-2}	7	6.74×10^{-3}			
Lack of fit	0.01	3	3.40×10^{-3}	0.37	0.78	Not significant
Pure Error	3.70×10^{-2}	4	9.25×10^{-3}			
Cor Total	0.79	16				

Footnote: A= GLD (%), B*= Temperature (°C), C= pretreatment time (min).

Table S6. Analysis of variance (ANOVA) of the optimized steam-assisted combined GLD and paper wastewater pretreatment model for glucose yields

Source	Sum of Squares	Degrees of freedom*(df)*	Mean Square	F-value	p-value (probability > F)	
Model	0.29	9	0.03	5.59	1.67×10^{-2}	Significant
A-GLD conc.	0.04	1	0.04	6.90	3.41×10^{-2}	
B*-Temperature	0.23	1	0.23	39.68	4.00×10^{-4}	
C-Pretreatment time	5.90×10^{-3}	1	5.90×10^{-3}	1.01	0.35	
AB*	8.94×10^{-4}	1	8.94×10^{-4}	0.15	0.71	
AC	0.01	1	0.01	2.04	0.20	
B*C	9.16×10^{-4}	1	9.16×10^{-4}	0.16	0.70	
A^2	1.76×10^{-3}	1	1.76×10^{-3}	0.30	0.60	
B^{*2}	9.88×10^{-5}	1	9.88×10^{-5}	0.02	0.90	
C^2	5.34×10^{-4}	1	5.34×10^{-4}	0.09	0.77	
Residual error	0.04	7	5.84×10^{-3}			
Lack of fit	0.02	3	5.99×10^{-3}	1.04	0.46	Not significant
Pure Error	0.02	4	5.74×10^{-3}			
Cor Total	0.34	16				

Footnote: A=GLD (%), B*=Temperature (°C), C=pretreatment time (min).

Table S7. Validation of the optimized conditions for the MGLD-PWW and SGLD-PWW pretreatment of corn cobs

Model	Input parameters				Output			
					Reducing sugar yield (g/g)		Glucose yield (g/g)	
	GLD concentration (%)	Power intensity (W)	Temperature (°C)	Pretreatment time (min)	Observed	Predicted	Observed	Predicted
MGLD-PWW	48.70	800	-	9	1.04±0.01	1.05	0.51±0.06	0.43
SGLD-PWW	49.89	-	118	5	1.53±0.36	1.02	0.85±0.16	0.63

Footnote: MGLD-PWW=microwave-assisted combined Green liquor dregs and paper wastewater, SGLD-PWW=Steam-assisted combined Green liquor dregs and paper wastewater.

Table S8. Compositional analysis of the native, MGLD-PWW and SGLD-PWW pretreated CCW samples

Sample	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Native	42.35	39.18	12.13
MGLD-PWW	53.30	26.59	16.13
SGLD-PWW	53.14	25.12	19.49

Footnote: MGLD-PWW=microwave-assisted combined Green liquor dregs-Paper wastewater, SGLD-PWW=Steam-assisted combined Green liquor dregs and paper wastewater.

Table S9. Comparison of the alkaline pretreatment methods on various lignocellulosic residues

Substrate	Microwave or steam heating	Pretreatment conditions	Sugar yield	Reference
Corn cobs	Microwave	48.70% GLD, 10% SL, 800 W, 9 min	1.04 g/g[a]	This study
Corn cobs	Microwave	48.70% GLD, 10% SL, 800 W, 9 min	0.51 g/g[b]	This study
Corn cobs	Steam	49.89% GLD, 10% SL, 118°C, 5 min	1.53 g/g[a]	This study
Corn cobs	Steam	49.89% GLD, 10% SL, 118°C, 5 min	0.85 g/g[b]	This study
Corn cobs	Steam	15.86% GLD, 3.97% SL, 121°C, 50.33 min	0.42g/g[b]	David et al. (2020)
Sorghum leaves	Microwave	1.67 M $ZnCl_2$, 400 W, 5 min, 1.52 M NaOH, 400 W, 5 min	1.29 g/g[b]	Moodley and Gueguim Kana (2017)
Napier grass	Steam	0.5 M NaOH, 6% SL, 121°C, 60 min	0.36 g/g[b]	Phitsuwan et al. (2016)
Sugarcane bagasse	Microwave	1% NaOH, 10% SL, 600 W, 4 min	0.67 g/g[a]	Binod et al. (2012)
Sugarcane leaf	Steam	1.36M NaOH, 1.73M $ZnCl_2$, 9.69% SL, 121°C, 30 min	1.17 g/g[a]	Moodley and Gueguim Kana (2017)
Oil palm trunk	Microwave	2.5M NaOH, 5% SL, 700 W, 80°C, 60 min	18 g/L[b]	Lai and Idris (2016)
Corn stover	Steam	6% NaOH, 12% urea, 4% SL, 80°C, 20 min	0.54 g/g[b]	Shao et al. (2020)

Footnote: [a] = Reducing sugar, [b] = Glucose, CCW= Corn cob waste, GLD= Green liquor dregs, SL= Solid loading.

Table S10. Chemical composition of the green liquor dregs (GLD)

Elements and compounds	GLD sample concentration (%)
Moisture, H_2O	45-55
Calcium as $CaCO_3$ and $Ca(OH)_2$	16-20
Sodium as Na_2CO_3 and NaOH	14-20
Elemental sulfur	1.4-1.7
Dissolve sulfur	0.8-1
Sulfur	0.4-0.6
Sodium sulfide/sulfate	1.3-1.7
Chromium oxide	0.2-0.35
Manganese as MnO	0.8-0.95

Table S11. Chemical composition of the paper wastewater (PWW)

Elements and compounds	PWW sample concentration (mg/L)
Arsenic	<0.0025
Boron	0.215
Barium	0.175
Cadmium	<0.0005
Cobalt	<0.002
Chromium	0.5351
Hexavalent chromium	<0.006
Copper	<0.007
Mercury	<0.001
Manganese	1
Molybdenum	<0.002
Nickel	0.009
Lead	<0.005
Antimony	0.007
Selenium	<0.003
Vanadium	0.0144
Zinc	0.031
Total dissolved solids	7 522
Chloride	1 114.1
Sulphate	448.5
Nitrate (NO_3) as Nitrogen	<0.05
Fluoride	<0.3
Cyanide total	0.06

Footnote: All trace elements and chemical substances that may present environmental and health concerns were shown to be below the individual total concentration threshold limit, thus meeting the minimum requirements for classification as a low toxicity waste according to the National norms and standards for assessment of waste for landfill disposal.

Chapter 4: Appendix

Data not shown as per journal specifications are included as supporting information.

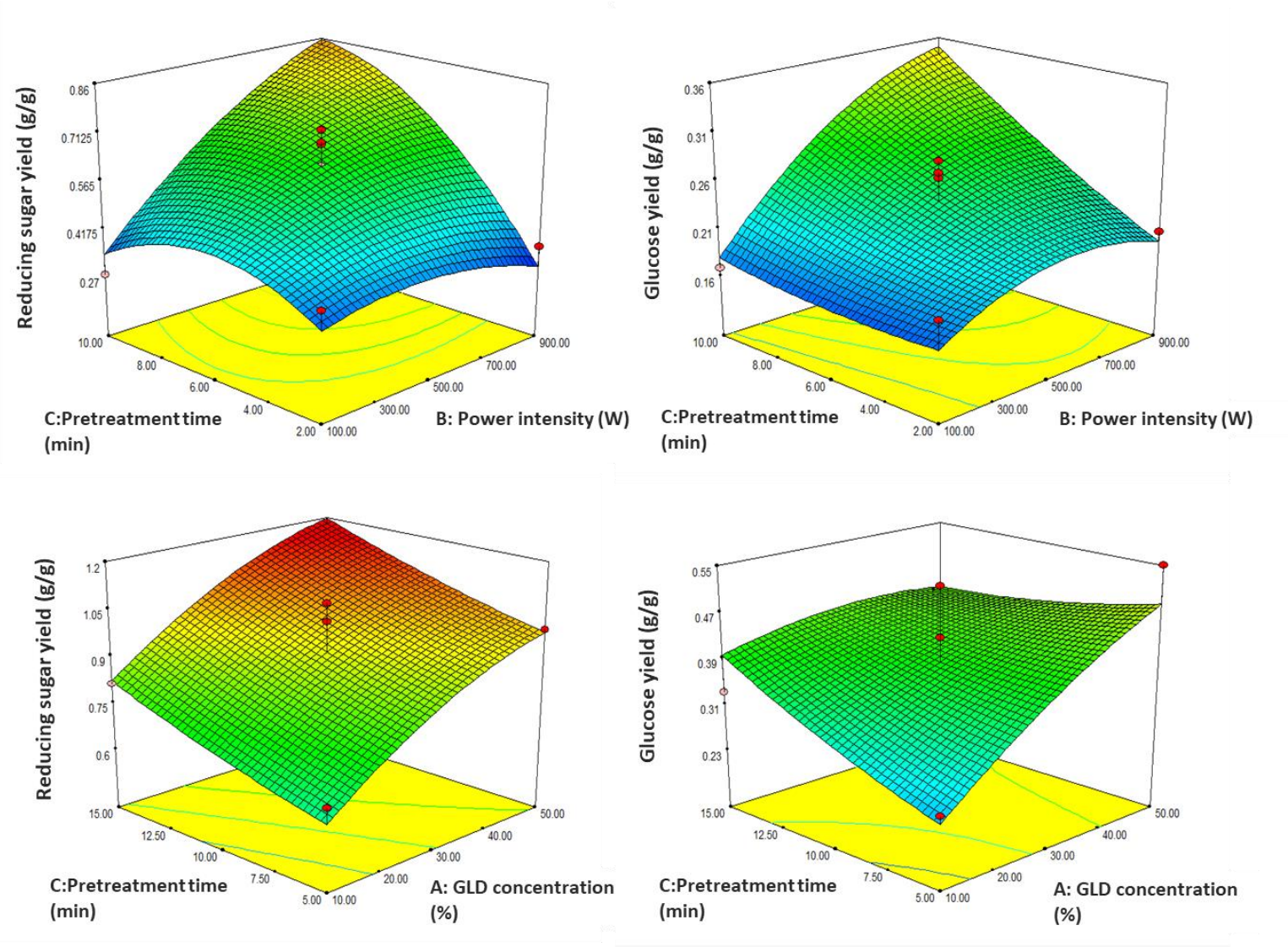

Fig. 1. 3D Response surface plots representing the effects of the various input parameters on the fermentable sugar recovery from corn cobs: (A) time (min) and power intensity (W) (MGLD-PWW_{rs}), (B) time (min) and power intensity (W) (MGLD-PWW_{g}), (C) GLD concentration (%) and time (min) (SGLD-PWW_{rs}), (D) GLD concentration (%) and time (min) (SGLD-PWW_{g}).

CHAPTER 5

Harnessing Artificial Neural Networks and large language models for bioprocess optimization: Predicting sugar output from Kraft waste-based lignocellulosic pretreatments

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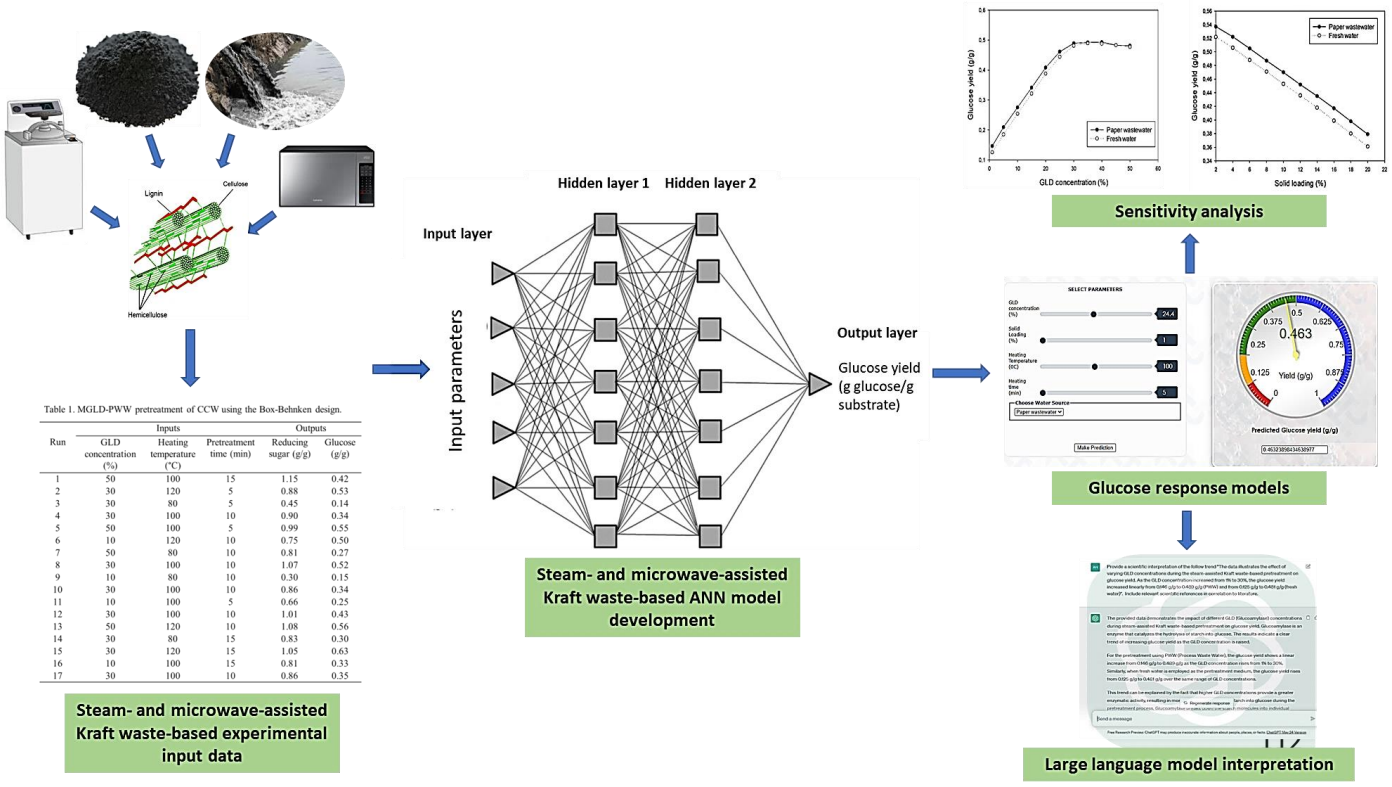
This chapter has been published in *Industrial Crops & Products* (206, 117686) with the title: Harnessing Artificial Neural Networks and large language models for bioprocess optimization: Predicting sugar output from Kraft waste-based lignocellulosic pretreatments. The published paper is presented in the following pages.

Highlights

- First report on artificial intelligence and ChatGPT for pretreatment optimization.
- Artificial Neural Network (ANN) models were developed with R2 >0.95.
- Green liquor dregs concentration and power intensity are key parameters.
- Process insights deduced by ChatGPT concurred with the authors' interpretation.
- Potential elimination of labour and resource intensive lignocellulosic pretreatments.

Graphical abstract



Industrial Crops & Products 206 (2023) 117686

Contents lists available at ScienceDirect

Industrial Crops & Products

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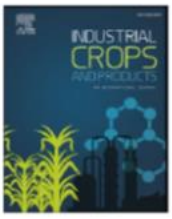

Harnessing Artificial Neural Networks and large language models for bioprocess optimization: Predicting sugar output from Kraft waste-based lignocellulosic pretreatments

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ARTICLE INFO

Keywords:
Artificial Neural Network
Bioprocess modelling and optimization
Sensitivity analysis
Large language models
Kraft waste-based pretreatment

ABSTRACT

This study implements Artificial Neural Network (ANN) models as predictive tools for glucose responses from Kraft waste-based pretreatments. The developed steam- and microwave-assisted ANN models achieved R^2 scores > 0.95 for the observed and predicted glucose responses. An in-depth sensitivity analysis revealed that the glucose responses for the steam and microwave models were highly susceptible to the stepwise variation in green liquor dregs concentration (>3.3-fold) and power intensity (>2.6-fold), respectively. Comparative assessment on the capability of the large language model, ChatGPT, to generate innovative and factually accurate insights based on the process data was carried out. The novel process insights deduced by ChatGPT concurred with the authors' findings of this study, underscoring the unique critical role of integrating advanced artificial intelligence and domain-specific knowledge to accelerate progression in lignocellulosic waste pretreatment. As such, these synergies align with global sustainable developmental objectives that leverage 4IR technologies, propelling this research field forward.

1. Introduction

The overall global demand of fossil fuel-derived energy and commodity chemicals has caused widespread issues rooted in natural resource scarcity, global warming and waste accumulation. This has exacerbated the mandate to progress from a largely reliant non-renewable economy towards a carbon-neutral bioeconomy to achieve energy independence and effective management of the environmental footprint (Patel and Shah, 2021). In this respect, the conversion of waste-derived-lignocellulosic biomass to high value bioproducts has become an emerging concept in the race towards a more sustainable future. Lignocellulosic biomass (LCB), especially agricultural waste residues are energy-dense, non-food-based feedstocks that regenerates itself annually and in abundance (Nunes et al., 2020). This inexhaustible and low-cost feedstock contains cellulose (38–55%), hemicellulose (23–32%) and lignin (15–25%) that account for approximately 90% of the dry matter present within the substrate and may be converted into value added products such as biochemicals and bioenergy (Kim et al., 2015; Muthuvelu et al., 2019; McKendry, 2002). More specifically, LCB such as corn cob waste (CCW) and banana pseudostems (BPS) have been recognized for its abundant annual output, high growth rate, rich carbohydrate and low lignin content, making these substrates attractive precursors for high sugar recovery (David et al., 2021; Laltha et al., 2022). The amorphous hemicellulose networks link the cellulose chains into microfibrils and form crosslinks with the non-carbohydrate lignin polymers to provide mechanical strength and intense impermeability to the LCB (Rebello et al., 2020). As a result, pretreatment is necessary prior to bioconversion processes in order to destabilize the cohesive lignin structure for enhanced amenability to enzymatic reactions followed by microbial fermentation.

Alkaline pretreatment strategies have been tagged as a gold standard due to its high depolymerization activity, but the extensive operational costs and energy requirements have jeopardized industrial applicability (David et al., 2021). For this reason, a pretreatment technology that: (1) minimizes waste, (2) uses low toxicity processes and, (3) employs energy saving approaches while preserving the key production output will lead to a functional, economically sound lignocellulosic bioprocess. In an effort to streamline the search for an effective pretreatment method that meet these criteria, the spotlight has turned towards industrial waste derived pretreatment chemicals such as green liquor dregs (GLD) and

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https://doi.org/10.1016/j.indcrop.2023.117686
Received 10 August 2023; Received in revised form 19 October 2023; Accepted 20 October 2023

paper wastewater (PWW) as viable alternatives. Both GLD and PWW are waste residues generated in abundance from the Kraft paper and pulp mill industry that plague the system with filter blockages, hydraulic conductivity issues, extensive landfilling and wastewater treatment costs (David et al., 2020). However, its alkaline nature shows promise of greater traction over commonly reported laboratory-grade alkaline chemicals such as NaOH, NH_4OH and $NaHCO_3$ amongst others (David et al., 2020; David et al., 2021; Laltha et al., 2022). For instance, GLD contains alkaline species (Na_2CO_3 and Na_2S) that modify the structure of the cell wall matrix, while PWW exhibits dual functionality as a pretreatment catalyst and freshwater substitute (David et al., 2021). Repurposing these waste residues will minimize waste disposal, mitigate invasive biohazardous treatments, relieve the current water crisis, reduce pretreatment chemical costs and valorize waste constituents into marketable products.

In most cases, chemical catalysts have been associated with physical pretreatment strategies to enhance productivity and enable high recovery of fermentable sugars due to lignocellulosic degradation. In particular, steam-assisted heating ionizes water at high temperatures and pressures to catalyse the hydrolysis of ester and ether linkages (Toscan et al., 2019). Conversely, microwave irradiation, an energy-efficient mechanism uses direct heating technology to cause fibre fragmentation through molecular collisions (Sorn et al., 2019). Despite the mechanistic advantages of each of these processes, the system is still inundated with issues surrounding economics, productivity and bioprocess scale-up.

Lignocellulosic processes are influenced by cost factors related to raw material acquisition, resource usage, energy consumption, downstream processing, yield generation, product purification and market demand amongst others. Furthermore, achieving high conversion rates of LCBs into the desired products, maximizing product yields and minimizing product losses are vital to improve productivity. More especially, scaling up the lignocellulosic pretreatment processes from laboratory-scale to commercial production has long been a significant challenge since it involves maintaining process performance, productivity and product quality during its transition from small-scale reactors to larger industrial-scale equipment. Scale-up also impacts the overall economics of the process as capital costs, energy requirements, and operational considerations are continuously being modified. Addressing the aforementioned challenges encountered with lignocellulosic pretreatment processes requires a comprehensive understanding of the underlying science, optimization of bioprocess conditions, improved resource performance and integration of advanced process control strategies. These elements collectively contribute to the development of efficient and logically coordinated pretreatment technologies. By optimizing process parameters, improving efficiency and implementing effective scale-up strategies, the economics and productivity of lignocellulosic processes can be enhanced, leading to more sustainable and commercially viable biorefinery systems. As a result, these challenges necessitate the development of effective modelling and optimization strategies to address the research gap.

Modelling plays a pivotal role in determining the key conditions that will favourably impact the optimization of fermentable sugar outputs at the early stages of bioprocess development (Sindhu et al., 2016). The fourth industrial revolution (4IR) together with global sustainability goals have steered research in the direction of artificial intelligence (AI) systems such as Artificial Neural Networks (ANN), enabling virtual experiments that curb alarming costs and intensive labour. ANNs imitate the cognitive responses of the human brain to mathematically model complex non-linear systems without prior knowledge of kinetics, metabolic fluxes or the bioprocessing medium. Data-driven tools, such as ANNs, utilize correlations between the process inputs and corresponding outputs to train machine learning algorithms for accurate yield prediction, leading to maximized product generation and reduced inhibitors (Sebayang et al., 2017). Furthermore, ANNs employ feature selection methods to identify and rank critical input features within a dataset, thus minimizing redundancy, reducing training time, mitigating the risk of overfitting and facilitating data interpretation (Chen et al., 2020). Sensitivity analysis, a notable feature selection method, identifies vital input parameters for prediction of the output variable and quantifies how the changes in these input values affects the resultant output. Consequently, these intelligent models expedite screening assessments of the fermentable sugar release from pretreated LCB, offering foresight into industrial scale applications. Few studies have reported on the prediction of fermentable sugar yields from pretreated LCB using intelligent modelling. For example, Moodley et al. (2019) developed microwave- and steam- inorganic salt-based ANN models for the prediction of reducing sugar yields from sugarcane leaves using experimental data from published studies. The reported study revealed coefficient of determination (R^2) values of 0.97 for both models (Moodley et al., 2019). Similarly, Vani et al. (2015) applied ANN modelling to predict the effect of various input variables on the glucose and xylose concentrations during the enzymatic hydrolysis of rice straw. The high R^2 value of > 0.97 and error lying close to 0 indicates reliability of the ANN model in predicting the sugar yields during hydrolysis (Vani et al., 2015). In another instance, Lee et al. (2020) optimized an ultrasonic-assisted organosolv pretreatment process on oil palm empty fruit bunches for improved reducing sugar concentration. The Leverburgh Marquee ANN model indicated that ultrasonic-assisted organosolv pretreatment was significantly affected by temperature, time and sonication power with good predictive accuracies ($R^2 = 0.91$) (Lee et al., 2020). In addition, Chang et al. (2011) subjected napiergrass to a two-stage pretreatment process of steam explosion (SE) followed by alkaline delignification. Three different methods were used, namely, Back-Propagation Neural Network (BPNN), the Multiple Linear Regression (MLR), and the Partial Least-Square regression (PLS) with enzymatic digestibility as the output. The data indicates that the BPNN model obtained a higher prediction performance ($R^2 > 0.98$) compared to the MLR and PLS models (Chang et al., 2011). Rego et al. (2018) presented a study that compares the optimization of sugarcane bagasse delignification subjected to alkaline H_2O_2 using ANN and Adaptive Network based Fuzzy Inference System (ANFIS) with glucose and xylose concentrations as outputs. The statistical quality of the models was significant due to the low error values and high R^2 values close to 1 (Rego et al., 2018). As it stands, to the best of our knowledge, the application of ANN on Kraft waste-based pretreatment modelling has not yet been documented. Such intelligence approaches will provide real-time estimations of process parameters, enhance workflow by eliminating various laboratory tasks and negate experimentation costs.

While ANN is a robust tool for prediction, pattern recognition and complex decision-making, there has been a growing interest in exploring the potential of advanced large language models (LLMs), such as ChatGPT to elucidate complex perceptions in the domain of bioprocess development. By harnessing the capabilities of deep learning algorithms, the LLM possesses the ability to process, analyse and synthesize intricate patterns from extensive and multifaceted scientific data information, generating human-like articulations (Ray, 2023). Unlike contextual knowledge in human interpretation, LLMs provide unbiased analysis based on statistical associations presented in the dataset (Ray, 2023). However, these models lack domain-specific expertise and comprehension, potentially hindering its ability to provide nuanced interpretations intrinsic to the specialized field. To address this challenge, an integrative approach that synergistically combines the vast computational and data processing capabilities of LLMs with human interaction can be employed to ensure accurate and contextually informed decisions. This collaborative method aids in identifying potential discrepancies and cognitive biases that may arise in both human and model interpretations, ultimately propelling research advancements in factual, scientific knowledge generation.

Therefore, this study aims to develop two ANN models, steam- and microwave-assisted models, built on existing experimental data from previously published Kraft waste-based pretreatment regimes on

lignocellulosic wastes (David, . et al., 2020; David et al., 2021; Laltha et al., 2022). The Kraft waste-based pretreatments included: (a) steam-assisted green liquor dregs (GLD) only in pure water (SGLD-W) (David et al., 2020), (b) steam-assisted combined GLD and paper wastewater (PWW) (SGLD-PWW) (David et al., 2021), (c) microwave-assisted combined GLD and PWW (MGLD-PWW) (David et al., 2021) and (d) microwave-assisted PWW (MPWW) (Laltha et al., 2022). Additionally, a sensitivity analysis was carried out to evaluate the impact of each input variable on the glucose yield output. These models will thereafter be able to identify data patterns and efficiently predict the glucose responses from new physicochemical input values that can be deployed as a virtual experimentation platform within a global public repository. In conjunction with ANN model development and contextual assessment, this study incorporates a unique element by introducing ChatGPT, a large language model (LLM) to the sensitivity analysis dataset. This explored the model's capability to recognize process data patterns, interpret data and generate factually sound insights in scientific reporting compared to already established human cognitive responses.

2. Materials and methods

2.1. Experimental data collection for ANN model development

The experimental data used for model development were obtained from our previous studies on Kraft waste-based pretreatment of corn cob waste (CCW) and banana pseudostem (BPS) (David et al., 2020; David et al., 2021; Laltha et al., 2022). These studies assessed the effect of various physicochemical parameters that enhanced the disintegration cross-linked lignin fractions of lignocellulosic waste for enzymatic conversion of cellulose to glucose. The pretreatments selected for model development were based on a: (a) steam-assisted green liquor dregs (GLD) only in pure water (SGLD-W) ((David et al., 2020), (b) steam-assisted combined GLD and paper wastewater (PWW) (SGLD-PWW) (David et al., 2021), (c) microwave-assisted combined GLD and PWW (MGLD-PWW) (David et al., 2021) and (d) microwave-assisted PWW (MPWW) (Laltha et al., 2022). A total of 96 experimental runs were used to develop the ANN model with the predictor being glucose yield. The selected input parameters and ranges for the microwave model included GLD concentration (0–50%), solid loading (10–30%), power intensity (100–900 W), heating time (1–10 min) and substrate type (corn cobs or banana pseudostem). For the steam model, the input variables consisted of GLD concentration (1–50%), solid loading (1–20%), heating temperature (80–121 °C), heating time (5–60 min) and water source (pure water or PWW). The resultant output for both models were glucose yield (g/g).

2.2. ANN model development for sugar yield prediction

Two ANN models, namely, steam-assisted and microwave-assisted Kraft waste-based models were structured on the Google Collaboratory platform using the Python version 2.4.1 (64-bit), with TensorFlow and Keras libraries. A feed-forward multi-layered perceptron (MLP) was used to model the non-linear relationship between the considered inputs and glucose yield from the Kraft waste pretreatments. The topology of both the steam- and microwave-assisted Kraft waste-based models consisted of one input layer to accommodate datasets with five predictor variables and two hidden layers with a dynamic number of nodes (20) within each layer (Table 1, Fig. 1A and B). With the aim of introducing non-linearity into the steam- and microwave-assisted Kraft waste-based models, the Rectified Linear Unit (ReLU) activation function was applied within these hidden layers. Lastly, the output layer consisted of a single node responsible for generating regression predictions. In particular, this layer applied no activation function, thus enabling the model to produce continuous numerical predictions without imposing any specific output limitations. For the feed forward training algorithm, the input data was scaled through weight assignment, which associated a real number quantity to the connection of two neurons. The neurons in the input layer transmitted the signals of the scaled data to the neurons in the hidden layer (Sewsynker-Sukai and Gueguim Kana, 2017a).

Table 1
Steam- and microwave-based model structure.

Layer (type)	Output shape	Number of parameters
Dense_256	None, 20	120
Dense_257	None, 20	420
Dense_258	None, 20	420
Dense_259	None, 1	21

2.3. ANN training and validation using backpropagation

Prior to using the data set, data normalization was carried out in the range [− 1 to − 1] according to Eq. (1).

$$\text{Normalized}(e_i) = \frac{e_i \quad -E_{min}}{E_{max} \quad -E_{min}} \tag{1}$$

where e_i is the normalized data and E_{min} and E_{max} denote the minimum and the maximum values.

Following data normalization, the input dataset was divided such that 80% of samples were assigned to the training set and the remaining 20% formed the validation set. Generally, the input layer processes the collated data and allocates each feature to an input node. The neurons are then stimulated through a forward-propagation approach such that the impact of each neuron's activation is regulated by the adjusted weights. These activators are propagated until the predicted output is achieved (Fig. 2). Subsequently, the back propagation algorithm employed to train the model adjusted the neuron's connections until the net error between the experimental and predicted data was reduced to a level below the target threshold. The weights were then revised based on their contribution to the error. The training process was repeated until the minimum mean square error was attained. A grid search approach was employed to optimize the model's hyperparameters for the number of epochs (400, 600, 1000), the optimizer (RMSprop, Adam) and the number of nodes (20, 30). The optimized hyperparameters were 600, RMSprop and 20 for the number of epochs, optimizer and number of nodes, respectively. The accuracy of the ANN models were evaluated using coefficients of determination (R^2), Mean Absolute Error (MAE), Mean Absolute Percentage Error (MAPE), Mean Squared Error (MSE) and Root Mean Squared Error (RMSE) on the validation data for each model (Eqs. 2–6). The trained and validated models were deployed on a globally accessible AISocket platform. The steam-assisted and microwave-assisted models can be located at https://www.aisocket.org/dashboard/005/ and https://www.aisocket.org/dashboard/007/, respectively.

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(d_i - O_i)^2}{\sum_{i=1}^{n}(O_i)^2} \tag{2}$$

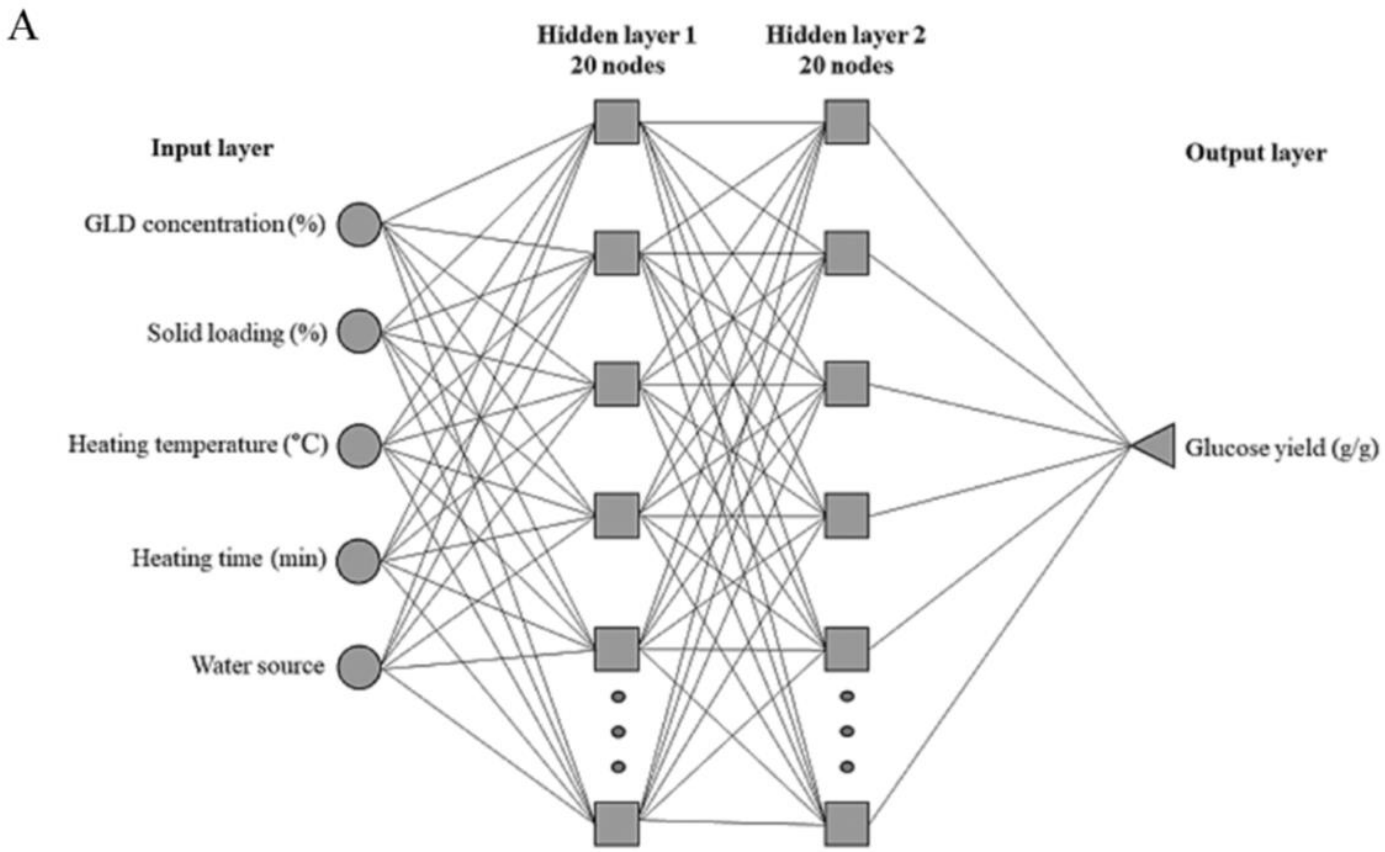
$$\text{MAE} = \frac{1}{n} \quad \sum_{i=1}^{n}|d_i - O_i| \tag{3}$$

$$\text{MAPE} = \frac{100}{n} \quad \sum_{i=1}^{n}\frac{O_i - d_i}{d_i} \tag{4}$$

$$\text{MSE} = \frac{\sum_{i=1}^{n}(d_i - O_i)^2}{n} \tag{5}$$

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(d_i - O_i)^2}{n}} \tag{6}$$

where d_i and o_i are the observed and predicted outputs, and n is the



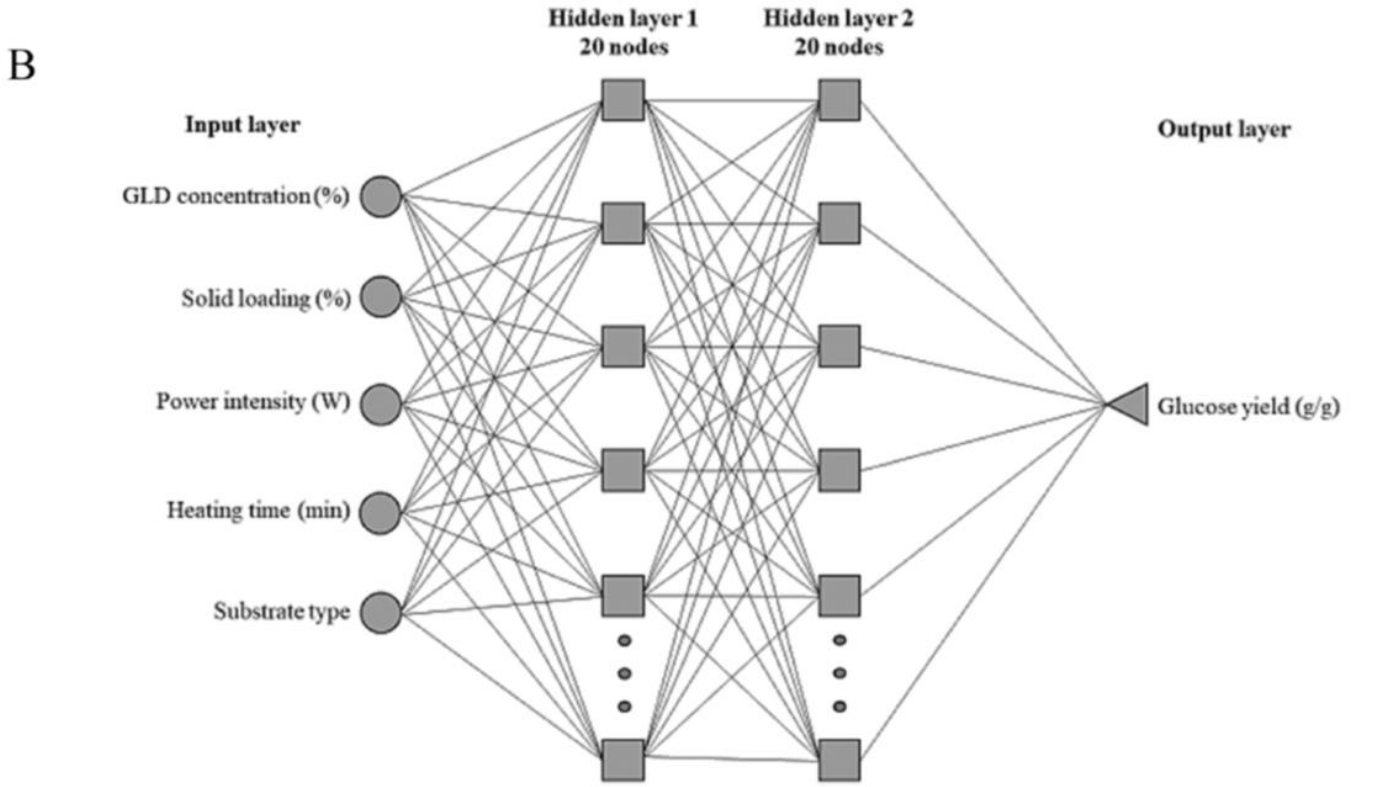


Fig. 1. Topology of Neural Networks used for the (A) steam- and (B) microwave-assisted Kraft waste-based model consisting of one input layer (five neurons), two hidden layers (20 nodes each) and one output layer (one neuron).

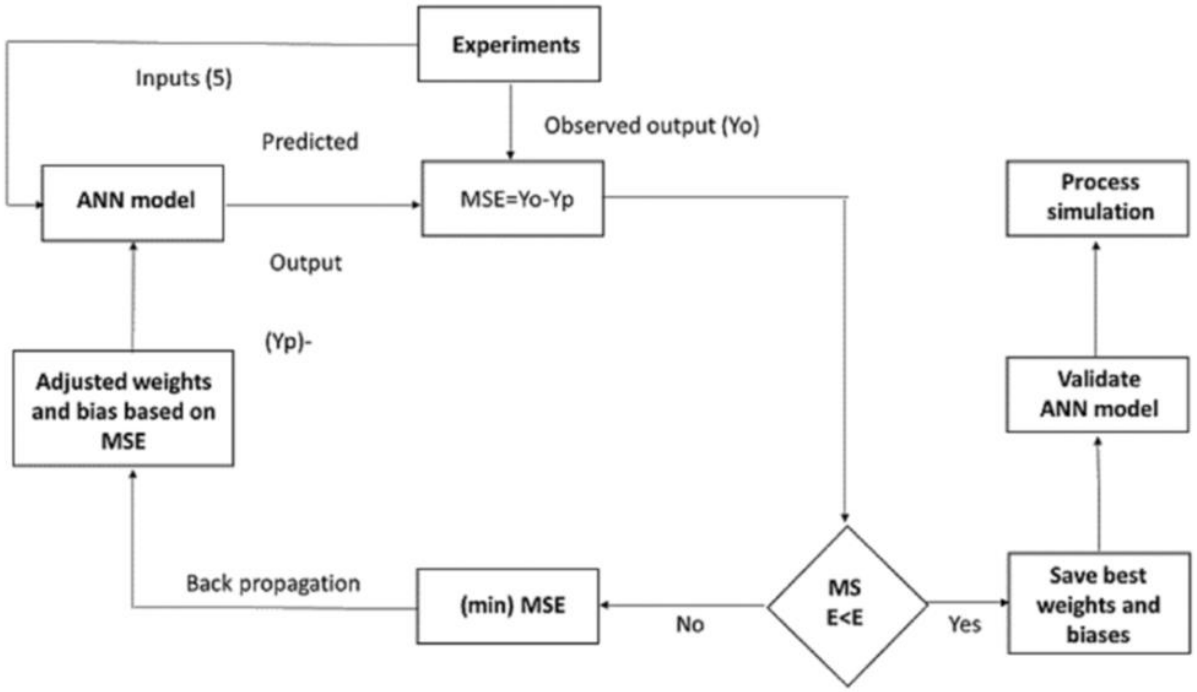


Fig. 2. The back propagation training flowchart for artificial neural network for the steam- and microwave-assisted Kraft waste-based model.

validation data set (20% of the data) for the ANN model.

2.4. Sensitivity analysis

Sensitivity analysis was utilized to determine the impact of fractional changes of the input parameters on the glucose response (Rorke et al., 2017). The variation of each input parameter ranged between its minimum (−1) and maximum (1) value, while maintaining all other inputs at their midpoint values (0). To explicate the functional relationships between pretreatment inputs and the glucose yield outputs, mathematical equations were derived from each model using curve fitting.

2.5. Analysis of data trends using large language model

The large language model, ChatGPT (version GPT-3.5 as of 24 May 2023, developed by OpenAI) was exposed to the datasets consisting of the fractional changes in the selected input parameters and its corresponding glucose yield outputs for both steam-assisted and microwave-assisted Kraft waste-based models of the present study. Search prompts were meticulously formulated and directed to the model in order to analyse the data trends of the glucose yield outputs in relation to its changes in input parameters. This approach enables interpretation of the relationships and patterns within the data through the perspective of the advanced LLM.

3. Results and discussion

3.1. Evaluation of developed ANN models

The developed ANN models were validated using the remaining 20% data sets not incorporated into the model training process. This validation focused on the prediction of glucose yield generation. The steam-based and microwave-assisted Kraft waste-based models yielded coefficients of determination (R^2) of 0.95 and 0.97, respectively as shown in Fig. 3A and B. This demonstrates the model's ability to effectively explain 95% and 97% of the variability observed in the data for the steam-based and microwave-based models, respectively. Typically, R^2 values falling within the range of 0.7–1 indicates a good model fit, as the R^2 value is a statistical measure of how well the regression line of the predicted data aligns with the actual data points. Furthermore, an additional metric analysis was conducted using the Mean Absolute Error (MAE), Mean Absolute Percentage Error (MAPE), Mean Squared Error (MSE) and Root Mean Squared Error (RMSE) to assess the adequacy of the model's predictive performance. For the steam- and microwave-assisted Kraft waste-based models, the MAE suggests that, on average, the model's predictions deviate by approximately 0.065 and 0.043 units, respectively from the actual values. Lower MAE values indicate higher predictive accuracy, and the MAE values in the present study are relatively low, indicating a reasonably accurate model. The MAPE expresses the prediction errors as a percentage of the actual values. Our results indicate that the model's predictions exhibit a relative error of about 25.25% and 17.18% for the steam- and microwave-assisted models, respectively, when compared to the actual values. This suggests a moderate level of accuracy. The low MSE values of 0.007 for the steam-assisted model and 0.003 for the microwave-assisted model imply a good model fit. A low RMSE suggests that the model's predictions deviate by approximately 0.082 and 0.056 units from the actual values for the steam- and microwave-assisted models respectively. Both models demonstrate a reasonably accurate predictive performance, as evidenced by the low MAE, MSE and RMSE values, indicating minimal average prediction errors and a good model fit. The slight variation between experimental and predicted results may be attributed to the inherent complexities of a dynamic biological system that is characterized by constant change in the enzymatic saccharification process over time. This is dependent on the enzyme-substrate interactions, active energy exchanges and physicochemical responses. For this reason, the ANN modelling approach may not completely capture the intricacies of the biological system, leading to slight divergences between predicted and observed output. Nevertheless, the statistical indices suggest that the developed models displayed a strong correlation between the observed and predicted glucose outputs. As evidenced in both figures (Fig. 3A and B), a large majority of the data points are congregated along the predictive trend line, illustrating high accuracy for predicting the glucose yield when subjected to new process conditions. The robust relationship between the predicted and observed yields of the developed models can facilitate an appropriate navigation space needed for the pretreatment design studies. As such, the ANN predictive system aims to implement an initial preliminary screening tool for optimizing process

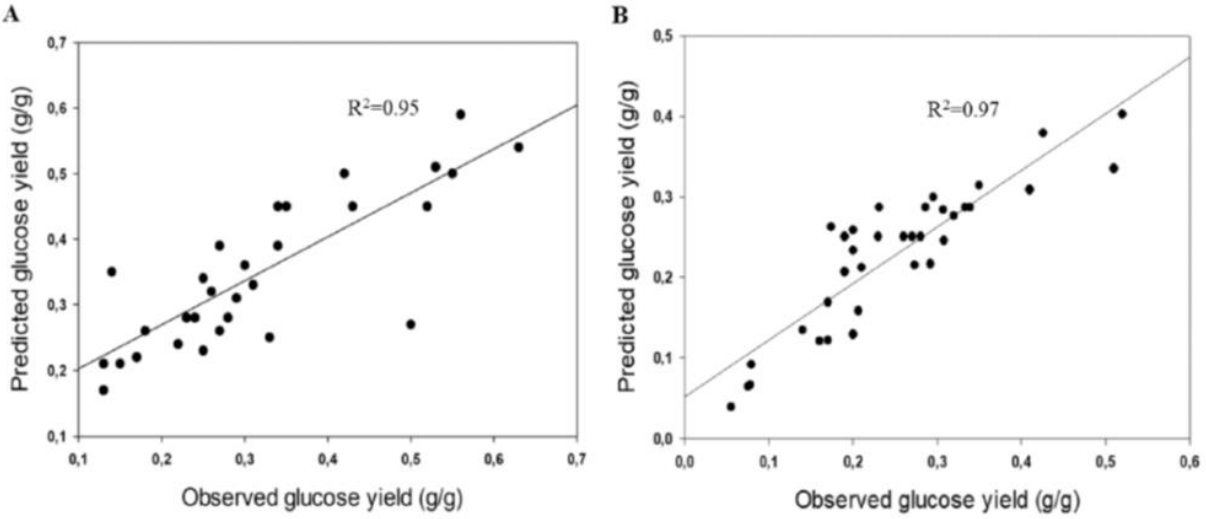


Fig. 3. Regression plots showing observed versus predicted glucose yield (g/g) for the (A) steam- assisted Kraft waste-based model and (B) microwave-assisted Kraft waste-based model. *Note*: The diagonal line depicts expectations under a one-to-one relationship for the predicted and observed yields.

parameters within lignocellulosic pretreatment technologies. These models have the potential to enhance resource efficiency, improve productivity and decrease process development costs. Kumari et al. (2019) optimized the pretreatment process for Leucaena leucocephala wood using three parameter inputs being catalyst concentration, duration and temperature with a total reducing sugar yield as its single response outcome. The results of these experiments were analyzed by three algorithms of neural networks such as Bayesian Regularization Neural Network (BRNN), Scaled Conjugate Gradient Neural Network (SCGNN), and Levenberg Marquardt Neural Network (LMNN) in which BRNN gave most accurate predictions for total reducing sugar yield with the lowest Root Mean Square Error (RMSE= 7.17×10^{-7}) and Standard Error of Prediction percentage (SEP=3.84×10^{-7}%). In a similar manner, Nikzad et al. (2015) reported on Response surface methodology (RSM) and ANN modelling of alkali pretreatment of rice husks for enhanced glucose and xylose yields. Both modeling methods were statistically compared by means of the R^2 and RSME. The RSM model gave R^2 (>0.83) and RMSE (>1.96) while the ANN model reported R^2 (>0.92) and RMSE (>1.36) value for the glucose and xylose yield prediction (Nikzad et al., 2015). It was therefore concluded that the ANN model observed a slightly higher prediction performance compared to RSM. Furthermore, Valim et al. (2017) reported on the use of ANN as prediction and fault detection tools for the delignification process of sugarcane bagasse subjected to alkaline H_2O_2. The performance of the ANN model was evaluated by the R^2 (0.99), MSE (0.004) and sum of squared errors (SSE=0.354). The values obtained for R^2 (close to 1) and the error indices (close to 0) indicated a good agreement of the theoretical and actual data.

3.2. *Effect of changes in the input parameters on glucose yield*

Sensitivity analysis was employed to determine the impact of the process input variables on the glucose yield and was carried out on the steam- (Fig. 4A-D) and microwave- (Fig. 4E-H) assisted Kraft waste-based pretreatment models. A sensitivity indicator represents the rate of change of the output, attributable to the fluctuations in the input parameters. An increased sensitivity to a parameter suggests that a slight variation in the input value can cause a significant change in the process output (Oh et al., 2003). On the other hand, a low sensitivity implies that even in the presence of large variations in the input parameter, a marginal change in the glucose output may occur. The functional relationships between the process inputs used within range and glucose output were derived from curve fitting for the steam- and microwave-assisted Kraft waste-based models (Table 2, Table 3).

For the steam-assisted Kraft waste-based model, the sensitivity analysis revealed that an increase in the GLD concentration from 1% to 30% resulted in a linear increase in the glucose yield from 0.146 g/g to 0.489 g/g and 0.125 g/g to 0.481 g/g when using PWW and pure water, respectively (Fig. 4A). This translates to a 3.3-fold and 3.8-fold increase from the baseline GLD concentration (1%) with the use of PWW and pure water, respectively. The exponential rise in glucose yield illustrates that it is largely dependent on the GLD concentration with a high sensitivity within this region. The high glucose yields obtained under the increasing GLD concentrations may be ascribed to the strong alkaline interactions of the GLD that cleaves specific bonds within the corn cob structure. This results in the disintegration of the lignocellulosic matrix and subsequent physical damage that facilitates easy penetration of the hydrolytic enzymes for improved sugar recovery (Kim et al., 2015). A further increase in the GLD concentration initiated a plateau in the glucose yield, followed by a slight decrease from 0.493 g/g to 0.481 g/g when the GLD concentration reached 40% (Fig. 4A). Given the primarily insoluble nature of GLD, its increase in concentration increases the viscosity by holding water tightly within the pretreatment slurry. This reduces the mass transfer capacity and hinders the diffusion of the GLD chemical to the CCW substrate. Furthermore, the temperature was maintained at its median value of 100 °C which may not be adequate for solubilization of the CCW substrate since heat transfer is impeded as a result of the high GLD concentrations (>30%). In a related study on alkaline pretreatment, Qing et al. (2016) observed an upward trend in the sugar yield when the Na_3PO_4 concentration was increased from 1% to 9%. A further increase in the Na_3PO_4 concentration above 9% resulted in a plateaued sugar yield (Qing et al., 2016). The relationship between the GLD concentration and glucose yield fit a rational model type for both the PWW and freshwater pretreatments (Table 2).

Conversely, a hyperbolic decline relationship (Table 2) was observed in Fig. 4B where an increase in the solid loading from 2% to 20% triggered a decrease in glucose yield from 0.537 g/g to 0.379 g/g and 0.522 g/g to 0.361 g/g when subjected to PWW and fresh water, respectively. The low SL (2%) facilitates a pretreatment medium that is relatively fluid in nature which ensures optimal GLD-substrate interactions by maximizing mass and heat transfer. As the SL increases (>2%), the liquid volume is reduced due to absorption, culminating in a viscous suspension with low diffusion capacity. This indicates that a SL < 2% enables a maximum glucose yield, resulting in a reduced resource utilization for improved process economics. Notably, Raghavi et al. (2016) showed that an incline in the NaOH pretreated sugarcane waste from 10% to 15% SL decreased the sugar yield from 0.70 g/g to< 0.50 g/g.

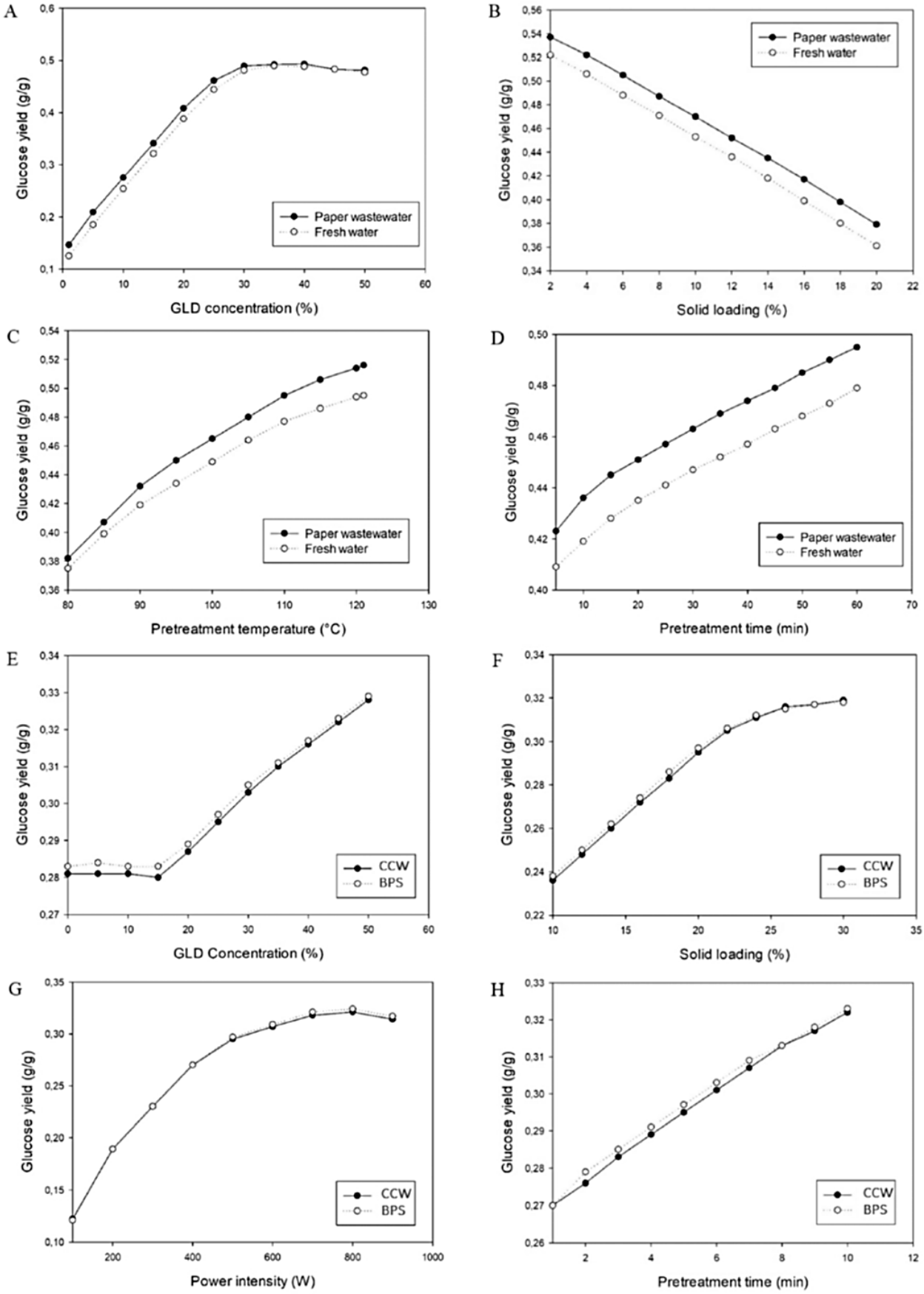


Fig. 4. Effect of fractional changes of input parameters from the steam-assisted (A-D) and microwave-assisted Kraft waste-based pretreatments (E-H) on the glucose yield.

Table 2
Steam-assisted Kraft waste-based model equations for the effect of fractional changes in the input parameters on the process output.

Water source	Input	Model equation	Model type	Fitted model	R^2
PWW	(a) GLD concentration	$y = \frac{a+bx}{1+cx+dx^2}$	Rational	$y = \frac{0.13+0.01x}{1-0.02x+0.001x^2}$	0.99
	(b) Solid loading	$y = q_0\left(1+\frac{bx}{a}\right)^{\left(-\frac{1}{b}\right)}$	Hyperbolic decline	$y = 0.55\left(1-\frac{1.44x}{68.49}\right)^{\left(\frac{1}{1.44}\right)}$	0.99
	(c) Temperature	$y = \frac{\theta x^{\eta}}{\kappa^{\eta}+x^{\eta}}$	DR-Hill-Zerbackground	$y = \frac{0.59x^{3.22}}{66.27^{3.22}+x^{3.22}}$	0.99
	(d) Pretreatment time	$y = \frac{a+bx}{1+cx+dx^2}$	Rational	$y = \frac{0.39+0.11x}{1+0.24x-0.0004x^2}$	0.99
Fresh water	(a) GLD concentration	$y = \frac{a+bx}{1+cx+dx^2}$	Rational	$y = \frac{0.11+0.01x}{1-0.02x+0.001x^2}$	0.99
	(b) Solid loading	$y = q_0\left(1+\frac{bx}{a}\right)^{\left(-\frac{1}{b}\right)}$	Hyperbolic decline	$y = 0.54\left(1-\frac{1.42x}{65.46}\right)^{\left(\frac{1}{1.44}\right)}$	0.99
	(c) Temperature	$y = \frac{\theta x^{\eta}}{\kappa^{\eta}+x^{\eta}}$	DR-Hill-Zerbackground	$y = \frac{0.57x^{2.93}}{64.39^{2.93}+x^{2.93}}$	0.99
	(d) Pretreatment time	$y = a+br^x+cx$	Exponential plus linear	$y = 0.42-(0,02)(0,89)^x+0.001x$	0.99

Footnote: PWW = paper wastewater, GLD= green liquor dregs, R^2 = Coefficient of determination.

Table 3
Microwave-assisted Kraft waste-based model equations for the effect of fractional changes in the input parameters on the process output.

Substrate type	Input	Model equation	Model type	Fitted model	R^2
CCW	(a) Time	$y = \frac{a+bx}{1+cx+dx^2}$	Rational	$y = \frac{2.27-17.18x}{1-60.29x+0.77x^2}$	0.99
	(b) Solid loading	$y = \frac{a}{(1+e^{b-cx})^{\frac{1}{d}}}$	Richards	$y = \frac{0.32}{(1+e^{0.46-0.42x})^{\frac{1}{17.56}}}$	0.99
	(c) Power intensity	$y = \frac{1}{A+B\ln(x)+C(\ln(x))^3}$	Steinhart-Hart equation	$y = \frac{1}{41.63-8.66\ln(x)+6.5(\ln(x))^3}$	0.99
	(d) GLD concentration	$y = a+\frac{\theta x^{\eta}}{\kappa^{\eta}+x^{\eta}}$	DR-Hill	$y = 0.28+\frac{0.06x^{3.65}}{34.65^{3.65}+x^{3.65}}$	0.99
BPS	(a) Time	$y = \gamma+(1-\gamma)\Gamma(\alpha,\beta x)$	DR-Gamma	$y = 0.26+(1-0.26)x0.26(0.74,0.003x)$	0.99
	(b) Solid loading	$y = \frac{a}{(1+e^{b-cx})^{\frac{1}{d}}}$	Richards	$y = \frac{0.32}{(1+e^{0.19-0.42x})^{\frac{1}{17.30}}}$	0.99
	(c) Power intensity	$y = a+br^x+cx$	Exponential plus linear	$y = 0.89-(0,85)(1)^x-0.0004x$	0.99
	(d) GLD concentration	$y = a+\frac{\theta x^{\eta}}{\kappa^{\eta}+x^{\eta}}$	DR-Hill	$y = 0.28+\frac{0.06x^{3.54}}{35.26^{3.54}+x^{3.54}}$	0.99

Footnote: CCW = Corn cob waste, BPS= Banana pseudostem, GLD= green liquor dregs, R^2 = Coefficient of determination.

On the other hand, a steady rise in the glucose yield from 0.382 g/g to 0.516 g/g (PWW) and 0.375 g/g to 0.495 g/g (fresh water) was shown when the pretreatment temperature was increased beyond 80 °C till 121 °C (Fig. 4C). This interaction between the temperature and glucose yield was illustrated by a DR-Hill-Zer background relationship (Table 2). Exposure to high temperature induces rapid reaction rates and enhanced solubilization, attributable to the change in chemical properties of water by ionization (Toscan et al., 2019).

Likewise, when the pretreatment time increased from 5 min to 60 min, the glucose yield increased linearly from 0.423 g/g to 0.495 g/g and 0.409 g/g to 0.479 g/g when using PWW and fresh water, respectively (Fig. 4D). An extended pretreatment time increases the contact time for the GLD, fostering a positive influence on the fractionation of the LCB. The rational model best illustrated the relationship between the pretreatment time and glucose yield with PWW as a water source (Table 2). On the other hand, the exponential plus linear model type expressed the relationship between the pretreatment time and glucose yield when fresh water was utilized in the pretreatment system (Table 2).

With regards to the sensitivity analysis for the microwave-assisted Kraft waste-based model, similar glucose yields of 0.281 g/g and 0.283 g/g from CCW and BPS, respectively, were depicted when the GLD concentration increased from 0% to 15% (Fig. 4E). The GLD concentration to SL ratio translates to < 1:1, suggesting that the pretreatment process may be limited by the low GLD concentrations and possibly permeates only a fraction of the total median biomass (20%) within solution. Thereafter, a sharp linear increase was observed in the glucose yield of the CCW (0.287 g/g to 0.328 g/g) and BPS (0.289 g/g to 0.329 g/g) when the GLD concentration was further elevated from 20% till 50% (Fig. 4E). Interestingly, when the GLD to SL ratio was initiated at a > 1:1 ratio, the glucose yield showed a strong positive correlation. Accordingly, the DR-Hill model was used to describe the relationship between the GLD concentration and glucose yield using both the CCW and BPS as substrate (Table 3).

In another instance, an incline in the solid loading from 10% to 24% initiated a linear increase in the glucose yield from 0.236 g/g to 0.311 g/g and 0.238 g/g to 0.312 g/g after pretreatment of CCW and BPS, respectively (Fig. 4F). The GLD to SL ratio when the SL range was between 10% and 24% was denoted at > 1:1, resulting in the induction of saturated GLD solutions for optimal lignocellulosic disbanding with significant cellulose release. A further increase in the SL from 24% to 30% caused the glucose release from CWW (0.311 g/g to 0.319 g/g) and BPS (0.312 g/g to 0.318 g/g) to increase slightly thereafter. Despite a higher SL (> 24%), the pretreatment threshold may have been reached due to GLD being the limiting factor. This interaction was depicted using the Richards model for both CCW and BPS (Table 3).

Remarkably, exponential increases in the glucose yields from 0.122 g/g to 0.318 g/g (CCW) and 0.121 g/g to 0.321 g/g (BPS) were shown when the power intensity was increased from 100 W to700 W (Fig. 4G). This translates to a 2.6-fold and 2.7-fold increase from the baseline power intensity (100 W) when using CCW and BPS, respectively. Microwave irradiation promotes the breakdown of the

lignocellulosic constituents by the introduction of molecular collisions caused by dielectric polarization on the chemical covalent bonding of the feedstock (Sorn et al., 2019). A further increase in the power intensity from 700 W to 800 W resulted in a slight plateau of the glucose yield where CCW only released 0.318 g/g to 0.321 g/g and BPS produced 0.321 g/g to 0.324 g/g. Interestingly, an increase beyond 800 W reduced the glucose yields for both the CCW (0.314 g/g) and BPS (0.317 g/g). In the same vein, Sewsynker-Sukai and Gueguim Kana (2018) depicted that an incline in power intensity beyond 700 W did not significantly influence the sugar yield. This was ascribed to the high microwave irradiation that accelerates the evaporation of water and adversely affects the homogeneity of the pretreatment slurry and penetration of the microwaves to the biomass. This correlation was best described by a Steinhart-Hart equation and exponential plus linear model types for the CCW and BPS substrates, respectively (Table 3).

Moreover, when the pretreatment time was adjusted from 1 min to 10 min, the glucose obtained from the CCW drastically increased from 0.27 g/g to 0.322 g/g while the BPS gave 0.27 g/g to 0.323 g/g (Fig. 4H). Lin et al. (2015) demonstrated a similar effect of pretreatment time on water hyacinth using microwave pretreatment and revealed that increments from 5 to 10 min resulted in a 51.85% increase in the reducing sugar yield. However, a further extension in the pretreatment time did not significantly impact the sugar yield and may be attributed to the degradation of monosaccharides into other by-products such as acetic acid and furfural. As a result, the rational model was used to illustrate the interaction between the pretreatment time and glucose yield using CCW while the DR-gamma model fit the association with BPS (Table 3).

3.3. Critical assessment of large language model interpretations: comparisons and insights in lignocellulosic pretreatment research

This study utilized a LLM to extract knowledge on the impact of several physicochemical input parameters on fermentable sugar yields during lignocellulosic pretreatment, specifically focusing on the complete Kraft waste-based lignocellulosic pretreatment. The model's responses were based on meticulously designed input prompts. Subsequently, the prompts were compared to the authors findings detailed in *Section 3.2* , drawn from domain expertise and direct analysis of study data. A selection of significant input parameters impacting Kraft waste-based pretreatment of CCW for improved glucose recovery, as interpreted by the LLM, are discussed below:

For instance, when prompted on the data trends with regards to the effect of varied solid loading during steam-assisted Kraft waste-based pretreatment on glucose yield, the LLM elucidated that the decrease in glucose yield with increasing solid loading can be attributed to several factors. One of the notable factors is the decrease in mass and heat transfer efficiency. As the solid loading increases, the liquid volume decreases due to absorption, which in turn results in a more viscous suspension with low diffusion capacity (See Supplementary material). The model interpretation aligns with previous studies underscoring that increased solid loading results in reduced substrate-pretreatment chemical contact and lower glucose yields (Raghavi et al., 2016; Sewsynker-Sukai and Gueguim Kana, 2017b). Moreover, the model provided additional insight, stating that high solid loadings result in a higher degree of substrate structural heterogeneity, which can potentially inhibit enzymatic hydrolysis, a known occurrence in LCB pretreatment (Jeoh et al., 2017). Similarly, ChatGPT interpreted the linear increase in glucose yield with increasing GLD concentration within the steam-assisted Kraft waste-based pretreatment. The enhanced delignification and biomass fractionation at higher GLD concentrations have been attributed to increased alkaline conditions, which facilitate the breakdown of lignin and hemicellulose, making cellulose more accessible for enzymatic hydrolysis. However, excessive GLD concentration can lead to increased slurry viscosity and reduced mass transfer, ultimately decreasing the overall efficiency of the pretreatment process (See supplementary material). The LLM highlights the importance of optimizing GLD concentration during pretreatment to achieve maximum glucose yields, while mitigating the negative effects associated with excessive GLD concentrations.

In the same vein, the model interpreted the effects of the fractional changes in the power intensity and its influence on the glucose yield during microwave-assisted Kraft waste-based pretreatment. It detailed that an increasing microwave power intensity can enhance biomass pretreatment effectiveness due to higher energy input, leading to improved sugar yields. The model further indicated that beyond an optimal power intensity, improvements in the glucose yield may be insignificant and could possibly lead to decreased yields (See supplementary material). In this context, the ChatGPT responses coincide with previous studies on the influence of microwave power intensity on the pretreatment of LCB (Sewsynker-Sukai and Gueguim Kana, 2018; Laltha et al., 2021).

The LLM knowledge generated from the search prompts were evaluated in light of the authors interpretations of the findings in this study (*Section 3.2*). When comparing the LLM responses to the contextual interpretation, significant parallels were observed. The model was able to effectively recognize and interpret the data trends presented for the parameter impact on the glucose yields of the steam and microwave-assisted Kraft waste-based pretreatment. This approach also provided a deeper understanding of the underlying mechanisms in the pretreatment process for improved predictive modelling in future research. Therefore, the LLM contributes substantially to the existing body of knowledge on lignocellulosic pretreatment available within the public domain.

Despite these remarkable insights, it is important to note that LLMs present certain limitations due to its reliance on the statistical associations with existing literature rather than depth of understanding and contextual awareness, possibly leading to misinterpretation of data (Ray, 2023). Furthermore, it lacks real-time information since its training is reliant on a static dataset that has a knowledge cut-off date, specifically the year 2021. For this reason, expert human knowledge inputs are necessary to augment LLM interpretations. This synergistic approach underscores the use of predictive LLM modelling technologies to identify patterns, extract key insights and provide broader context, while human experts use critical thinking and domain knowledge to validate and verify its sources to ensure factual accuracy and reliability.

4. Conclusion

The developed models demonstrated R^2 values > 0.95 for the predicted and observed glucose outputs. Sensitivity analysis exhibited increased response to GLD concentration and power intensity for the steam and microwave models, respectively. Furthermore, ChatGPT presented key insights that aligned with the study's contextual interpretations, substantiating its value as a complementary analytical tool. By harnessing the objectivity of virtual tools, valuable knowledge on behavioural patterns and outcomes associated with waste-based lignocellulosic pretreatment prior to process development, optimization and scale-up will be generated. This facilitates informed decision making, pretreatment design improvement, automated assessments of economic outlooks and productivity within lignocellulosic biorefineries.

CRediT authorship contribution statement

Anthea N. David: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Y. Sewsynker-Sukai:** Validation, Data curation, Writing - review & editing, Supervision, Project administration. **E.L. Meyer:** Writing - review & editing. **E.B. Gueguim Kana:** Validation, Data curation, Writing - review & editing, Resources, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgments

This work is based on research supported in part by the National Research Foundation (NRF) of South Africa (Grant number 122080). Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the NRF.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2023.117686.

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CHAPTER 6

Co-valorization of corn cobs and dairy wastewater for simultaneous saccharification and lactic acid production: Process optimization and kinetic assessment

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This chapter has been published in *Bioresource Technology* (348, 126815) with the title: Co-valorization of corn cobs and dairy wastewater for simultaneous saccharification and lactic acid production: Process optimization and kinetic assessment.

The manuscript and supplementary material are presented in the following pages.

Bioresource Technology 348 (2022) 126815

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Co-valorization of corn cobs and dairy wastewater for simultaneous saccharification and lactic acid production: Process optimization and kinetic assessment

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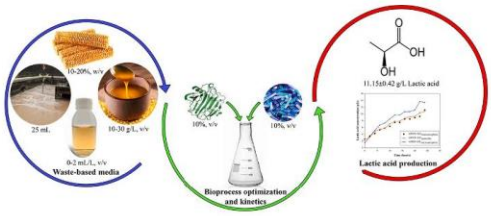
HIGHLIGHTS

- Co-valorization of dairy wastewater and corn cobs for lactic acid (LA) production.
- Optimization using waste-based medium gave maximum LA of 11.15 g/L.
- Waste-based bioprocess revealed comparable μ_{max} (0.35 h^{-1}) and P_m (13.01 g/L).
- Microaerophilic conditions enhanced microbial cell growth and lactic acid formation.
- Potential elimination of expensive media components and fresh water in bioprocesses.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Lactic acid
Dairy wastewater formulated media
Corn cob waste
Simultaneous saccharification and fermentation

ABSTRACT

This study optimized the co-valorization of corn cob wastes (CCW) and dairy wastewater for simultaneous saccharification and lactic acid (LA) production (sDWW-SSF). Subsequently, the kinetics of *Lactobacillus plantarum* growth and LA production was assessed using the optimized conditions under microaerophilic (sDWW-$SSF_{microaerophilic}$) and anaerobic (sDWW-$SSF_{anaerobic}$) conditions, and thereafter compared to De Man, Rogosa and Sharpe (MRS) medium modified with pretreated CCW (mMRS-$SSF_{microaerophilic}$). Optimized sDWW-SSF conditions produced maximum LA concentration and conversion of 11.15 ± 0.42 g/L and 18.90 ± 0.75%, respectively. Kinetic studies revealed that although the mMRS-$SSF_{microaerophilic}$ system obtained a higher maximum specific growth rate (μ_{max}) and maximum potential LA concentration (P_m) compared to the wastewater-based bioprocesses, the data obtained for the latter were comparable when taking the resources and costs into consideration. These findings represent the potential to eliminate the use of valuable resources in lignocellulosic bioprocesses and provide insights on innovation towards driving a sustainable economy in line with the food-energy-water nexus.

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https://doi.org/10.1016/j.biortech.2022.126815
Received 7 December 2021; Received in revised form 31 January 2022; Accepted 1 February 2022
Available online 5 February 2022

1. Introduction

Microbial conversion of lignocellulosic-derived feedstocks into high value-added products is an emerging trend towards ensuring sustainable economic development with reduced environmental implications (Coelho et al., 2020). Lactic acid (LA) is among these platform chemicals that has high commercial value, due to its versatile applications in the food, chemical, cosmetics and pharmaceutical industries (Li et al., 2021). The production of lactic acid can be achieved either by chemical synthesis or microbial fermentative routes. The latter accounts for approximately 90% of the lactic acid production worldwide and gives rise to either D-lactic acid, L-lactic acid or a mixture of the two, based on the microbial strain, fermentation conditions, and its desired use in industry (Carpinelli Macedo et al., 2020). Lactic acid producers (LABs) include bacteria belonging to the genera *Lactococcus, Lactobacillus, Bifidobacterium* and *Streptococcus,* amongst others. *Lactobacillus* strains are characterized as facultative anaerobes that are known to be the most commonly used for lactic acid production, attributable to their high acid tolerance, economic viability, enhanced yield and productivity as well as amenability to genetic engineering (Abedi and Hashemi, 2020; Sano et al., 2020). Based on their carbon metabolism, LABs tend to follow either the homofermentative or heterofermentative lactic acid pathway. The homolactic pathway converts monosaccharides almost exclusively to lactic acid, however, heterolactic fermentation results in the metabolism of glucose into lactic acid, acetic acid, ethanol and carbon dioxide (Song et al., 2022). Homofermentative LABs are favoured due to their high yield (almost 100%), high optical purity (>99%) and productivity (Abedi and Hashemi, 2020). Lignocellulosic biomass (LCB) substrates are considered renewable and may serve as an attractive carbon source for the fermentative production of valuable industrial commodities such as lactic acid. As such, corn cob waste (CCW) represents a potential feedstock for fermentable sugar recovery, owing to its abundant global output, rich carbohydrate content and low lignin content (Potumarthi et al., 2012). However, the commercialization of lignocellulosic lactic acid production has been limited by expensive and resource-intensive processes that often lead to low product yields and costly downstream operations (Kumar and Sharma, 2017). To overcome the above drawbacks, the pursuit of processes that reduce costs, energy and resources, while producing high sugar yields are needed. In line with this, complete waste-based pretreatments have emerged (David et al., 2021) as suitable methods for use in lignocellulosic lactic acid production. The simultaneous saccharification and fermentation (SSF) process type has been touted as efficient systems since it combines both the enzymatic hydrolysis and fermentation reactions in a single bioreactor (Jiang et al., 2019; Li et al., 2021). This could potentially reduce overall energy inputs, operational costs, risk of contamination and alleviate the effect of glucose inhibition, while enhancing product yield and productivity (Sewsynker-Sukai and Gueguim Kana, 2018). In the context of microbial fermentation, LABs are auxotrophic, i.e., they require an exogenous source of complex nutrients such as carbon, nitrogen, phosphorus, vitamins and minerals for growth, since they lack many biosynthetic capabilities (Chen et al., 2020). The standard De Man, Rogosa and Sharpe (MRS) media is a nutrient-rich source that is routinely used for the cultivation of various LABs. However, considering the growth requirements of *Lactobacilli*, the expense attached to the complex supplementation has restricted MRS media use to laboratory-scale cultivation and fermentation. Hence, the strategic selection of raw ingredients and formulation of the optimum fermentation media that is both effective and economically viable is considered one of the most important steps in the development of lactic acid processes towards large-scale production. Within biological processes such as lactic acid fermentation, nitrogen source is an indispensable component, due its pivotal role in the biosynthesis of key constituents such as amino acids, proteins and nicotinamide adenine dinucleotide (NAD) that are needed for cell functioning (Martinez-Burgos et al., 2021). The standard MRS media contains nitrogen sources in the form of meat extract, peptone and yeast extract with the latter being the main nitrogen contributor. However, nitrogen source accounts for approximately 30% of the production costs, thus impairing the economics of lactic acid production (Tang et al., 2013). Nitrogen sources that have previously been studied as cheaper alternatives include soybean meal, protein hydrolysate and corn steep liquor (CSL), amongst others (Mis Solval et al. (2019); Jiang et al., 2019; Li et al., 2021). The latter is a by-product of the corn starch industry that consists of rich amino acids, inorganic salts, polypeptides and considerable amounts of vitamin B-complexes (Hofer et al., 2018). The composition of CSL may be sufficient to satisfy the nutritional needs of LABs for lactic acid production and is approximately one fifth of the cost of conventionally used yeast extract (Tan et al., 2016). CSL can therefore be used for the partial or total replacement of yeast extract within the fermentation medium. Another component of importance within commercial MRS media is the presence of Tween 80, a non-ionic surfactant that contains a mono-unsaturated fatty acid (oleate). The presence of Tween 80 has shown to have a multi-fold effect within lignocellulosic bioprocesses by: (1) resulting in improved uptake of nutrients surrounding the cell body (Taoka et al., 2011), (2) acting as an emulsifying agent for the addition of water to insoluble compounds (Taoka et al., 2011) and (3) increasing stability and reducing unproductive enzyme binding during enzymatic hydrolysis (Zhang et al., 2018). With the above requirements in mind for lignocellulosic fermentation, the solvent in which these media components are dissolved is crucial to the bioprocess. The water footprint of a lignocellulosic bioprocessing system has placed major constraints on sustainability, capital costs and the environment. In view of these aspects, the application of untreated industrial wastewater streams as a freshwater substitute within the fermentation process of the biorefinery system may be a more logical and feasible approach. The dairy industry production had reached 906 million tons in 2020, and its volume is expected to increase in order to satisfy the ever-growing market (FAO, 2021). It consumes high volumes of fresh water and generates large quantities of dairy wastewater (DWW), approximately 0.2–10 L of wastewater/L of processed milk (Coelho et al., 2020). The DWW outputs require stringent treatments to reduce contaminants prior to discharge into water bodies. However, these methods are considered a burden industrially, due to its high capital investments, energy consumption, tedious nature and a major source of greenhouse gas emissions (Gogoi et al., 2021). The application of DWW as a freshwater substitute allows the dairy industry to completely by-pass its effluent treatment process, while supplying bioprocessing units with sufficient water, without the implications of consuming freshwater resources. Interestingly, DWW streams also consists of residual nutritional components such as soluble organic compounds, carbohydrates, nitrogen and protein that assist in stimulating the growth of several LABs (Kaur, 2021). Therefore, the optimization of key input parameters such as nitrogen concentration, surfactant concentration and substrate solid loading for a waste-based medium is imperative to overcome challenges associated with lignocellulosic lactic acid production. The response surface methodology (RSM) model can be used to obtain reliable, consistent and statistically significant data on the basis of experimental outputs, without knowing the details of the complex reactions occurring during the fermentation process (Kucharska et al., 2018). On the other hand, kinetic bioprocess models such as the logistic and modified Gompertz provide major insights on the regulatory function and dynamics of microbial metabolic processes regarding cell growth and product formation, respectively (Muloiwa et al., 2020; Sewsynker-Sukai and Gueguim Kana, 2018). These modelling tools are deemed useful for process scale up and optimization due to enhanced product yield and productivity, reduction of undesired by-products and improved resource management. There has been a scarcity of knowledge on the optimization of a feasible waste-based formulation using cost effective raw materials and wastewater streams as medium constituents and a solvent, respectively, for simultaneous saccharification and lactic acid production. Moreover, assessing the kinetics of the microbial cell growth and lactic acid formation within

the waste-based medium under microaerophilic and anaerobic conditions and comparing the outcomes with a modified MRS media formulation may provide insight to improve production and economic payback for its industrial scale trajectory. With respect to the above-mentioned viewpoints, the present study aims to model and optimize the co-valorization of pretreated corn cobs and dairy wastewater for simultaneous saccharification and lactic acid (LA) production using the response surface methodology (RSM). For the SSF optimization, the dairy wastewater was supplemented with corn steep liquor (CSL), Tween 80 and pretreated CCW (solid loading) that were used as the input parameters while lactic acid concentration and conversion were the responses. Following optimization, the logistic and modified Gompertz models were used to determine the kinetics of cell growth and lactic acid production , respectively, for: (1) supplemented dairy wastewater under SSF bioprocess-microaerophilic conditions (sDWW-$SSF_{microaerophilic}$), (2) supplemented dairy wastewater under SSF bioprocess-anaerobic conditions (sDWW-$SSF_{anaerobic}$), and (3) conventional MRS medium modified with pretreated CCW under SSF bioprocess-microaerophilic conditions (mMRS-$SSF_{microaerophilic}$).

2. Materials and methods

2.1. Materials

All chemicals used in this study were purchased from Merck, South Africa. The corn cob waste (CCW) used in this study was harvested, dried, milled and stored according to David et al. (2021). The green liquor dregs (pH 10–12) and paper wastewater (pH ~ 8) used during pretreatment were supplied with the chemical compositions (David et al., 2021) by a local paper Kraft pulp and paper mill industry (Mondi, Richards Bay, South Africa). The dairy wastewater was provided by a local dairy (Fairfield, Howick, South Africa).

2.2. Lactobacillus species revival and storage

The *Lactobacillus rhamnosus* ATCC 9595, *Lactobacillus plantarum* ATCC 14917 and *Lactobacillus casei* ATCC 393 strains employed in this study were purchased from Microbiologics Incorporated. All three strains were derived from a collection of ATCC® type cultures. For each bacterial strain, the lyophilized pellet was hydrated in the supplied hydrating fluid and aseptically swabbed onto MRS agar growth medium. The MRS agar as supplied by Merck, South Africa, consisted of 10 g/L special peptone, 5 g/L meat extract, 5 g/L yeast extract, 20 g/L dextrose (glucose), 1 mL/L Tween 80 (Polysorbate 80), 2 g/L tri-ammonium citrate, 5 g/L sodium acetate, 0.1 g/L magnesium sulfate, 0.05 g/L manganese sulfate, 2 g/L dipotassium hydrogen phosphate and 12 g/L bacteriological agar. The cells were incubated at 37 °C for 48 hrs. The isolated colonies from each MRS agar plate were cultured in 100 mL MRS broth (Merck, South Africa). The optimally grown bacterial suspensions were stored in 20% glycerol (v/v) at −80 °C. For short term storage, an isolated colony was cultured on double-strength MRS agar slants, incubated at 37 °C for 24 hrs and thereafter stored at 4 °C.

2.3. Screening of microorganism

A preliminary screening was carried out to determine the most suitable *Lactobacillus* species (*L. rhamnosus*, *L. plantarum* and *L. casei*) for the optimization and kinetic studies. A single isolated colony grown for 48 hrs at 37 °C was transferred into 100 mL of sterilized MRS broth. *L. rhamnosus* and *L. plantarum* were incubated for 14 hr and *L. casei* incubated for 22 hrs, according to a pre-determined growth curve. All cultures were subjected to incubation at 37 °C in an orbital shaker (120 $_{rpm}$). Exponentially growing *Lactobacillus* cells (*L. plantarum, L. rhamnosus* or *L. casei*) were inoculated at 10 % (v/v) into 90 mL MRS broth. All fermentation experiments were incubated at 37 °C and 120 rpm over a period of 24 hrs. For the lactic acid analysis, 1 mL aliquots were removed every 6 hrs.

2.4. Pretreatment of CCW

The milled CCW was optimally pretreated according to a steam-assisted combined green liquor dregs and paper wastewater (SGLD-PWW) pretreatment method established in a previous study (David et al., 2021). The CCW substrate at a 10% (w/v) solid loading (SL) was immersed in 150 mL of PWW containing 49.89% GLD and subjected to autoclave heating at 118 °C for 5 min. The pretreated slurry was filtered using a domestic sieve (<1mm) and washed thoroughly with deionized water. The resultant solid residue was dried at 60 °C for 24 hrs and thereafter used for the SSF bioprocess. The composition of the optimized pretreated CCW biomass (53.14% cellulose, 25.12% hemicellulose and 19.49% lignin) (David et al., 2021).

2.5. Modelling and optimization of the SSF process using response surface methodology

The Box-Behnken design (Design Expert V12, Stat Ease Inc, USA) was applied for the optimization of lactic acid by supplementing dairy wastewater as the water source in the fermentation medium under the SSF bioprocess (sDWW-SSF). The fermentation input variables selected for the sDWW-SSF model consisted of corn steep liquor (CSL) concentration (10 to 30 g/L, v/v), Tween 80 concentration (0 to 2 mL/L, v/v) and substrate solid loading (SL) (10 to 20%, w/v). The response variables included the lactic acid concentration and the lactic acid conversion resulting in two process response surface models that were evaluated and designated as follows: :(1) supplemented dairy wastewater under SSF bioprocess-lactic acid concentration (sDWW-$SSF_{concentration}$), (2) supplemented dairy wastewater under SSF bioprocess-lactic acid conversion (sDWW-$SSF_{conversion}$). The input ranges were selected according to previous studies (Coelho et al., 2010; Carpinelli Macedo et al., 2020). Seventeen experimental runs were generated and carried out in duplicate according to the RSM design. The resultant experimental data were used to fit the polynomial model equations to investigate the interactive effect of the independent input variables for enhanced lactic acid production.

2.6. SSF process

2.6.1. Cellic CTec 2 enzyme and enzyme activity

Cellic CTec 2, a commercial cellulase-based enzyme blend with an enzyme activity of 160 FPU/mL was generously provided by Novozyme (Novozymes A/S, Denmark).

2.6.2. Microorganism inoculum preparation

Based on the screening of the *Lactobacillus* species, *L. plantarum* was selected for further process optimization. Prior to fermentation, the *L. plantarum* stock culture (stored at 4 °C) was streaked onto MRS agar media and incubated at 37 °C for 48 hrs. Thereafter, a single isolated colony was aseptically inoculated into sterilized MRS broth and incubated at 37 °C and 120 rpm for 14 hrs. The exponentially grown suspension contained 7.98×10^7 cells/mL of *L. plantarum* cells. The initial cell concentration was determined using a Neubauer cell counting chamber (Neubauer, Germany).

2.6.3. Dairy wastewater (DWW) citrate buffer preparation

The citrate buffer (pH 4.8, 0.05 M) was prepared using DWW in place of deionized water. The buffer was sterilized at 121 °C for 15 min for use in the SSF bioprocess.

2.6.4. Lactic acid production

The lactic acid fermentation was performed in 100 mL Erlenmeyer flasks. The reaction solution of a constant 25 mL working volume contained the SGLD-PWW pretreated CCW with solid loading (10–20%), CSL (10–30 g/L), Tween 80 (0–2 mL/L) (Table 1), standard enzyme loading (10 FPU/g), DWW citrate buffer (pH 4.8, 0.05 M) and *L. plantarum* cells (10%, v/v).

A constant temperature (37 °C) and shaker speed (120 rpm) was maintained for all SSF experiments. During the incubation, 1 mL aliquots were aseptically removed from the flasks at the 24 hr time point. The optimization experiments were characterized as microaerophilic processes due to the initial exposure to molecular oxygen without subsequent aeration (Sewsynker-Sukai and Gueguim Kana, 2018). All seventeen fermentation experiments were performed in duplicate.

2.7. Validation of the optimized sDWW-SSF bioprocess and kinetic modelling

The validation conditions of the optimized lactic acid production using supplemented dairy wastewater under SSF bioprocess (sDWW-SSF) were determined using the developed RSM model. The reaction volume of DWW citrate buffer was maintained at 25 mL containing 10% SGLD-PWW pretreated CCW, 25 g/L CSL and 2 mL/L Tween 80 with a constant enzyme loading of 10 FPU/g and inoculum concentration of 10% (v/v). In addition to the validated sDWW-SSF model, the optimized conditions for lactic acid production using supplemented dairy wastewater under SSF bioprocess was subjected to anaerobic conditions. Furthermore, the commercial MRS medium, containing no glucose was adapted with pretreated CCW and Cellic CTec2 enzyme as a reference experiment. Each process was denoted as follows: (1) lactic acid production using supplemented dairy wastewater under SSF bioprocess-microaerophilic conditions (sDWW-$SSF_{microaerophilic}$), (2) lactic acid production using supplemented dairy wastewater under SSF bioprocess-anaerobic conditions (sDWW-$SSF_{anaerobic}$), and (3) lactic acid production using conventional MRS medium modified with SGLD-PWW pretreated CCW under SSF bioprocess-microaerophilic conditions (mMRS-$SSF_{microaerophilic}$). After inoculation with the *L. plantarum* culture, the anaerobic experimental flasks (sDWW-$SSF_{anaerobic}$) were flushed with nitrogen (N_2) gas for 1 min in order for anaerobiosis to occur. The experimental flasks void of N_2 gas flushing were considered as microaerophilic due to the exposure of molecular oxygen (O_2) within the flask headspace. All experiments were incubated at 37 °C and 120 rpm for 24 hrs. The control experiments (uninoculated) were subjected to the same conditions as per the corresponding experimental sets. All experiments were performed in duplicate. Samples of 1 mL was aseptically removed from each experimental set and its corresponding controls (uninoculated) every 2 hrs over a period of 24 hrs. The aliquoted sample was thereafter used to determine lactic acid, biomass and glucose concentrations as described below.

2.8. Analytical methods

2.8.1. Analysis of lactic acid, biomass and glucose concentration

The total lactic acid concentration (g/L) was quantified using the Megazyme lactic acid assay kit, product code (K-DLATE) (©Megazyme, Wicklow, Ireland) according to specified protocols as stated by the manufacturer. The VERSAmax tuneable microplate reader (Molecular Devices, California, USA) was used to spectrophotometrically analyse the lactic acid content. The lactic acid conversion yield was calculated according to the following equation (1).

$$\text{Lactic acid conversion } (\%) = \frac{[LA]f}{1(f[biomass]1.111)} \text{x}100 \quad (1)$$

Where the numerator expressed as [LA]f is the highest lactic acid concentration produced during the fermentation process (g/L) and the denominator expression represents the theoretical lactic acid concentration, where [biomass] is the dry lignocellulosic biomass concentration at the beginning of the fermentation (g/L), f is the cellulose content of dry biomass (0.531 g/g in this study), 1.111 is the conversion factor of cellulose to equivalent glucose (NREL, 2001) and 1 is a conversion factor for glucose to lactic acid based on the reaction stoichiometry (1 mol glucose → 2 mol lactic acid) (Abedi and Hashemi, 2020).

The glucose concentration of the experimental and control (uninoculated) samples for the kinetic assessment was determined by the Megazyme glucose kit, product code (K-GLUC) (©Megazyme, Wicklow, Ireland). Glucose analysis was performed as per the manufacturers specified protocol. The control experiments (uninoculated) were conducted to estimate the initial glucose concentration that was produced at each time point. The test experimental glucose concentration was subtracted from the control glucose concentration to determine the glucose utilization (%) as per Eq. (2).

$$\text{Glucose utilization } (\%) = \frac{control_{glucoseconcentration(g/L)} - experimental_{glucoseconcentration(g/L)}}{control_{glucoseconcentration_{(g/L)}}} \text{x}100 \quad (2)$$

Table 1
Lactic acid concentration and conversion for the sDWW-SSF process using the Box-Behnken design.

Run	Inputs			Outputs	
	Solid loading (%)	Corn steep liquor concentration (g/L)	Tween 80 concentration (mL/L)	Lactic acid concentration (g/L)	Lactic acid conversion (%)
1	20	20	2	10.31	8.74
2	10	30	1	10.19	17.27
3	15	10	0	8.79	9.94
4	10	20	2	10.41	17.64
5	10	20	0	9.12	15.45
6	10	10	1	8.80	14.92
7	15	20	1	9.44	10.67
8	15	10	2	9.09	10.27
9	15	20	1	9.60	10.85
10	20	30	1	9.68	8.20
11	15	20	1	9.24	10.44
12	20	10	1	8.61	7.30
13	15	20	1	8.88	10.04
14	15	30	0	9.86	11.14
15	15	30	2	10.11	11.42
16	20	20	0	9.24	7.83
17	15	20	1	9.44	10.67

Where the expression as $control_{glucose\ concentration(g/L)}$ is the initial glucose concentration available at a specific time point and the $experimental_{glucose\ concentration(g/L)}$ is the final glucose concentration present at a specific time point.

The *L. plantarum* cell concentration (g/L) was quantitatively evaluated by relating the cell count as a function of the dry cell weight. The *L. plantarum* culture was grown exponentially in MRS broth and diluted accordingly. Each diluted sample (1, 1/2, 1/4, 1/8 and 1/16) was decanted with a total volume of 10 mL and centrifuged at 9000 rpm for 5 min. The resultant biomass pellet was oven dried at 90 °C, until a constant mass was reached. The dry weights (g/L) of each sample were plotted against its corresponding cell counts (cells/mL) to produce a standard curve. To determine the experimental biomass concentration (g/L), the cell counts (cells/mL) were substituted into the equation generated from the standard curve.

2.8.2. Dairy wastewater (DWW) analysis

For the elemental analysis (see supplementary material), the DWW sample was prepared by filtering through a 0.45 μm filter syringe prior to analysis. The Ca, K, Na, Mg, P and S concentrations were tested using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES) according to standard methods for the examination of water and wastewater as specified by APHA (1998a). The Cu, Fe, Mn and Zn concentrations were tested using the Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) according to standard methods for the examination of water and wastewater previously described by APHA (1998b). The total organic carbon (TOC) was determined by oxidizing the wastewater sample using high temperature oxidation with a platinum catalyst to produce carbon dioxide. The carbon dioxide was thereafter measured by a Non-Dispersive Infrared (NDIR) detector. The total nitrogen was assessed according to the Kjeldahl method based on the accumulative concentration of Kjeldahl nitrogen, nitrates and nitrites.

2.8.3. Fermentative pathway detection

To distinguish between the homofermentative and heterofermentative pathway (Abdel-Rahman et al., 2011) in *Lactobacillus plantarum* ATCC 14917, a diagnostic carbon dioxide (CO_2) gas detection test using the portable multi-gas analyzer (SKZ industrial. Shandong, China) was performed.

2.9. Kinetic modelling

2.9.1. Logistic model for cell growth

The logistic model in the differential form of Eq. (3) represents the exponential and stationary phases of growth. Eq. (3) was integrated to form Eq. (4). This logistic model illustrates the relationship of cell biomass (X) to initial cell concentration (X_0), maximum cell concentration (X_{max}) and maximum specific growth rate (μ_{max}) at specific times (t) during the exponential and stationary phases of *L. plantarum* growth. However, the model does not predict the death phase of microorganisms after the stationary phase.

$$\frac{dX}{dt} = \mu_{max}\left(1 - \frac{X}{X_{max}}\right)X \tag{3}$$

$$X = \frac{X_0 \exp(\mu_{max} t)}{1 - \left[\left(\frac{X_0}{X_{max}}\right)(1 - exp(\mu_{max} t))\right]} \tag{4}$$

2.9.2. Modified Gompertz model for product formation

The lactic acid production data generated from the various SSF process experiments ($sDWW_{microaerophilic}$, $sDWW_{anaerobic}$, $mMRS_{microaerophilic}$) were used to fit the modified Gompertz model by using the least squares method (CurveExpert V1.5.5, MyBiosource, Inc., USA). The model reveals the relationship of the lactic acid concentration (P) to the potential maximum lactic acid concentration (P_m), maximum lactic acid production rate ($r_{p,m}$) and lag time (t_L) from the beginning of fermentation to exponential lactic acid production as shown in Eq. (5).

$$P = P_m.exp\left\{-exp\left[\frac{r_{p,m}.exp(1)}{P_m}\right].(t_L - t) + 1\right\} \tag{5}$$

3. Results and discussion

3.1. Preliminary screening of microorganisms

According to the preliminary screening results, *L. plantarum* produced the highest lactic acid concentration of 10.01 g/L, followed by *L. casei* (8.54 g/L) and *L. rhamnosus* (7.51 g/L), and was therefore selected for SSF optimization and kinetic studies.

3.2. sDWW-SSF model development

The suitability of the mDWW-SFF model was evaluated by the Analysis of Variance (ANOVA) (see supplementary material). P-values < 0.05 illustrate model and parameter significance, while high F-values indicate that response trends can be interpreted by the regression equations (Chaganti et al., 2012). The $sDWW\text{-}SSF_{concentration}$ and $sDWW\text{-}SSF_{conversion}$ models presented low p-values of 0.037 and < 0.0001, respectively. Additionally, high F-values of 4.15 and 95.36 was obtained for $sDWW\text{-}SSF_{concentration}$ and $sDWW\text{-}SSF_{conversion}$ models, respectively. Among the studied SSF input parameters, the CSL concentration ($p = 0.002$) and Tween 80 concentration ($p = 0.019$) demonstrated a notable effect on the $sDWW\text{-}SSF_{concentration}$ model. For the $sDWW\text{-}SSF_{conversion}$ model, all three variables, CCW solid loading ($p=<0.0001$), CSL concentration ($p = 0.0023$) and Tween 80 concentration ($p = 0.018$) had a significant impact on the production of lactic acid. The CSL consists of a concentrated source of nitrogen, amino acids, vitamins B-complexes and minerals that participate in various metabolic pathways for lactic acid production (Hofer et al., 2018). Furthermore, free amino nitrogen present in the CSL solution may stimulate the rate at which reducing sugars are converted into lactic acid (Carpinelli Macedo et al., 2020). Like CSL, Tween 80 has also been shown to positively influence lactic acid production. Tween 80, a non-ionic surfactant, acts as a vehicle in which water insoluble compounds are emulsified into media so that microorganisms can utilize the nutrients present in the substrate. Moreover, the non-ionic surfactant interferes with the cell membrane permeability to enhance the nutrient uptake into the cell for increased biomass production, thus leading to higher lactic acid yields (Taoka et al., 2011). On the other hand, the substrate solid loading typically affects the viscosity of the fermentation medium. A high solid loading has the capacity to hold more water, which increases the viscosity of the solution. As a result, enzyme diffusion through the medium is hindered, adversely affecting the conversion of cellulose to glucose, subsequently reducing its accessibility to the microorganism for growth and product formation (Aguilar-Reynosa et al., 2017). Apart from the p- and F-values, RSM model fitness is assessed using the coefficient of determination (R^2) value. R^2 values in the range of 0.7 to 1 indicates a good model fit. The $sDWW\text{-}SSF_{concentration}$ and $sDWW\text{-}SSF_{conversion}$ models gave high R^2 values of 0.84 and 0.99, respectively. These statistical indices suggest that the developed models displayed a good correlation between the input parameters and the lactic acid output. The polynomial model Equations (6) and (7) represented below were generated using the lactic acid concentration and conversion responses from the sDWW-SSF experiments (Table 1). The model equations relate the interaction between the significant input parameters and the lactic acid response.

$$\text{Lactic acid}_{concentration}\ (g/L) = 9.32 - 0.084A + 0.57B + 0.36C - 0.08AB - 0.056AC - 0.013BC + 0.15A^2 - 0.15B^2 + 0.29C^2 \tag{6}$$

$$\text{Lactic acid}_{\text{conversion}}\ (\%) = 10.53 - 4.15A + 0.70B + 0.46C - 0.36AB - 0.32AC - 0.015BC + 1.56A^2 - 0.17B^2 + 0.33C^2 \quad (7)$$

where, A, B and C are CCW solid loading (%), CSL concentration (g/L) and Tween 80 concentration (mL/L), respectively.

3.3. Effects of process variables on the lactic acid concentration and conversion

The fermentation input parameters along with its corresponding lactic acid concentrations and conversions are represented in Table 1. The sDWW-SSF model observed a minimum lactic acid concentration and conversion of 8.61 g/L and 7.30%, respectively for run 12 (20% SL, 10 g/L CSL, 1 mL/L Tween 80). Conversely, the maximum lactic acid concentration (10.41 g/L) and conversion (17.64%) was obtained for run 13 (10% SL, 20 g/L CSL, 2 mL/L Tween 80). For run 7, 9, 11, 13 and 17, all the input variables were maintained at their median values of 15% SL, 20 g/L CSL and 1 mL/L Tween 80. These experiments resulted in a lactic acid concentration range from 8.88 g/L to 9.60 g/L, while the lactic acid conversion varied between 10.04% and 10.85%. The variation in these responses showed that the lactic acid production was dependent on the CSL and Tween 80 concentration.

The response surface plots (Fig. 1A-F) evaluate the interactive effects of the input variables on the lactic acid concentration and conversion. These outputs are illustrated in Fig. 1A and 1B, respectively, as a function of the CSL concentration and SL, while maintaining the Tween 80 concentration constant at its median value (1 mL/L). Fig. 1A shows that an increase in CSL concentration from 10 g/L to 30 g/L, while the SL was maintained at its minimum value of 10% resulted in an increase in the lactic acid concentration from 8.75 g/L to 10.05 g/L. Further increase in the SL (10% to 20%) negatively impacted the lactic acid concentration with a decrease from 10.05 g/L to 9.7 g/L. In the same vein, Fig. 1B illustrated that an increase in the CSL concentration (10 g/L to 30 g/L) while the SL is maintained at 10% observed lactic acid conversion ranging from 14.95% to 17.05%. Simultaneous increase in the CSL concentration (10 g/L to 30 g/L) and SL (10% to 20%) led to a decline in the lactic acid conversion from 14.95% to 8.13%. The glucose derived from the pretreated CCW as well as CSL are valuable carbon and nutrient sources respectively, that control the biosynthesis of lactic acid. The substrate solid loading is a major factor that influences the release of glucose into the broth medium. At a low SL (10%), the fluidity of the medium is increased for maximum mass and heat transfer. This allows ease of flow for the enzyme to access the organic solids and adequate heat transfer to ensure optimal enzymatic activity, thus increasing the conversion efficiency of cellulose to glucose. The higher glucose concentrations present in the medium has been reported to promote glycolytic flux towards lactic acid formation (Zhang et al., 2021). At a high SL, there is an increase in the hydrodynamic interactions of solid matter and the surrounding fluid as well as interactions between the particles (Viamajala et al., 2009). With an increase in these interactions, the liquid volume is reduced leading to a dense suspension. Challenges associated with viscous solutions include reduced diffusion of enzymes to the lignocellulosic biomass and nutrients to the microorganism as well as poor heat transfer and aeration resulting in inefficient hydrolysis and fermentation product formation. This may account for the lower lactic acid concentration and conversion observed at high solid loadings. Macedo et al. (2020) observed a similar trend in which the lactic acid concentration was at its maximum of approximately 32 g/L when the CSL concentration was increased from 8% to 12 % while the substrate SL remained at its minimum of 14%. Likewise, Wee et al. (2006) demonstrated that supplementing wood hydrolysate with increasing CSL concentration from 15 to 60 g/L produced a lactic acid concentration of up to 48.6 g/L. The pairwise effect of the Tween 80 concentration and SL on the lactic acid concentration and conversion when CSL concentration is kept at its middle value (20 g/L) is depicted in Fig. 1C and 1D, respectively. An increasing trend in the Tween 80 concentration (0 to 2 mL/L) and SL (10% to 20%) exhibited a lactic acid concentration increase from 9.4 g/L to 9.99 g/L (Fig. 1C). However, increases in the Tween 80 concentration from 0 mL/L to 2 mL/L when the SL is maintained at 10% increases the lactic acid concentration from 9.4 g/L to 10.26 g/L. In the same vein, Fig. 1D noted that a proportional change in the Tween 80 concentration (0 mL/L to 2 mL/L) and SL (10% to 20%) revealed a negative relationship for the lactic acid conversion (15.62% to 8.4%). However, when the Tween 80 concentration was increased to its maximum (2 mL/L) while the SL was kept at 10%, the lactic acid conversion ranged from 15.62% to 17.2%. The results showed that a 10% SL is the threshold to obtain higher lactic acid concentrations and conversions. The water availability within the medium is reduced at a higher SL, which leads to a high viscosity broth that affects the mixing ability and homogeneity of the environment. High solid content accompanied by non-uniform mixing creates nutrient depletion zones at a microscopic level where nutrients cannot be accessed by the microorganism, resulting in stagnant microbial activity and product formation (Staley et al., 2011). Although Tween 80 positively influences the lactic acid bioprocess, the reduced nutrient access to the microorganism at a higher solid loading may result in a decrease in lactic acid concentration and conversion. Qi et al. (2009) investigated the effect of Tween 80 on the production of lactic acid using *L. casei* and observed an 8% higher lactic acid concentration and yield when the medium was supplemented with 0.1% Tween 80 (Qi et al., 2009). Fig. 1E and 1F depicts the interaction of the Tween 80 concentration and CSL concentration on the lactic acid concentration and conversion, respectively, when the SL is kept at its centre point value. An increase in both the CSL concentration from 10 g/L to 30 g/L and Tween 80 concentration from 0 mL/L to 2 mL/L led to a maximum lactic acid concentration of 10.37 g/L (Fig. 1E). Similarly, a simultaneous increase in the Tween 80 concentration from 0 mL/L to 2 mL/L and CSL concentration from 10 g/L to 30 g/L resulted in a slight increment in the lactic acid conversion (9.51% to 11.84%) (Fig. 1F). The results show a strong interaction between CSL and Tween 80 on the lactic acid production. LABs such as *L. plantarum* are nutritionally fastidious microorganisms that require various vitamins and amino acids for growth (Yu et al., 2008). Increased production of lactic acid at high CSL concentrations may be attributed to the complex source of nitrogen, amino acids (ala, arg, cys, asp, glu, trp, gly, his, lle, leu, lys, tyr, met, phe, pro, ser, thr, val), vitamins (biotin, choline, inositol, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine) and minerals (Ca, Cu, Mn, Fe, Mg, K, Na, P, Se, Z, S) present in CSL (Hofer et al., 2018). These components play a fundamental role in the biological processes by activating major enzymes involved in metabolism, promoting organic acid synthesis and improving microbial cell growth (Ye et al., 2018). However, these nutrients require an avenue for its uptake into the cell for use in important metabolic routes. Tween 80 is a surface-active agent that exerts its mechanistic effect by combining with phospholipid components of the microbial cell membrane, causing disorganization and disruption of its permeability properties (Waller and Lichstein, 1967). These configurational changes render the cells transport system vulnerable for rapid uptake of nutrients (Waller and Lichstein, 1967). In an earlier study, Coelho et al. (2010) optimized the lactic acid production by varying the CSL (11.8–68.2 mL/L) and Tween 80 (0.15–1.85 mL/L). A maximum lactic acid concentration of 41.65 g/L under optimal conditions (CSL = 65.4 mL/L and Tween 80 = 1.27 mL/L) was reported (Coelho et al., 2010).

3.4. RSM model validation

Validation of the developed models were carried out using optimum process conditions of 10% SL, 25 g/L CSL concentration and 2 mL/L Tween 80 concentration. The model predicted a lactic acid concentration and conversion of 10.55 g/L and 17.84%, respectively. In comparison, a lactic acid concentration and conversion of 11.15 g/L and 18.90% were reported for the experimental validation, thus 5.38% and 5.61% higher, respectively, compared to the model predictions, which were considered negligible.

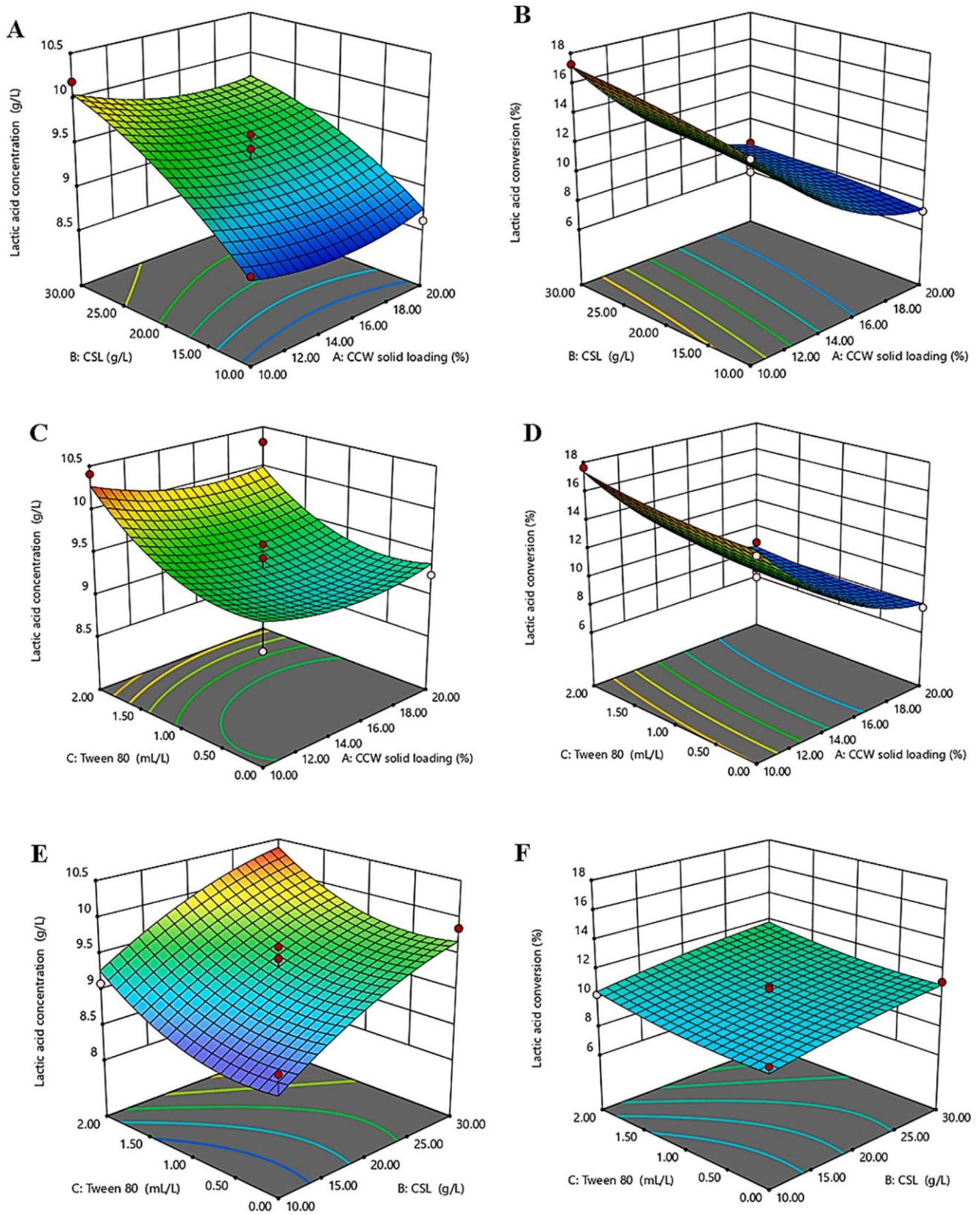

Fig. 1. 3D Response surface graphs illustrating the interactive effect of the various input parameters on the lactic acid production: (A) CSL concentration (g/L) and CCW solid loading (%) (sDWW-SSF$_{concentration}$), (B) CSL concentration (g/L) and CCW solid loading (%) (sDWW-SSF$_{conversion}$), (C) Tween 80 concentration (mL/L) and CCW solid loading (%) (sDWW-SSF$_{concentration}$), (D) Tween 80 concentration (mL/L) and CCW solid loading (%) (sDWW-SSF$_{conversion}$), (E) CSL concentration (g/L) and Tween 80 concentration (mL/L) (sDWW-SSF$_{concentration}$), (F) CSL concentration (g/L) and Tween 80 concentration (mL/L) (sDWW-SSF$_{conversion}$).

3.5. Comparison of the lactic acid production with previous studies

Results from the present study were comparatively assessed with previous reports on lignocellulosic and dairy waste-derived lactic acid production (see supplementary material). Higher lactic acid concentrations of 57%, 56% and 65% were achieved with the sDWW-SSF$_{microaerophilic}$, sDWW-SSF$_{anaerobic}$ and mMRS-SSF$_{microaerophilic}$ processes, respectively, in comparison to a study by Cizeikiene et al. (2018), when two different subspecies of *Lactobacillus delbrueckii* metabolized wheat straw hydrolysate. On the other hand, Cui et al. (2011), Zhang and Vadlani (2015), Oonkhanond et al. (2017) and Liu et al. (2018) investigated modified MRS-based media with lignocellulosic or dairy wastes for lactic acid production and reported higher concentrations compared to the sDWW-SSF$_{microaerophilic}$, sDWW-SSF$_{anaerobic}$ and mMRS-SSF$_{microaerophilic}$ processes in the present study. The differences in the lactic acid production between the present study and previous reports may be ascribed to the various factors such as the microbial strain, physiological parameters (temperature, pH, agitation), the type and concentration of substrate and enzyme. Despite the higher lactic acid concentrations produced by previous studies, the implementation of MRS media in the fermentation system negatively impacts its economic viability (Tang et al., 2013). Furthermore, the water footprint of these lignocellulosic bioprocessing systems places strain on the capital costs, finite resources as well as the environment.

3.6. Kinetic modelling of *L. plantarum* cell growth for the sDWW-SSF$_{microaerophilic}$, sDWW-SSF$_{anaerobic}$ and mMRS-SSF$_{microaerophilic}$ processes

For an in-depth comparison of the different culture conditions concerning nutrients (DWW and modified MRS) and aeration (microaerophilic and anaerobic), the kinetics of microbial cell growth and lactic acid production were evaluated using the logistic and modified Gompertz models, respectively. The evolution of the *L. plantarum* cell growth over time assessed under the previously optimized process conditions for the sDWW-SSF$_{microaerophilic}$ system, was thereafter applied to the sDWW-SSF$_{anaerobic}$ and mMRS-SSF$_{microaerophilic}$ processes (Fig. 2A). All three systems experienced exponential phases that occurred almost instantaneously between 0 hrs-10 hrs for the sDWW-SSF$_{microaerophilic}$ and mMRS-SSF$_{microaerophilic}$ processes and 0 hrs-16 hrs for the sDWW-SSF$_{anaerobic}$ process. The increment in the *L. plantarum* cell growth corresponds to glucose utilization of 37.39%, 16.74% and 13.37% for the sDWW-SSF$_{microaerophilic}$, sDWW-SSF$_{anaerobic}$ and mMRS-SSF$_{microaerophilic}$ bioprocesses, respectively (Fig. 2B). Similar biomass concentration values were achieved for the sDWW-SSF$_{anaerobic}$ (2.27 g/L), sDWW-SSF$_{microaerophilic}$ (2.30 g/L) and mMRS-SSF$_{microaerophilic}$ (2.32 g/L) bioprocesses. The biomass concentration began to plateau after 10 hrs for the sDWW-SSF$_{microaerophilic}$ and mMRS-SSF$_{microaerophilic}$ process compared to 16 hrs for the sDWW-SSF$_{anaerobic}$ process. The *L. plantarum* cell biomass concentration over a 24 hr period (Fig. 2A) were used to fit the logistic models with high correlation coefficients (R^2) of 0.98, 0.99, and 0.98 for the sDWW-SSF$_{microaerophilic}$, sDWW-SSF$_{anaerobic}$ and mMRS-SSF$_{microaerophilic}$ processes, respectively. The highest maximum specific growth rate (μ_{max}) of 0.64 h^{-1} was obtained for the mMRS-SSF$_{microaerophilic}$ process, while the sDWW-SSF$_{microaerophilic}$ and sDWW-SSF$_{anaerobic}$ fermentation processes showed lower μ_{max} values of 0.35 h^{-1} and 0.34 h^{-1}, respectively (see supplementary material). The lower μ_{max} values for the sDWW-SSF$_{microaerophilic}$ (0.35 h^{-1}) and sDWW-SSF$_{anaerobic}$ (0.34 h^{-1}) processes may be due to a nutrient deficiency present in the wastewater-based medium formulations. In particular, complex MRS media used in mMRS-SSF$_{microaerophilic}$ process consists of manganese (Mn^{2+}) and magnesium ions (Mg^{2+}) that are essential growth factors for LABs. These act as cofactors of enzymatic reactions and synergistically improve the binding affinity of the enzyme-substrate complex in carbohydrate metabolism (Yu et al., 2008, Lew et al., 2013). Moreover, both Mg^{2+} and Mn^{2+} play fundamental roles in biosynthesis of lipoteichoic acid and peptidoglycan in microbial cell walls (Lew et al., 2013). The DWW analysis revealed significantly lower concentrations of magnesium (<0.63 mg/L) and manganese (2.56 μ/L) in comparison to the standard MRS media that contained 0.1 g/L magnesium and 0.05 g/L manganese in the form of magnesium sulfate and manganese sulfate, respectively. With regards to the mMRS-SSF$_{microaerophilic}$ system, during microaerophilic conditions, the available oxygen may alter the carbon metabolism away from the lactate dehydrogenase reaction, thus, suppressing lactate production (Zotta et al., 2017). The O_2 activates a minimal electron transport chain and acts as an electron acceptor in a non-glycolytic pathway to achieve high ATP yields (32 ATP) for accelerated cell growth. The combined effect of the high energy metabolism along with sufficient growth factors in MRS media may account for the significantly higher μ_{max} value attained for the mMRS-SSF$_{microaerophilic}$ (0.64 h^{-1}) process. The highest maximum cell concentration (X_{max}) was

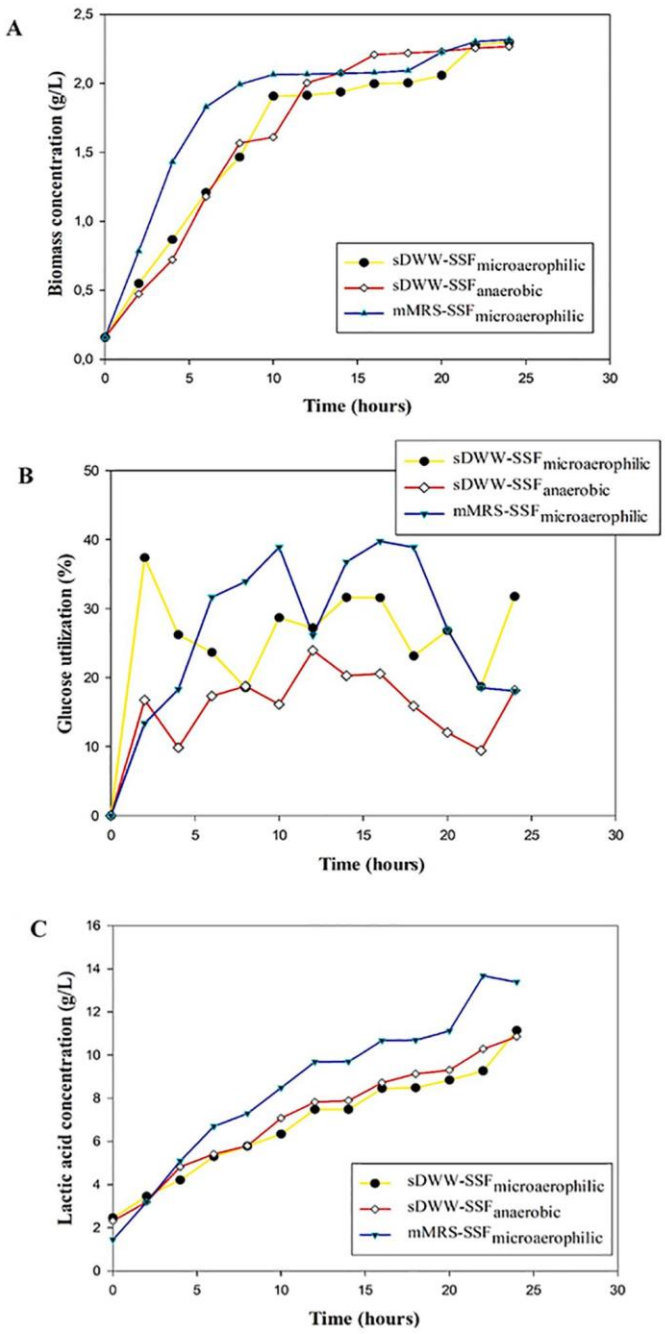

Fig. 2. *Lactobacillus plantarum* ATCC 14,917 biomass concentration (A), glucose utilization (B) and lactic acid concentration (C) for the sDWW-SSF$_{microaerophilic}$, sDWW-SSF$_{anaerobic}$ and mMRS-SSF$_{microaerophilic}$ processes.

achieved for the sDWW-$SSF_{anaerobic}$ (2.26 g/L) process, followed by the mMRS-$SSF_{microaerophilic}$ (2.15 g/L) and the sDWW-$SSF_{microaerophilic}$ (2.14 g/L) processes. The slightly lower X_{max} values reported for the mMRS-$SSF_{microaerophilic}$ and sDWW-$SSF_{microaerophilic}$ processes may be ascribed to microaerophilic environments employed for each process. Although microaerophilic conditions promote *L. plantarum* cell growth, the alternative metabolic pathways (arginine deiminase pathway or NADH oxidase pathway) are repressed in the presence of high glucose concentrations in batch fermentation (Sano et al., 2020). As a result, the biomass accumulation will decrease due to the reduction in ATP generation. To account for the slightly higher X_{max} during the sDWW-$SSF_{anaerobic}$ process, it has been reported that *L. plantarum* is a facultative anaerobe, therefore in an anaerobic environment, its NAD^+ regeneration mechanism is as a result of the glycolytic pathway (Sano et al., 2020). This particular pathway is not repressed in the high glucose concentrations, consequently, the microorganism could potentially consume more sugars for increased cell biomass. In an earlier study, Ha et al. (2003) reported on the kinetics of *Lactobacillus casei* growth and lactic acid production from MRS media supplemented with yeast extract (0.697–2.091%), CSL (1.708–5.123%) and glucose (2–4%) under anaerobic conditions. The optimum concentrations of yeast extract (1.276%), CSL (3.505%) and glucose (2.390%) were observed for the growth of lactic acid bacteria, which resulted in the highest μ_{max} and X_{max} values of 0.99 h^{-1} and 6.37 g/L, respectively. The variation observed in the kinetic coefficients of the aforementioned studies to the present study may be due to factors relating to lignocellulosic substrate type and concentrations, fermentable sugar type and configuration, nutrients, microbial strains and process conditions.

3.7. *Kinetic modelling of lactic acid production for the sDWW-$SSF_{microaerophilic}$, sDWW-$SSF_{anaerobic}$ and mMRS-$SSF_{microaerophilic}$ processes*

The lactic acid production for the sDWW-$SSF_{microaerophilic}$, sDWW-$SSF_{anaerobic}$ and mMRS-$SSF_{microaerophilic}$ processes are illustrated in Fig. 2C. Similar to cell growth, the lag phase was minimal in all three processes for lactic acid production, suggesting that lactic acid was produced at the onset of the SSF bioprocess. This coincided with the increase in lactic acid concentration from 1.44 g/L to 13.68 g/L occurring within 0 hr to 22 hrs for the mMRS-$SSF_{microaerophilic}$ bioprocess. Similarly, a sharp progression in the lactic acid concentration up to 11.15 g/L and 10.86 g/L was observed for the sDWW-$SSF_{microaerophilic}$ and sDWW-$SSF_{anaerobic}$ processes, respectively, during a time lapse from 0 hrs to 24 hrs. The increase in lactic acid concentration occurred all through the duration of the exponential growth phase in all three bioprocesses and continued after exponential into the stationary phase. Even though the cell growth plateaued after 10 hrs (sDWW-$SSF_{microaerophilic}$ and mMRS-$SSF_{microaerophilic}$) and 16 hrs (sDWW-$SSF_{anaerobic}$), there was still sufficient fermentable sugars in the medium to continue metabolism towards non-growth associated lactic acid production. This was elucidated by the glucose utilization observed at 10 hrs and 16 hrs for the sDWW-$SSF_{microaerophilic}$ (29%), sDWW-$SSF_{anaerobic}$ (21%) and mMRS-$SSF_{microaerophilic}$ (40%) processes (Fig. 2B). The cellulase-based enzyme, Cellic CTec2 used in the present study, is a high performing and robust enzyme that displays high saccharification efficiencies, which may lead to the conversion of cellulose to fermentable sugar at a rate that is faster than microbial consumption, thus causing sugar accumulation in the medium. Moreover, high glucose levels in the medium promote increased glycolytic rates that encourage the lactate dehydrogenase reaction, resulting in enhanced lactate production (Sano et al., 2020). The resultant experimental data from the production of lactic acid (Fig. 2C) were used to fit the modified Gompertz model with high R^2 values for the sDWW-$SSF_{microaerophilic}$ (0.97), sDWW-$SSF_{anaerobic}$ (0.99) and mMRS-$SSF_{microaerophilic}$ (0.97) fermentation processes. A lag time (t_L) of 0 hrs was obtained for the sDWW-$SSF_{microaerophilic}$, sDWW-$SSF_{anaerobic}$ and mMRS-$SSF_{microaerophilic}$ processes (see supplementary material). The lag times of 0 hrs displayed for the sDWW-$SSF_{microaerophilic}$ and sDWW-$SSF_{anaerobic}$ may be accounted for by the addition of CSL in the medium formulation at the start of the fermentation process. An advantage of CSL addition in both the sDWW-$SSF_{microaerophilic}$ and sDWW-$SSF_{anaerobic}$ systems is the initial carbohydrates available (in the form of water-soluble carbohydrates) for *L. plantarum* metabolism, giving the enzyme sufficient time to hydrolyse the cellulose to fermentable sugars for utilization. For the mMRS-$SSF_{microaerophilic}$ process, the seed inoculum may contain residual glucose to sustain the metabolic activity of the *L. plantarum* cells until fermentable sugars become available. Furthermore, the MRS media contains three different nitrogen sources (peptone, meat extract, and yeast extract) which are imperative in the biosynthesis of key constituents such as amino acids, proteins and NAD required for cell function (Martinez-Burgos et al., 2021). The yeast extract along with dipotassium phosphate are sources of phosphorus that is involved in cellular energy supply through the exergonic phosphate bonds of cellular ATP (Amrane, 2000). This energy supply drives the glycolysis cycle for rapid formation of lactic acid (Amrane, 2000). Moreover, the efficient saccharification of the pretreated CCW by the Cellic CTec2 enzyme provide the microorganisms with carbohydrate in a short period of time (<2 hrs), thus, drastically reducing the lag phase. This was evident by the glucose utilization at the 2 hr interval for the mMRS-$SSF_{microaerophilic}$ (37.39%), sDWW-$SSF_{microaerophilic}$ (16,74%) and sDWW-$SSF_{anaerobic}$ (13.37%) processes and imply that the time period for the lag phase is highly influenced by the availability of fermentable sugars and nutrients in the initial fermentation medium. The highest maximum lactic acid production rates ($r_{p.m}$) was reported for the mMRS-$SSF_{microaerophilic}$ (0.65 g/L/h) process, followed by the sDWW-$SSF_{anaerobic}$ (0.44 g/L/h) and the sDWW-$SSF_{microaerophilic}$ (0.37 g/L/h) bioprocesses. The higher $r_{p.m}$ observed for the mMRS-$SSF_{microaerophilic}$ (0.65 g/L/h) system may be attributed to the growth factors Mn^{2+} and Mg^{2+} ions that aid in cell growth and product formation. They act as cofactors of enzymatic reactions within cell division, stabilization of nucleic acids (DNA and RNA), peptide hydrolysis and synthesis of the Gram-complex and enhance the binding affinity of the enzyme-substrate complex in metabolic processes (Yu et al., 2008, Lew et al., 2013). The high μ_{max} of 0.64 h^{-1} observed for the standard MRS-based process (mMRS-$SSF_{microaerophilic}$) coincides with the higher lactic acid production rates. With regards to sDWW-$SSF_{microaerophilic}$ and sDWW-$SSF_{anaerobic}$ processes, the reduced concentrations of Mg^{2+} (<0.63 mg/L) and Mn^{2+} (2.56 μg/L) within the DWW, may have significantly impacted the $r_{p.m}$ for both these processes. The deficiency of Mg^{2+} and Mn^{2+} may result in slower cell division and product formation since the catalytic action of these metal ions on various types of metalloenzymes cannot be carried out efficiently. On the other hand, during anaerobic lactic acid fermentation, the absence of oxygen may have led to metabolic shifts within the LAB towards lactic acid formation, thereby supporting the higher $r_{p,m}$ value (0.44 g/L/h) achieved for the sDWW-$SSF_{anaerobic}$ bioprocess. The mMRS-$SSF_{microaerophilic}$ process also generated a high maximum potential lactic acid concentration (P_m) of 14.01 g/L while the sDWW-$SSF_{microaerophilic}$ and sDWW-$SSF_{anaerobic}$ process showed slightly lower P_m values of 13.01 g/L and 12.01 g/L, respectively. According to LAB metabolism, the Embden-Meyerhof Parnas (EMB) glycolytic pathway is followed to produce ATP under anaerobic culture conditions. During this process, oxidized NAD^+ is consumed in the glyceraldehyde triphosphate dehydrogenase (GAPDH) reaction in order to produce pyruvate (Sano et al., 2020). The regeneration of NAD^+ lost in the GAPDH reaction is imperative to drive glycolysis. Therefore, the microorganism converts pyruvate to lactate by the lactate dehydrogenase (LDH) reaction, thus producing NAD^+ (Sano et al., 2020), which favours lactic acid production under anaerobically cultured conditions. However, the data illustrated that the higher P_m values were observed for the microaerophilic bioprocesses (sDWW-$SSF_{microaerophilic}$ = 13.01 g/L and mMRS-$SSF_{microaerophilic}$ = 14.01 g/L). Since the microaerophilic (sDWW-$SSF_{microaerophilic}$ and mMRS-$SSF_{microaerophilic}$) processes obtain its O_2 from the headspace of the reactor and takes place in a closed system with no exogenous supplementation of O_2, the initial O_2 present is depleted at a

fast rate toward a high energy metabolism. Upon depletion of the initial O_2, the sDWW-$SSF_{microaerophilic}$ and mMRS-$SSF_{microaerophilic}$ processes may assume the position of anaerobic metabolism and thus, follows the glycolytic pathway resulting in higher lactic acid concentrations. In addition to metabolic shifts, the initial oxygen available to the *L. plantarum* cells act as an electron acceptor in a non-glycolytic pathway to obtain high ATP for accelerated cell growth and replication (Zotta et al., 2017). The change in the metabolic routes of the microorganism could potentially yield high biomass and lactic acid concentration. Both these aspects are elucidated by the higher μ_{max} and P_m that were obtained for the sDWW-$SSF_{microaerophilic}$ and mMRS-$SSF_{microaerophilic}$ processes. In a recent study, Sharma et al. (2021) assessed the kinetics of lactic acid production in standard MRS and modified MRS (whey permeate instead of glucose) media and observed slightly lower P_m(16.40 g/L), shorter t_L (4.76 h) and higher $r_{p,m}$ (2.6 g/L/h) values for the standard MRS in comparison to the modified MRS medium ($P_{m=}$17.69 g/L, $r_{p.m\ =}$1.68 g/L/h, t_L = 8.56 h) (Sharma et al., 2021). According to the kinetic data from the present study, the optimized dairy wastewater-based processes (sDWW-$SSF_{microaerophilic}$ and sDWW-$SSF_{anaerobic}$) observed a comparable lactic acid concentration with the mMRS-$SSF_{microaerophilic}$ process. In view of a sustainable economy and the food-energy-water nexus, this study addresses the pending issues regarding lignocellulosic bioprocesses by highlighting the potentiality of a wastewater formulation to produce a high value product. The findings from the present study demonstrated that the sDWW-SSF (microaerophilic) process alleviate the costs and water usage by: (1) utilizing a cheaper nitrogen source, (2) including a surfactant that serves a multifold purpose, (3) substituting dairy wastewater streams to reduce freshwater consumption, (4) engaging in a SSF process that negates separate processing units, and (5) circumventing anaerobiosis in the SSF process. This waste-based system could potentially speed up the trajectory of commercial scale bioconversion of waste to high-value commodities such as lactic acid and will help forge quadruple helix-like collaborations between multidiscipline stakeholders and the industrial sector.

4. Conclusion

This study presents a novel perspective for lactic acid (LA) production from supplemented dairy wastewater (DWW) media under simultaneous saccharification and fermentation (SSF) (sDDW-SSF). The sDWW-SSF process obtained maximum LA concentration (11.15 ± 0.42 g/L) and conversion (18.90 ± 0.75%). Following LA optimization, the kinetics of cell growth and product formation was assessed. The sDWW-$SSF_{microaerophilic}$ system (μ_{max} = 0.35 h^{-1}, P_m = 13.01 g/L) observed slightly lower kinetic coefficients compared to the mMRS-$SSF_{microaerophilic}$ (μ_{max} = 0.64 h^{-1}, P_m = 14.01 g/L) and interestingly higher values than the sDWW-$SSF_{anaerobic}$ bioprocess (μ_{max} = 0.34 h^{-1}, P_m = 12.01 g/L). These comparable outcomes may initiate the circular economy scheme and sustainable bioconversion within lignocellulosic processes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work is based on research supported in part by the National Research Foundation (NRF) of South Africa (Grant numbers 122080 and 129556). Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the NRF.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2022.126815.

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Chapter 6: Supplementary material

Table S1. Analysis of variance (ANOVA) of the optimized supplemented dairy wastewater under the SSF process for lactic acid concentrations

Source	Sum of Squares	Degrees of freedom *(df)*	Mean Square	F-value	p-value (probability > F)	
Model	4.28	9	0.48	4.15	3.70×10^{-2}	significant
A-CCW solid loading	0.06	1	0.06	0.50	0.50	
B-CSL concentration	2.57	1	2.57	22.42	2.10×10^{-3}	
C-Tween 80 concentration	1.06	1	1.06	9.23	1.90×10^{-2}	
AB	0.03	1	0.03	0.23	0.65	
AC	0.01	1	0.01	0.11	0.75	
BC	6.66×10^{-4}	1	6.66×10^{-4}	5.81×10^{-3}	0.94	
A^2	0.10	1	0.10	0.86	0.38	
B^2	0.10	1	0.10	0.88	0.38	
C^2	0.37	1	0.37	3.19	0.12	
Residual	0.80	7	0.11			
Lack of fit	0.49	3	0.16	2.12	0.24	not significant
Pure error	0.31	4	0.08			
Cor total	5.08	16				

Footnote: A= CCW solid loading (%), B=CSL concentration (g/L), C= Tween 80 concentration (mL/L).

Table S2. Analysis of variance (ANOVA) of the optimized supplemented dairy wastewater under the SSF process for lactic acid conversion

Source	Sum of Squares	Degrees of freedom *(df)*	Mean Square	F-value	p-value (probability > F)	
Model	155.43	9	17.27	95.36	$< 1 \times 10^{-4}$	significant
A-CCW solid loading	137.90	1	137.90	761.47	$< 1 \times 10^{-4}$	
B-CSL concentration	3.93	1	3.93	21.71	2.30×10^{-3}	
C-Tween 80 concentration	1.73	1	1.73	9.53	1.80×10^{-2}	
AB	0.53	1	0.53	2.91	0.13	
AC	0.41	1	0.41	2.28	0.17	
BC	8.50×10^{-4}	1	8.50×10^{-4}	4.7×10^{-3}	0.95	
A^2	10.21	1	10.21	56.36	1×10^{-4}	
B^2	0.12	1	0.12	0.66	0.44	
C^2	0.45	1	0.45	2.47	0.16	
Residual	1.27	7	0.18			
Lack of fit	0.87	3	0.29	2.94	0.16	not significant
Pure error	0.40	4	0.099			
Cor total	156.70	16				

Footnote: A= CCW solid loading (%), B=CSL concentration (g/L), C= Tween 80 concentration (mL/L).

Table S3. Elemental analysis of dairy wastewater

Element	Concentration
Dissolved calcium	3.06 mg/L
Potassium	16.90 mg/L
Dissolved magnesium	<0.63 mg/L
Sodium	684 mg/L
Dissolved copper	54 μg/L
Dissolved iron	91 μg/L
Dissolved manganese	2.56 μg/L
Dissolved sulfur	3.80 mg/L
Dissolved zinc	21 μg/L
Total phosphorus	6.96 mg/L
Total nitrogen	36 mg/L
Total organic carbon	421 mg/L

Table S4. Comparisons of the lactic acid production from lignocellulosic and dairy wastes using *Lactobacillus* species

Microorganism	Substrate/carbon source	Medium type	Process conditions	Lactic acid concentration (g/L)	Lactic acid conversion (%)	Reference
Lactobacillus plantarum ATCC 14917	Corn cob waste	Supplemented dairy wastewater	10% (w/v)[a], 10% (w/v)[b], Cellic CTec2[c], 10 FPU/g[d], 37°C[e], 120 rpm[f]	11.15	18.90	This study
Lactobacillus plantarum ATCC 14917	Corn cob waste	Supplemented dairy wastewater	10% (w/v)[a], 10% (w/v)[b], Cellic CTec2[c], 10 FPU/g[d], 37°C[e], 120 rpm[f]	10.85	18.40	This study
Lactobacillus plantarum ATCC 14917	Corn cob waste	Modified MRS	10% (w/v)[a], 10% (w/v)[b], Cellic CTec2[c], 10 FPU/g[d], 37°C[e], 120 rpm[f]	13.68	23.19	This study
Lactobacillus delbrueckii subsp. *bulgaricus DSM* 20081	Wheat straw hydrolysate	-	0.01% (v/w)[a], CeluStar XL[c], 42°C[e]	4.81	ND	Cizeikiene et al. (2018)
Lactobacillus delbrueckii subsp. *bulgaricus* MI	Wheat straw hydrolysate	-	0.01% (v/w)[a], CeluStar XL[c], 42°C[e]	4.74	ND	Cizeikiene et al. (2018)

Footnote: [a]=microorganism concentration, [b]=solid loading, [c]=enzyme type, [d]=enzyme concentration, [e]=temperature, [f]=agitation speed, MRS= de Man, Rogosa and Sharpe.

Table S4. Continued…

Microorganism	Substrate/carbon source	Medium type	Process conditions	Lactic acid concentration (g/L)	Lactic acid conversion (%)	Reference
Lactobacillus brevis ATCC 367	Corn stover	Modified MRS	10% (v/v)[a], 4% (w/v)[b], Cellic CTec2[c], 8 FPU/gd, 37°C[e], 150 rpm[f].	16.3	ND	Zhang and Vadlani (2015)
Lactobacillus plantarum ATCC 21028	Corn stover	Modified MRS	10% (v/v)[a], 4% (w/v)[b], Cellic CTec2[c], 8 FPU/g[d], 37°C[e], 150 rpm[f].	21	ND	Zhang and Vadlani (2015)
Lactobacillus rhamnosus	Corn stover	Modified MRS	2% (v/v)[a], 3% (w/v)[b], cellulase[c], 25 FPU/g[d], 37°C[e], 100 rpm[f]	17.70	ND	Cui et al. (2011)
Lactobacillus casei TISTR 390	Sugarcane bagasse hydrolysate	Modified MRS	10% (v/v)[a], 10% (w.v)[b], accellerase 1500[c], 200 FPU/g[d], 37°C[e], 30–35 rpm[f]	21.30	ND	Oonkhanond et al. (2017)
Lactobacillus bulgaricus CGMCC 1.6970	Cheese whey powder	Modified MRS	10% (v/v)[a], 59.25 g/L[b], 42°C[e], 200 rpm[f]	27.34	ND	Liu et al. (2018)

Footnote: [a]=microorganism concentration, [b]=solid loading, [c]=enzyme type, [d]=enzyme concentration, [e]=temperature, [f]=agitation speed, MRS= de Man, Rogosa and Sharpe.

Table S5. Microbial growth and lactic acid formation kinetic parameters for the sDWW-$SSF_{microaerophilic}$, sDWW-$SSF_{anaerobic}$ and mMRS-$SSF_{microaerophilic}$ processes

Kinetic parameter	Bioprocess		
	sDWW-$SSF_{microaerophilic}$	sDWW-$SSF_{anaerobic}$	mMRS-$SSF_{microaerophilic}$
μ_{max} (h^{-1})	0.35	0.34	0.64
X_o (g/L)	0.29	0.26	0.27
X_{max} (g/L)	2.14	2.26	2.15
P_m (g/L)	13.01	12.01	14.01
$r_{p.m}$ (g/L/h)	0.37	0.44	0.65
t_L (h)	0	0	0

Footnote: μ_{max}=maximum specific growth rate, X_0=initial cell concentration, X_{max}=maximum cell concentration, P_m=maximum potential lactic acid concentration, $r_{p,m}$ =maximum lactic acid production rate, t_L=lag time.

CHAPTER 7

Novel Strategies for High-Efficiency Lactic Acid Production: Process Optimization and Scale-Up on Corn Cob Waste and Supplemented Dairy Wastewater medium

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This chapter has been submitted to the journal *Industrial Crops and Products* with the title: Novel Strategies for High-Efficiency Lactic Acid Production: Process Optimization and Scale-Up on Corn Cob Waste and Supplemented Dairy Wastewater medium

The manuscript and supplementary material are presented in the following pages.

Novel Strategies for High-Efficiency Lactic Acid Production: Process Optimization and Scale-Up on Corn Cob Waste and Supplemented Dairy Wastewater medium

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Abstract

This study investigated strategies for enhancing lactic acid (LA) yield and sugar conversion in a supplemented dairy wastewater medium, focusing on buffer agents, micronutrient enrichment, pH adjustments, and bioprocess types. The optimized process conditions were scaled up to 0.5 L in a continuous stirred tank reactor, based on constant impeller tip speed (V_{tip}) and constant power input per unit volume (P/V), as mixing criteria. The optimized mixing regime was scaled-up to 5 L with corresponding kinetic assessment. The resultant waste effluent was analysed as a component for animal feed and biofertilizer potential. The simultaneous saccharification and fermentation process in the presence of $CaCO_3$ and MnO nanoparticle-supplemented medium demonstrated high LA concentration (31.12 g/L) and sugar utilization (up to 46.27%) at flask scale. LA production in a 0.5 L bioreactor revealed an 18.25% higher LA concentration and 40% reduced production time for constant P/V compared to the constant V_{tip}. Process scale- up at 5 L scale with constant P/V showed that the LA concentration peaked at 31.43 g/L while achieving up to 43.55% sugar utilization, corresponding to 0.26 h^{-1} maximum specific growth rate (μ_{max}) and 35.11 g/L maximum LA potential (P_m). Furthermore, the waste effluent rich in essential elements demonstrated a

suitable nutritive profile for animal feed and biofertilizer application. These findings underscore the critical role of media formulation that eliminates chemical and feedstock costs, reduces water consumption, energy and time, with significant emphasis on improved pH control, increased production rates and enhanced mixing efficiency. The study also provides key insights on industrial bioprocesses for the transformation of lignocellulosic, Kraft and dairy wastes into valuable commodities, and highlights the sustainability and efficacy of developing waste-based bioprocesses for LA production.

Keywords

Bioprocess scale-up, Calcium carbonate supplementation, Kinetic modelling, Lactic acid production, Nanoparticles

7.1. Introduction

To meet the rising global lactic acid (LA) demand, microbial fermentative production is considered the mainstream technology, accounting for approximately 90% of its production (Macedo et al., 2020). Lactic acid microbial producers (LABs), more specifically, *Lactobacillus* strains are commonly used for LA production due to its desired characteristics in industrial processes such as high acid tolerance, enhanced yield and amenability to genetic engineering (Sano et al., 2020). Nevertheless, LABs are auxotrophic microorganisms that rely on exogenous sources of complex nutrients including carbon, nitrogen, trace elements, amino acids and growth factors that are crucial for growth, given its limited biosynthetic capabilities (Chen et al., 2020). The De Man, Rogosa and Sharpe (MRS) is a standard defined media that has been used commercially for the cultivation of LABs and LA production, due to its specific nutrient-rich composition, several micro and macro-nutrients as well as pH regulators (Chen et al., 2020). However, its complex composition challenges the economic feasibility

and practicality of large-scale LA production due to the expenses attached to nutrient sources such as yeast extract, protein and refined sugars, contributing to approximately 30% of the bioprocess costs (Tang et al., 2013; Canon et al., 2021). Research efforts are directed towards developing minimally supplemented media that enables precise control over metabolic pathways, product specificity, and overall process optimization, thereby reducing costs. The three key factors influencing LA production include nitrogen source, carbon source, and surfactants, each being critical for optimizing microbial growth and product formation (Martinez et al., 2013; Reitermayer et al., 2018).

Nitrogen plays a fundamental role in the synthesis of key biomolecules such as amino acids and proteins involved in LABs metabolism (Ye et al., 2018). Over the years, researchers have promoted the adoption of corn steep liquor (CSL), a rich emulsion of nutrients that approximates only one fifth of the cost of conventionally employed yeast extract (Tan et al., 2016). Carbon is another integral building block in the derivation of energy and macromolecule biosynthesis. Lignocellulosic biomass (LCB), like corn cob waste (CCW) has emerged as a sustainable carbon source that necessitates efficient pretreatment technologies to release fermentable sugar (Chakraborty et al., 2024). Cost-effective alternatives like industrial waste-derived chemicals, green liquor dregs (GLD) and paper wastewater (PWW) have come to the forefront as pretreatment catalysts, thus circumventing expensive lab-grade chemicals (David et al., 2021). These waste-derived solutions offer a promising avenue for modifying the rigid CCW cell wall structure to extract valuable cellulose moieties for bioconversion to glucose, that can be utilized by the microorganism. In addition, surfactant-assisted fermentation, exemplified by the inclusion of Tween 80 in MRS media, has gained prominence due to the potential enhancement of nutrient uptake, medium emulsification, and enzyme stability during substrate saccharification (Taoka et al., 2011; Zhang et al., 2018). To enhance the absorption capacity, substrate solubility and reaction rates of the interactive

nitrogen, carbon and surfactant sources; selecting an appropriate solvent for optimizing the fermentation medium design is imperative.

The repurposing of untreated dairy wastewater streams offers a sustainable alternative to traditional dairy industry treatments, reducing environmental impact, capital expenses, and conserving freshwater resources in bioprocessing. This approach marks a significant shift towards environmentally friendly and cost-effective bioprocesses. Building on this innovative strategy, our previous research focused on the optimization of a supplemented DWW medium for simultaneous saccharification and LA fermentation (sDWW-SSF) (David et al., 2022). By supplementing the DWW with CSL, pretreated CCW using Kraft waste, and Tween 80 as sources of nitrogen, carbon, and surfactant, respectively, the study aimed to develop a cost-effective and sustainable waste-based LA fermentation technology. Nevertheless, findings from the study observed a residual glucose content (~37.84 g/L) at the end of the sDWW-SSF process, indicating the potential for further improvement in the LA production (David et al., 2022). Several bioprocess development strategies may therefore be explored to enhance the LA production and sugar utilization, aiming to comprehensively valorize the pretreated CCW and minimally supplemented DWW medium. These approaches include the use of buffering agents, micronutrients, pH variations, and bioprocess types. Buffer agents play a crucial role in the LA synthesis processes by maintaining pH stability within its optimal range (pH 4.5 to 6.5) and providing a conducive environment for LABs to thrive (Anagnostopoulou et al., 2022). For this reason, meticulous selection of buffers such as K_2HPO_4, $NaHCO_3$, $Na_2HPO_4.12H_2O$ and $CaCO_3$ through lab-scale experiments are essential to establish their compatibility and efficiency prior to industrial application. The defining characteristics of buffer agents in LA processes are its ability to (1) readily accept hydrogen ions (H^+), (2) remain stable without undergoing chemical reactions or decomposition, (3) be non-toxic to LABs, (4) be compatible with the microorganism in terms of viability,

productivity or LA yield, and (5) maintain the pH within a specific range in the presence of external fluctuations. In particular, commonly used $CaCO_3$ acts as a buffer agent in LA media by reacting with LA to form calcium lactate salt (neutralized form of LA) and CO_2 gas, thus preventing excessive acidification that can be detrimental to the viability of the LAB (Wang et al., 2014; Yanga et al., 2015). These lactate salts are soluble in aqueous solution and do not contribute to acidity or alkalinity of the solution. Moreover, in its initial form, $CaCO_3$ is alkaline in nature, creating an environment less favourable for the growth of contaminating organisms that may compete with LABs during fermentation.

Apart from buffer systems, micronutrient supplementation is a significant factor to optimize bacterial growth, biomass growth rates, overall fermentation performance and yield generation. More specifically, $MgSO_4$ (Mg^{2+}) and $MnSO_4$ (Mn^{2+}) are micronutrients that act as cofactors to enhance the catalytic activity of enzymatic reactions in biosynthetic pathways, synergistically improving the enzyme-substrate binding affinity in carbohydrate metabolism and increasing stress tolerance of LABs (Yu et al., 2008, Lew et al., 2013). Such micronutrient capabilities have led to the development of nanoparticles that offer several potential advantages over its chemical counterpart. More so, Manganese oxide (MnO) nanoparticles exhibit unique physicochemical properties at a nanoscale, with its high surface-to-volume ratio and unique surface properties that enable efficient adsorption and activation of reactant molecules for enhanced LA production (Sanusi et al., 2020). MnO has gained traction for its recovery potential and reusability, thus contributing to cost-effectiveness and sustainability of the production process. Notably, the application of MnO nanoparticles in LA production is still an active area of research since the utilization of micronutrient nanoparticles opens new possibilities for precise and efficient delivery of essential elements to LABs. This promotes specific target delivery, better nutrient absorption, and overall effectiveness in LA bioprocessing (Powell et al., 2010; Abdelsalam et al., 2016).

Bioprocess types are integral in the production of various bioproducts, including LA and these different types of bioprocesses impact the process time, saccharification, biomass production, product formation and overall efficiency of the fermentation system. Among the bioprocess types commonly employed, simultaneous saccharification and fermentation (SSF) as well as prehydrolysis combined with SSF (PSSF) are noteworthy methods in terms of process efficiency, substrate utilization and product yields (Carrillo-Nieves et al., 2017). The SSF process integrates the simultaneous lignocellulosic conversion to fermentable sugar and subsequent LA production in a single bioreactor, thus eliminating the separate hydrolysis and fermentation steps, reducing contamination risk and potentially increasing yield generation due to continuous fermentable sugar availability (Sewsynker-Sukai et al., 2023). This concurrent system asserts several benefits of reduced process time, energy and costs (~20% reduction) (Wingren et al., 2003). For the PSSF process, a short prehydrolysis phase exhibits significant sugar release within a shorter time frame (6–24 hr) at optimal temperature (50-60 °C), followed by inoculation of the microbial culture into the vessel where the remaining complex carbohydrates are saccharified and fermented to LA (Sewsynker-Sukai and Gueguim Kana, 2018). This system strikes a balance between inhibitory and rate-controlling factors. However, inclusion of the initial prehydrolysis stage increases process complexity, utilizes additional equipment and extends the overall process duration, contributing to higher energy consumption and operational costs in the grand scheme of commercial scale-up (Wingren et al., 2003).

In addition to the factors previously discussed, a comprehensive understanding of the kinetics of microbial cell growth and LA production, along with the mastery of scale-up processes, are crucial for the successful development of bioprocesses aimed at achieving commercial-scale industrialization. Kinetic models like the logistic and modified Gompertz have been instrumental in describing microbial growth and product formation in batch systems

(Phukoetphim et al., 2017). The logistic model predicts microbial growth patterns based on rate as well as initial and maximum concentrations (Muloiwa et al., 2020), while the modified Gompertz model focuses on product formation by estimating lag time, maximum production rate, and concentration (Dodić et al., 2012). These models are crucial for enhancing LA yield and productivity, while reducing undesired by-products (Kucharska et al., 2018). Still, gaps remain in the understanding of LA bioprocess kinetics, particularly with minimally supplemented waste-derived media. Scaling up a bioprocess from flask to reactor necessitates careful consideration of kinetics and mixing efficiency. Efficient mixing is essential for ensuring uniform physicochemical conditions, homogenous nutrient distribution, and microorganism dispersion while avoiding concentration gradients that could impair microbial growth and product formation or lead to unwanted by-products (Xia et al., 2015). Key parameters for optimizing bioreactor design include constant power input per unit volume (P/V) and constant impeller tip speed (V_{tip}), which are crucial for ensuring effective agitation and overall bioprocess efficiency. Constant P/V represents the power needed relative to the medium's volume, and V_{tip} denotes the impeller blade tip's velocity during agitation. As bioprocess scale increases, adjusting these parameters is vital for preserving mixing efficiency, temperature regulation and microbial cell integrity.

Considering the challenges above and the critical importance of sustainability alongside cost-efficiency in industrial processes, this research focuses on the development of innovative and eco-friendly media formulations for LA production. Leveraging on a previously optimized sDWW-SSF process utilizing pretreated CCW and supplemented DWW (David et al., 2022), this study aims to enhance LA yield and sugar utilization for complete valorization of the feedstock. It investigates the impact of various buffer systems in conjunction with MnO nanoparticles on the efficiency, sugar utilization and stability of the LA bioprocess. Additionally, the interactive impact of the buffer agent type, micronutrient supplementation,

and pH adjustments on the LA concentration and sugar utilization were investigated. A comparative assessment of different bioprocess modes, such as SSF and PSSF were conducted to evaluate their efficacy in enhancing sugar release and quantification, product yield and pH control. The optimized conditions were then scaled up to 5 L in a bench-top bioreactor, where the kinetic studies of *Lactobacillus plantarum* ATCC 14917 cell growth and LA production were investigated. Finally, the potential of the fermentation effluent as animal feed and biofertilizer were explored through compositional and nutritional analysis, aiming for complete valorization.

7.2. Materials and methods

7.2.1. Materials

All chemicals required during this study were purchased from Merck, South Africa. The milled corn cob waste (CCW) in this study was pretreated according to our previously optimized steam-assisted combined green liquor dregs and paper wastewater (SGLD-PWW) pretreatment strategy using optimum parameters (10% CCW, 49.89% GLD, 118°C, 5 min) (David et al., 2021). The chemical composition of the optimized pretreated CCW biomass (46.91% cellulose, 29.90% hemicellulose, 20.50% lignin) was analysed according to the Van Soest method (Van Soest and McQueen, 1973). The commercial cellulase-based enzyme blend, Cellic CTec 2, was generously provided by Novozyme (Novozymes A/S, Denmark) and its enzyme activity (160 FPU/mL) was determined according to the Laboratory Analytical Procedure (LAP) of the National Renewable Energy Laboratory (NREL, 2008). The dairy wastewater (DWW) obtained after the product processing and washing step was collected from a local dairy (Howick, South Africa) and its elemental analysis is presented in

Table S7.7. The MnO nanoparticles were prepared using the co-precipitation method according to Sanusi et al. (2019) as described below.

7.2.2. Preparation of MnO nanoparticles

The MnO nanoparticles were synthesized using the co-precipitation method. Initially, 6.76 g $MnSO_4.H_2O$ was dissolved in 40 mL deionized water and thereafter, NH_3 was added gradually to the solution to obtain pH 11. The solution was continuously stirred at 60°C for 2 h to precipitate the MnO nanoparticles. The resultant brown precipitate was then washed three times with deionized water and oven dried at 70°C for 12 h.

7.2.3. Lactobacillus plantarum inoculum preparation

The 20% glycerol (v/v) stock culture of *L. plantarum* (stored at -80°C) was streaked onto standard MRS agar growth media. The MRS agar was incubated at 37°C for 48 h. Subsequently, a single colony was aseptically transferred into 100 mL sterile MRS broth and incubated at 37°C and 120 rpm for 14 h till the cell suspension was exponentially grown, as previously determined in our previous study (David et al., 2022).

7.2.4. Dairy wastewater citrate buffer preparation

The standard citrate buffer (0.05 M) was modified using DWW in place of deionized water. The buffer pH was adjusted accordingly based on the test variables where applicable. Thereafter, the DWW citrate buffer was sterilized at 121°C for 15 min for use in each bioprocess experimental run.

7.2.5. Screening of different buffer agents and MnO nanoparticle-assisted technology for enhanced lactic acid production and sugar utilization

To enhance the existing optimized CCW and supplemented DWW-based SSF process designated as the sDWW-SSF bioprocess reported in our previous study (David et al., 2022),

the potential of various buffer systems (K_2HPO_4, $NaHCO_3$, $Na_2HPO_4.12H_2O$, and $CaCO_3$) were evaluated to support complete sugar utilization and increase LA production. The SSF fermentation process was conducted in 100 mL Erlenmeyer flasks, with a total of 8 different experimental runs. The base reactions were maintained using the optimized sDWW-SSF bioprocess conditions (David et al., 2022) for all experiments. Each flask consisted of a constant working volume of 25 mL with 25 g/L CSL, 2 mL/L Tween 80, 10% SL and 10 FPU/g standard enzyme loading in the DWW citrate buffer (0.05 M, pH 4.8). The medium was inoculated with 10% (v/v) exponentially grown *L. plantarum* inoculum concentration (Petrides, 2000; Derabli et al., 2022; Sudhakar and Dharani, 2022) by measuring the optical density at 580 nm (OD580) using a spectrophotometer. Four experiments were subjected to different buffering agents, including 2 g/L K_2HPO_4, 15 g/L $NaHCO_3$, 15 g/L $Na_2HPO_4.12H_2O$ or 15 g/L $CaCO_3$, designated as sDWW-SSF_{KH2PO4}, sDWW-SSF_{NaHCO3}, sDWW-$SSF_{Na2HPO4.12H2O}$ and sDWW-$SSF_{CaCO3(15)}$, respectively. The concentration of KH_2PO_4 (2 g/L) selected for the process was based on the standard concentration used in commercial MRS media (HG000C87) (@Merck, Darmstadt, Germany). The $NaHCO_3$, $Na_2HPO_4.12H_2O$ and $CaCO_3$ buffer agents were added in a 4:1 ratio (glucose: buffer) as a baseline concentration, determined by Karnaouri et al. (2020), that studied the effect of different concentrations of buffer agent on the LA concentration. The glucose concentration (~60 g/L) for the glucose: buffer ratio was attained from our previous study using the sDWW-SSF process (David et al., 2022). In parallel with each buffer system, four corresponding experiments containing 0.025 g/L MnO nanoparticle supplementation (Table S7.1) were carried out. These experiments were designated as sDWW-$SSF_{KH2PO4+MnO}$, sDWW-$SSF_{NaHCO3+MnO}$, sDWW-$SSF_{Na2HPO4.12H2O+MnO}$ and sDWW-$SSF_{CaCO3(15)+MnO}$, respectively. The experimental conditions for the eight supplemented flasks including the type of buffer, buffer concentration and nanoparticle selection were informed by the literature (Sanusi et al., 2019;

Mis Solval et al., 2019; Karnaouri et al., 2020). To determine the sugar consumption, each experimental run had a corresponding control that remained uninoculated and was used to determine the initial sugar concentration that was produced. Both the uninoculated control and inoculated test experiments for each buffer agent were carried out in duplicate. All fermentation runs were incubated at 37°C and 120 rpm for a duration of 48 h, with samples aliquoted every 12 h to determine LA and reducing sugar concentrations using the methods described below (*Section 7.2.9.*).

7.2.6. Lactic acid production

7.2.6.1. Evaluating the effect of using various physicochemical enhancement parameters under SSF bioprocess: buffer agent, pH changes and micronutrient supplementation

The initial screening using different buffering agents (K_2HPO_4, $NaHCO_3$, $Na_2HPO_4.12H_2O$, and $CaCO_3$) in *section 7.2.5* demonstrated that the $CaCO_3$ addition yielded higher LA concentration and sugar utilization. $CaCO_3$ was therefore used in further experiments carried out on various physicochemical methods to the sDWW-SSF bioprocess. These involved assessing the singular impact of $CaCO_3$ agent, addition of micronutrients ($MgSO_4$ and $MnSO_4$ or MnO nanoparticle), effect of pH changes (pH 4.8, pH 5.5), or a combination of these parameters. The LA fermentation was carried out in 100 mL Erlenmeyer flasks with a total of 8 experiments. The base reactions maintained a constant working volume of 25 mL with 25 g/L CSL, 2 mL/L Tween 80, 10% SL, 10 FPU/g standard enzyme loading, and 10% (v/v) exponentially grown *L. plantarum* inoculum concentration, in the DWW citrate buffer (0.05 M). The DWW citrate buffer was adjusted to pH 4.8 or pH 5.5 according to Table S7.2. Each flask was subjected to a different variable, namely, 0.05 g/L Mn^{2+} ($MnSO_4$) and 0.5 g/L Mg^{2+} ($MgSO_4$) ions supplementation, 0.025 g/L nanoparticle supplementation (MnO), pH change (pH 4.8 or 5.5), 30 g/L $CaCO_3$ buffering or an interactive combination of the above

variables (Table S7.2). All fermentation runs were incubated at 37°C and 120 rpm for 84 h. The change in $CaCO_3$ concentration from a 4:1 ratio (*Section 2.5*) to 2:1 ratio was based on a study by Karnaouri et al. (2020). The aforementioned authors evaluated the addition of different concentrations of glucose to $CaCO_3$ ratios, 4:1 (low concentration), 2:1 (medium concentration) and 4:3 (high concentration) on the LA production and found that the 2:1 ratio was able to ensure proper pH regulation and buffering capacity without comprising the fluidity of the medium (Karnaouri et al., 2020). The experimental conditions for the eight supplemented flasks including the micronutrient selection and concentration, nanoparticle selection, and pH selection were informed by the literature (Abdulsattar et al., 2020; Sanusi et al., 2019; Mis Solval et al., 2019).

7.2.6.2. Comparative assessment of the optimized buffer and nanoparticle assisted lactic acid production using the PSSF bioprocess

In light of the results obtained from the evaluation of various physicochemical parameters to the sDWW-SSF bioprocess, the supplementation of $CaCO_3$ only (sDWW-$SSF_{CaCO3(30)}$) and $CaCO_3$ with MnO nanoparticles at pH 5.5 (sDWW-$SSF_{CaCO3(30)+MnO,\ pH\ 5.5}$) yielded high LA concentrations and sugar utilization. Nevertheless, during fermentation, $CaCO_3$ reacts with LA to produce calcium lactate, resultantly increasing the pH of the fermentation medium and affecting enzymatic saccharification. For this reason, the current research phase explored a prehydrolysis with SSF (PSSF) approach, focusing on optimizing pH during enzymatic saccharification and fermentation to enhance LA concentration and sugar utilization. It also assessed Tween 80 and/or MnO nanoparticle effects on prehydrolysis and fermentation systems.

To determine which bioprocess type was most effective under the varying experimental conditions based on the sDWW-$SSF_{CaCO3(30)}$ and sDWW-$SSF_{CaCO3(30)+MnO,\ pH\ 5.5}$ process using

the SSF mode, the PSSF bioprocess was also implemented and comparatively assessed. The PSSF fermentation process was carried in 100 mL Erlenmeyer flasks with a total of 5 experiments. Prior to the SSF process, a 24-h prehydrolysis stage was carried out in a 20 mL volume of DWW citrate buffer (pH 4.8, 0.05 M) containing SGLD-PWW pretreated CCW (10%, w/v) and 10 FPU/g enzyme loading. The prehydrolysis reactions were also supplemented with either Tween 80 (2 mL/L), MnO nanoparticle (0.025g/L) or a combination of the two where applicable (Table S7.3), to determine its effect on the enzymatic saccharification and fermentation processes, respectively. The flasks were incubated at 50°C and 120 rpm for 24 h. Following prehydrolysis, 25 g/L CSL and 30 g/L $CaCO_3$ in DWW citrate buffer as well as 10% (v/v) exponentially grown *L. plantarum* inoculum concentration was added to make up the constant 25 mL working volume. All fermentation runs introduced $CaCO_3$ in the fermentation stage of the process only, to ensure optimum pH regulation (pH 4.8) in the enzymatic hydrolysis stage. For the fermentation process, 2 mL/L Tween 80 and/or 0.025 g/L MnO nanoparticles were added (where applicable), according to Table S7.3. All fermentation reactions were incubated at 37°C and 120 rpm for 84 h.

For both the SSF and PSSF processes, the control experiments (uninoculated) were subject to the same conditions as their corresponding test experiments. Both the uninoculated control and inoculated test experiments for each variable were carried out in duplicate. A 1 mL sample was aseptically removed from each experimental set and its corresponding controls (uninoculated) every 12-h interval throughout the 84-h fermentation period. The aliquoted samples were thereafter used to determine LA and reducing sugar concentrations using the methods described below (*Section 7.2.9*).

7.2.7. Optimization of the scale-up assessment of lactic acid production at 0.5 L

Following the three-step screening process under the different experimental conditions, the optimized $CaCO_3$ buffer and nanoparticle supplemented LA production under the SSF process gave the maximum LA concentration and was selected for the scale-up assessment, with designation as MDWW-SSF. Two experimental scale-up processes were carried out in a 2 L bioreactor (Kori instruments, DF 1L, China) with a 0.5 L working volume (Table S7.4). Each reaction experiment was used to determine the optimum mixing speed for enhanced LA production based on the constant power input per unit volume (P/V) and constant impeller tip speed (V_{tip}) mixing regimes. The glass vessel was fed with 25 g/L CSL, 2 mL/L Tween 80, 10% SL (SGLD-PWW pretreated CCW), 10 FPU/g standard enzyme loading and 30 g/L $CaCO_3$ in the DWW citrate buffer (0.05 M, pH 4.8). The medium was inoculated with 10% (v/v) *L. plantarum* inoculum grown to exponential phase (14 h). The constant optimum operational temperature of 37°C was maintained for both bioprocesses over a duration of 48 h. The constant V_{tip} (58 rpm) and constant P/V (74 rpm) were determined using eq. 1 and 2, respectively, for each reaction. The control experiments (uninoculated) were subject to the same conditions as per the corresponding test experiments. Samples of 3 mL were aseptically removed from the reaction vessel of the experimental and corresponding control (uninoculated) sets every 6 h over a period of 48 h. The aliquoted samples were thereafter used to determine LA and reducing sugar concentrations as described below.

$$n_2 = n_1 (di_1/di_2) \quad (1)$$

$$n_2 = n_1 (di_1/di_2)^{2/3} \quad (2)$$

where *n* is the rpm and *di* the vessel diameter.

7.2.8. 5 L Scale-up assessment for lactic acid production

The MDWW-SSF process using the constant P/V mixing criteria at 74 rpm gave maximum LA production as previously determined for the 0.5 L scale-up assessment in the 2 L bioreactor. Therefore, the constant P/V mixing regime was applied to the 13 L bioreactor (Labfors INFORS HT, Switzerland) with a 5 L working volume. The vessel contained 25 g/L CSL, 2 mL/L Tween 80, 10% SL (SGLD-PWW pretreated CCW), 10 FPU/g standard enzyme loading and 30 g/L $CaCO_3$ in the DWW citrate buffer (0.05 M, pH 4.8) at a constant working volume of 5 L. The medium was inoculated with a 14-h exponentially grown *L. plantarum* culture of 10% (v/v) as determined by a growth curve of optical density at 580 nm (OD580) using a spectrophotometer over a duration of time. The constant optimum operational temperature of 37°C was maintained over a duration of 24 h. The constant P/V (54 rpm) was calculated using eq. 2. Identical conditions corresponding to the test experiment were maintained for the control experiment (uninoculated). Aliquots (3 mL) from experimental and corresponding control (uninoculated) sets were sampled aseptically from the reaction vessel every 2 h over a period of 24 h to determine LA, biomass and reducing sugar concentrations as described below (*Section 2.9*).

7.2.9. Analytical methods

7.2.9.1. Determination of LA concentration

The total LA concentration (g/L) was quantified using the Megazyme LA assay kit, designated by product code (K-DLATE) (©Megazyme, Wicklow, Ireland) according to specified protocols as stated by the manufacturer. The VERSAmax tuneable microplate reader (Molecular Devices, California, USA) was employed to spectrophotometrically analyse the LA concentration.

7.2.9.2. Determination of reducing sugar concentration

The reducing sugar concentration of the experimental and control (uninoculated) samples were quantified using the 3.5-dintrosalicylic acid method (Miller, 1959). The control experiments (uninoculated) were conducted to estimate the initial sugar concentration that was produced. The test experimental reducing sugar concentration was subtracted from the control experiment sugar concentration to determine the sugar utilization (%) as per Eq. (3).

$$\text{Sugar utilization (\%)} = \frac{\text{Initial sugar concentration (g/L)} - \text{Final sugar concentration (g/L)}}{\text{Initial sugar concentration (g/L)}} \times 100 \qquad (3)$$

7.2.9.3. Determination of microbial biomass concentration

The *L. plantarum* cell concentration (g/L) was quantitatively evaluated by relating the microbial cell count as a function of the dry cell weight. The *L. plantarum* culture was grown exponentially in MRS broth and diluted accordingly (1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$). The respective diluted sample was decanted with a total volume of 10 mL and centrifuged at 7607 g for 5 min. The resultant biomass pellet was oven dried at 90°C, until a constant mass was observed. The dry weights (g/L) of each sample were plotted against its corresponding cell counts (cells/mL) to produce a standard curve. To determine the experimental biomass concentration (g/L) for the MDWW-SSF bioprocess using the constant P/V mixing criteria at the 5 L working volume in the 13 L bioreactor scale-up experiment, the cell counts (cells/mL) were substituted into the equation generated from the standard curve.

7.2.10. Kinetic modelling

7.2.10.1. Logistic model for cell growth

The biomass concentration data obtained for the 5 L experimental scale-up was used for the logistic model. The logistic model in the differential form of Eq. (4) represents the exponential and stationary phases of growth. Eq. (4) was integrated to form Eq. (5). This

logistic model illustrates the relationship of cell biomass (X) to initial cell concentration (X_0), maximum cell concentration (X_{max}) and maximum specific growth rate (μ_{max}) at specific times (t) during the exponential and stationary phases of *L. plantarum* growth.

$$\frac{dX}{dt} = \mu_{max}\left(1 - \frac{X}{X_{max}}\right)X \quad (4)$$

$$X\,X = \frac{X_0 \exp(\mu_{max}t)}{1-\left[\left(\frac{X_0}{X_{max}}\right)(1-exp(\mu_{max}t))\right]} \quad (5)$$

7.2.10.2. Modified Gompertz model for lactic acid formation

The LA production data generated from the 5 L scale-up process experiment was used to fit the modified Gompertz model by using the least squares method (CurveExpert V1.5.5, MyBiosource, Inc., USA). The model revealed the lag time (t_L), maximum LA production rate ($r_{p,m}$), and the maximum potential LA concentration (P_m) as shown in Eq. (6).

$$P = P_m\,.exp\left\{-exp\left[\frac{r_{p,m}.exp(1)}{P_m}\right].(t_L - t) + 1\right\} \quad (6)$$

where P is LA concentration (g/L), P_m is potential maximum LA concentration (g/L), $r_{p,m}$ is maximum LA production rate (g/L/h) and t_L is the time from the beginning of fermentation to exponential LA production (hr).

7.2.11. Elemental analysis of dairy wastewater and MDWW-SSF process effluent

For the elemental analysis, the DWW sample and LA fermentation effluent (before and after fermentation) were prepared by filtering through a 0.45 μm nylon filter syringe prior to analysis. The concentrations of Ca, K, Na, Mg, P and S were tested using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES), following the standard methods for the examination of water and wastewater (APHA, 1998a). The Cu, Fe, Mn and Zn concentrations were analysed using the Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) according to standard water and wastewater examination protocols (APHA,

1998b). The total organic carbon (TOC) was measured by oxidizing the wastewater samples using high temperature oxidation with a platinum catalyst, generating carbon dioxide that was then detected by a Non-Dispersive Infrared (NDIR) detector. The total nitrogen was assessed according to the Kjeldahl method, based on the accumulative concentration of Kjeldahl nitrogen, nitrates and nitrites (Goyal et al., 2022).

7.2.12. Feed analysis of the MDWW-SSF process solid biomass

The moisture content was quantified by heating the sample overnight in an oven set at 75°C. The ash content was determined gravimetrically using residues that remain after incineration at 450°C. The compositional analysis of the fermentation effluent was determined according to the Van Soest method (Van Soest and McQueen, 1973). The neutral detergent fiber (NDF) analysis was achieved by boiling the sample in a neutral detergent fiber solution (pH 7.0). The NDF portion consisted of cellulose, hemicellulose and lignin. The acid detergent fiber (ADF) component was carried out by boiling the sample in an acid detergent fiber solution containing concentrated H_2SO_4 and cetyl trimethylammonium bromide to remove the soluble portion with the resultant components being cellulose and lignin. According to the Official Methods of Analysis for Fat (Crude) or Ether Extract in Animal Feed (1990), the dried, ground sample was extracted with diethyl ether to dissolve fats, oils, pigments and other fat-soluble substances. The ether was then evaporated from the fat solution at low temperatures and the resulting residue known as the ether extract or crude fat was weighed. For the elemental analysis, the solid MDWW-SSF residue was assessed for the Ca, K, Na, Mg, P, Cu, Fe, Mn and Zn concentration using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES) according to the Hunters methods for plant nutrients determination (Hunter, 1975). The total nitrogen was evaluated using sample combustion coupled with thermal conductivity (Leco TruMac analysers).

7.3. Results and discussion

7.3.1. Effects of different buffer agents and MnO nanoparticle-assisted strategies for enhanced lactic acid production and sugar utilization

From the buffer screening assessment in the sDWW-SSF medium, the sDWW-SSF$_{CaCO3(15)}$ process produced the highest LA concentration of 18.61 g/L, followed by sDWW-SSF$_{Na2HPO4.12H2O}$ (15.01 g/L), sDWW-SSF$_{KH2PO4}$ (11.90 g/L) and sDWW-SSF$_{NaHCO3}$ (10.19 g/L) (Table S7.1). Alongside the highest LA concentration, the sDWW-SSF$_{CaCO3(15)}$ experiment produced the highest reducing sugar utilization of 27.88% g/L, followed by sDWW-SSF$_{Na2HPO4.12H2O}$ (19.34%), sDWW-SSF$_{KH2PO4}$ (16.49%) and sDWW-SSF$_{NaHCO3}$ (14.48%). The efficacy of these buffering agents can be attributed to potential pH-dependent effects and kinetic reactions. At a fermentation medium pH of 4.8, such conditions can enhance the dissolution of $CaCO_3$, leading to the formation of calcium lactate (Seifan et al., 2017). Moreover, the rate of ion release may influence the duration required for pH adjustment and stabilization throughout the fermentation process. This, in turn, could improve LA production and overall process efficiency. Regarding the reduced LA concentration observed with sDWW-SSF$_{Na2HPO4.12H2O}$ and sDWW-SSF$_{NaHCO3}$ processes, the presence of Na^+ ions may induce osmotic stress on the microbial cells, leading to intracellular pH disruptions and ion imbalances. These conditions can negatively affect enzymatic activities and cellular functions, consequently lowering LA production (Zhu et al., 2007). In a similar context, the study by Zhu et al. (2007), showed that the use of Ca^{2+} as a base counter ion for pH adjustment yielded higher LA formation (90 g/L) compared to Na^+ (75.1 g/L) when added to the medium during LA production using engineered *Escherichia coli* strains.

In addition, the effect of the MnO nanoparticle-assisted fermentation alongside each buffer agent was also investigated. It was found that the sDWW-SSF$_{CaCO3(15)+MnO}$ produced the

highest LA concentration of 19.25 g/L, followed by sDWW-SSF$_{Na2HPO4.12H2O+MnO}$ (14.22 g/L), sDWW-SSF$_{NaHCO3+MnO}$ (13.23 g/L) and sDWW-SSF$_{KH2PO4+MnO}$ (11.05 g/L) (Table S7.1). Interestingly, the addition of $CaCO_3$ or $NaHCO_3$, respectively, together with the MnO nanoparticle slightly increased LA concentration when compared to the presence of $CaCO_3$ or $NaHCO_3$ only. On the other hand, despite the high LA concentrations obtained with the sole inclusion of $Na_2HPO_4.12H_2O$ (15.01 g/L LA), its combination with MnO nanoparticles resulted in a reduced LA concentration of 14.22 g/L. The same was observed for the combination of KH_2PO_4 and MnO nanoparticle (11.05 g/L) when compared to KH_2PO_4 only (11.90 g/L). The sDWW-SSF$_{CaCO3(15)+MnO}$ experiment produced the highest reducing sugar utilization of 28.92% g/L, followed by sDWW-SSF$_{Na2HPO4.12H2O+MnO}$ (17.91%), sDWW-SSF$_{NaHCO3+MnO}$ (16.70%) and sDWW-SSF$_{KH2PO4+MnO}$ (15.36%) during LA production. Given the increased LA concentration and efficient utilization of reducing sugars, $CaCO_3$ was selected as the buffering agent for subsequent optimization, scale-up and kinetic analyses. At this stage, comprehensive laboratory evaluations revealed that incorporating $CaCO_3$ as a buffer agent during the SSF bioprocess posed challenges for enzymatic glucose measurement using the Megazyme glucose assay kit (K-GLUC) (©Megazyme, Wicklow, Ireland). Specifically, these challenges arise when the concentrations of calcium and lactate ions exceeds their solubility limit, leading to the precipitation of solid calcium lactate complexes (Alhamad and Miskimins, 2022). The solubility limit is influenced by several factors including temperature, pressure, pH, and specific concentrations of calcium and lactate ions present in the solution (Alhamad and Miskimins, 2022). This precipitation reaction can interfere with enzyme specific glucose assays that rely on the activity of glucose oxidase or hexokinase enzymes, since calcium ions inhibit the activity of these enzymes, leading to irregularities in the glucose measurements. Moreover, production of calcium lactate increases the pH of the fermentation medium above the threshold for glucose oxidase function (optimal

activity pH 5.5) leading to enzyme inhibition or malfunction (Tsuge et al, 1975). Furthermore, calcium lactate precipitates in the reaction mixture may interfere with the enzymatic reaction by physically blocking enzyme-substrate interactions and reducing the availability of reactants, including glucose, to the enzymes. Apart from the above reaction effects, the calcium lactate precipitates may scatter light, which in turn interferes with spectrophotometric measurements commonly used in enzymatic glucose assays. These interferences can lead to reduced enzymatic activity and inaccurate glucose readings. As a result, the 3.5-dintrosalicylic acid (DNS) method, an assay commonly used in lignocellulosic bioprocessing research to quantify the total reducing sugars (glucose, fructose, xylose, sucrose amongst others) was adopted for subsequent quantification of the fermentable sugars in the media. Compared to the enzymatic glucose kit, the DNS colorimetric method was not influenced by the pH of the medium and did not cause calcium lactate precipitation since the DNS method relies on chemical reactions with the reducing sugars, specifically the reduction of 3,5-dinitrosalicylic acid (DNS), whereas the Megazyme glucose kit involves enzymatic reactions that are dependent on specific glucose oxidase and peroxidase enzymes. To standardize the sugar analysis, the DNS method was maintained throughout the research for experimentation containing $CaCO_3$.

7.3.2. Evaluating the effect of various physicochemical enhancement parameters under the SSF bioprocess: buffer agent, pH changes and micronutrient supplementation.

Fig. 7.1A and 1B illustrates the progression of LA production and the utilization of reducing sugars, respectively, under the SSF bioprocess across various physicochemical conditions tested. These conditions include the singular effect of the $CaCO_3$ buffering agent, the addition of micronutrients ($MgSO_4$ and $MnSO_4$ or MnO nanoparticles), alterations in pH levels (pH 4.8 and pH 5.5), or a combination thereof (Table S7.2). The findings indicate that the

sDWW-SSF$_{CaCO3(30)+MnO,\ pH\ 5.5}$ process yielded the highest LA concentration of 31.12 g/L. This was closely followed by sDWW-SSF$_{CaCO3(30)}$ (30.76 g/L), sDWW-SSF$_{CaCO3,\ pH\ 5.5}$ (28.69 g/L) and sDWW-SSF$_{CaCO3+Mn2+,\ Mg2+,\ pH\ 5.5}$ (26.94 g/L). Lower LA concentrations were observed for sDWW-SSF$_{pH\ 5.5}$ at 17.50 g/L, sDWW-SSF$_{MnO}$ at 15.43 g/L, sDWW-SSF$_{Mn2+\ and\ Mg2+}$ at 14.76 g/L and sDWW-SSF$_{control}$ at 13.85 g/L.

The sDWW-SSF$_{control}$ experiment was carried out as a control with optimum conditions determined in our previous study (David et al., 2022) and was maintained as the baseline for further experimentation. The sDWW-SSF$_{control}$ gave a LA concentration of 13.85 g/L and corresponding reducing sugar utilization up to 25.13%. In comparison, sDWW-SSF$_{Mn2+\ and\ Mg2+}$, sDWW-SSF$_{MnO}$, and sDWW-SSF$_{pH\ 5.5}$ evaluated the singular effect of Mg^{2+} and Mn^{2+} supplementation, MnO nanoparticle inclusion and pH change (pH 5.5), respectively in the sDWW-SSF bioprocess. The LA concentration obtained for the sDWW-SSF$_{Mn2+\ and\ Mg2+}$, sDWW-SSF$_{MnO}$, and sDWW- SSF$_{pH\ 5.5}$ processes were 14.76 g/L, 15.43 g/L and 17.50 g/L, respectively. The increase in LA concentrations for the sDWW-SSF$_{Mn2+\ and\ Mg2+}$ (6.17% increase) and sDWW-SSF$_{MnO}$ (10.24% increase) process in comparison to sDWW-SSF$_{control}$ may be attributed to the positive influence of micronutrient supplementation that acts as cofactors to enhance the catalytic activity of enzymes involved in biosynthetic pathways (Yu et al., 2008, Lew et al., 2013). In addition, the 20.86% increase in LA concentration for sDWW-SSF$_{pH\ 5.5}$ in comparison to sDWW-SSF$_{control}$ may be due to the pH change from 4.8 to 5.5. This change may result in a more favorable environment for *L. plantarum* metabolism since it was closer to the upper limit of the reported optimum range (pH 4.5 to 6.5) for LA production (Anagnostopoulou et al., 2022). It is also noted that the reducing sugar available for microbial metabolism in sDWW-SSF$_{control}$, sDWW-SSF$_{Mn2+\ and\ Mg2+}$, sDWW-SSF$_{MnO}$, and sDWW- SSF$_{pH\ 5.5}$ were 88.89 g/L, 104.99 g/L, 91.58 g/L and 90.91 g/L, respectively. Even with the high reducing sugar concentration within the fermentation medium, the sugar

utilized by the microorganism translated up to 25.13% only, for the duration of the process. This may be due to the high saccharification efficiency of the Cellic Ctec 2 enzyme that led to the conversion of cellulose to fermentable sugar at a rate that is faster than microbial consumption, thus causing sugar accumulation in the medium (Aguilar-Reynosa et al., 2017). In addition, the high reducing sugar available post-fermentation may not necessarily indicate high glucose concentration since the reducing sugar yield incorporates various monosaccharides alongside some disaccharides, oligosaccharides and polysaccharides. For instance, the xylose concentration was quantified as ~22.27 g/L of the total reducing sugar after fermentation.

Despite the marginally higher LA concentrations observed for sDWW-$SSF_{Mn2+ \text{ and } Mg2+}$, sDWW-$SSF_{MnO}$, and sDWW- $SSF_{pH\ 5.5}$ processes, the production of LA in the medium leads to a decrease in pH, which induces end-product inhibition (Othman et al., 2017). This phenomenon occurs as the product itself acidifies the culture medium, adversely affecting the viability of *L. plantarum* cells and the regulation of the microbial culture (Karnaouri et al., 2020). Typically, the yield and productivity of LA production are influenced by a pH range of 4.5 to 6.5, with many LABs unable to maintain optimal metabolic activity when the pH falls below 4 (Abedi and Hashemi, 2020; Anagnostopoulou et al., 2022). This was illustrated by the pH range of 3.48-3.57 recorded for the sDWW-$SSF_{control}$, sDWW-$SSF_{Mn2+ \text{ and } Mg2+}$, sDWW-$SSF_{MnO}$, and sDWW- $SSF_{pH\ 5.5}$ bioprocesses after fermentation. Consequently, this led to reduced consumption of fermentable sugars and a decrease in LA production.

Interestingly, the sDWW-$SSF_{CaCO3(30)}$ process involving the sole addition of $CaCO_3$ to the optimized medium at a pH of 4.8, achieved a peak LA concentration of 30.76 g/L at 48 hours, with reducing sugar utilization ranging from 26.90% to 45.48%. Notably, the sDWW-$SSF_{CaCO3(30)}$ process exhibited a 2.22-fold increase in LA concentration with the exclusive

addition of $CaCO_3$ compared to the sDWW-$SSF_{control}$. Introducing $CaCO_3$ into the fermentation medium neutralizes its pH by forming a basic salt (calcium lactate) in solution (Karnaouri et al., 2020). This aligns the pH value closer to the optimal range for LA bacterial cell metabolism (pH 4.5-pH 6.5), thus maintaining cell survival and maximizing LA yield (Wang et al., 2014; Anagnostopoulou et al., 2022). The pH range for sDWW-$SSF_{CaCO3(30)}$ was maintained between pH 5.5 and pH 5.83. Similarly, sDWW-$SSF_{CaCO3+Mn2+,\ Mg2+,\ pH\ 5.5}$ observed a 26.94 g/L LA concentration with a 28.52% to 43.27% reducing sugar utilization after 48 h with Mg^{2+}, Mn^{2+} and $CaCO_3$ supplementation at pH 5.5. The sDWW-$SSF_{CaCO3+Mn2+,\ Mg2+,\ pH\ 5.5}$ bioprocess resulted in a 45.21% increase in LA concentration compared to the sDWW-$SSF_{Mn2+\ and\ Mg2+}$ process, which involved the supplementation of only Mg^{2+} and Mn^{2+} at a pH of 4.8. This revealed that the addition of $CaCO_3$ was directly related to the improvement in LA concentration. Nevertheless, when sDWW-$SSF_{CaCO3+Mn2+,\ Mg2+,\ pH\ 5.5}$ process was compared to sDWW-$SSF_{CaCO3(30)}$ (supplemented with $CaCO_3$), it resulted to a 12.42% lower LA concentration. The inclusion of these ions may not necessarily impact the yield since the CSL and dairy wastewater contains residual amounts of Mg^{2+} and Mn^{2+} that may be sufficient for catalysis in enzymatic reactions within the carbohydrate metabolism. In the same vein, the data for sDWW-$SSF_{CaCO3(30)+MnO,\ pH\ 5.5}$ showing the interactive effect of MnO nanoparticles and $CaCO_3$ at pH 5.5 gave a LA concentration of 31.12 g/L at 36 h, with a reducing sugar utilization ranging from 28.97 to 46.27%. The sDWW-$SSF_{CaCO3(30)+MnO,\ pH\ 5.5}$ process observed a 50.24% incline in LA in comparison to sDWW-SSF_{MnO} containing the MnO nanoparticle only, underscoring the significance of $CaCO_3$ in the bioprocess. On the other hand, sDWW-$SSF_{CaCO3(30)+MnO,\ pH\ 5.5}$ process gave comparable results to sDWW-$SSF_{CaCO3(30)}$ which contained $CaCO_3$ only at a pH of 4.8 with a 0.81% increase. However, it is important to note that sDWW-$SSF_{CaCO3(30)}$ obtained its maximum LA concentration at 48 h while sDWW-$SSF_{CaCO3(30)+MnO,\ pH\ 5.5}$ observed its

maximum concentration at 36 h. This indicates that the nanoparticle inclusion enhanced the production rate of the LA by potentially reducing the process time. According to Sanusi et al. (2020), nanoparticles have the ability to alter the rate of reaction due to its chemical stability, catalytic properties, surface-to-volume ratio and specificity. In addition, sDWW-SSF$_{CaCO3,\ pH\ 5.5}$ produced a LA concentration of 28.69 g/L after 48 h with a 24.50 to 43.05% reducing sugar utilization within the fermentation medium containing $CaCO_3$ at pH 5.5. The higher pH of 5.5 at the beginning of fermentation increased the initial cellular metabolism of *L. plantarum*, thus increasing yield and productivity of the microorganism. However, the sDWW-SSF$_{CaCO3,\ pH\ 5.5}$ process observed a marginally lower LA concentration of 28.69 g/L compared to sDWW-SSF$_{CaCO3(30)}$ (30.76 g/L) and sDWW-SSF$_{CaCO3(30)+MnO\ nanoparticle,\ pH\ 5.5}$ (31.12 g/L), translating to a 6.73% and 7.81% decrease in LA, respectively. Even though the pH of 5.5 was within the optimal range for LA production, it is also the threshold pH for the cellulase-based Cellic CTec 2 enzyme during cellulose conversion to glucose (Abdulsattar et al., 2020), thus reducing the stability of the enzyme since LA production causes pH variation during the bioprocess. The optimal pH range for the Cellic CTec2 enzyme is 5-5.5, with pH 4.8 or 5.0 being the most commonly reported in the literature for effective use (Abdulsattar et al., 2020). Given these findings, a pH of 4.8 creates a controlled environment favourable for LA production and enzymatic saccharification. Henceforth, to ensure the stability of the Cellic CTec2 enzyme and optimal LA production, a pH of 4.8 was consistently maintained throughout the study.

According to the findings, the sDWW-SSF$_{CaCO3(30)+MnO,\ pH\ 5.5}$ process yielded the highest LA concentration of 31.12 g/L, followed by sDWW-SSF$_{CaCO3(30)}$, which resulted in a LA concentration of 30.76 g/L. This represents a more than 2-fold increase in LA concentration. These results provided valuable insights into nutrient supplementation of waste-based LA fermentation medium, and the potential for reduced process times and costs. In light of the

high concentration of LA achieved for the sDWW-SSF$_{CaCO3(30)}$ and sDWW-SSF$_{CaCO3(30)+MnO,\ pH\ 5.5}$ bioprocesses, efforts were prompted towards an investigation into the PSSF approach. This evaluation aimed to compare the effectiveness of both different bioprocess types (SSF and PSSF), in terms of pH control, LA yield and sugar utilization.

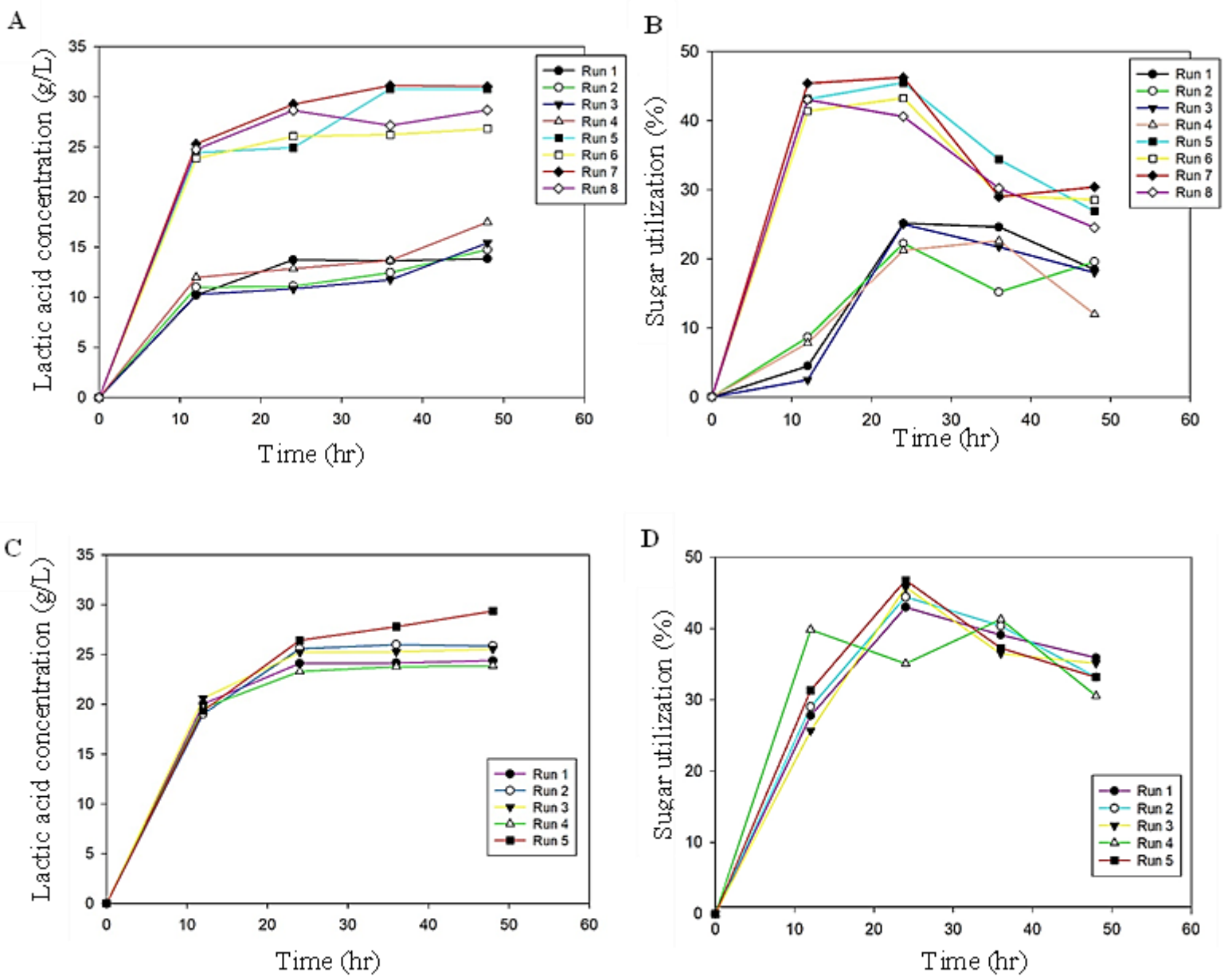

Fig. 7.1. Lactic acid production and sugar utilization of the modified sDWW-SSF (A, B) and sDWW-PSSF (C, D) bioprocesses, respectively.

7.3.3. Comparative assessment of $CaCO_3$ and/or nanoparticle-assisted lactic acid production using the PSSF bioprocess

Fig. 7.1C and 1D depicts the progression of LA concentration and reducing sugar utilization, respectively for the minimally supplemented DWW medium under the PSSF bioprocess (sDWW-PSSF). The prehydrolysis of the sDWW-$PSSF_{CaCO3}$ (containing $CaCO_3$) and sDWW-$PSSF_{CaCO3+MnO}$ (containing $CaCO_3$ and MnO nanoparticle) bioprocesses were carried out under standard conditions (10% SL, 10% enzyme, DWW citrate buffer, pH 4.8 for 24 h) and gave a reducing sugar concentration of 100. 88 g/L and 101.25 g/L, respectively. Thereafter, the sDWW-$PSSF_{CaCO3}$ process produced a LA concentration of 24.38 g/L with a corresponding sugar utilization up to 43% when 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* and 30 g/L $CaCO_3$ were added. The sDWW-$PSSF_{CaCO3+MnO}$ process containing 0.025 g/L MnO nanoparticle in addition to 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* and 30 g/L $CaCO_3$ gave LA concentration of 25.99 g/L and a corresponding sugar utilization up to 44.43%. The 6.19% increase in LA concentration for the sDWW-$PSSF_{CaCO3+MnO}$ when compared to the sDWW-$PSSF_{CaCO3}$ may be attributed to the improved bioactivity of microorganisms as a result of the presence of the MnO nanoparticle in the fermentation system (Kim et al., 2014). The sDWW-$PSSF_{PH\ with\ Tween\ 80,\ CaCO3}$ process evaluated the impact of Tween 80 to the standard prehydrolysis stage (Tween 80 omitted from fermentation stage) and resulted in a reducing sugar concentration of 102.18 g/L. The data for sDWW-$PSSF_{PH\ with\ Tween\ 80,\ CaCO3}$ illustrates negligible increase in reducing sugar recovery in comparison to the standard prehydrolysis stage that omits Tween 80 in the sDWW-$PSSF_{CaCO3}$ and sDWW-$PSSF_{CaCO3+MnO}$ bioprocesses. The fermentation process containing 25 g/L CSL, 10% *L. plantarum* and 30 g/L $CaCO_3$ for sDWW-$PSSF_{PH\ with\ Tween\ 80,\ CaCO3}$ process gave a LA concentration of 25.55 g/L with a corresponding sugar utilization of up to 45.79%. On the other hand, sDWW-$PSSF_{PH\ with\ MnO,\ CaCO3}$ evaluated the impact of MnO nanoparticle addition

on the standard prehydrolysis sugar release. The reducing sugar recovery was 103.26 g/L. The initiated fermentation process (25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* and 30 g/L $CaCO_3$) resulted in a LA concentration and sugar utilization of 23.86 g/L and up to 41.31%, respectively. Interestingly, even with the slightly higher reducing sugar concentration available after the prehydrolysis stage, the concentration of LA observed under the PSSF bioprocess was more than 15% lower than the LA concentrations observed for the SSF system. It is noted that the higher reducing sugar accumulation in PSSF processes may cause inhibition of the *Lactobacillus* culture, attributed to the sugar osmotic effects on the microbial cells, thus halting metabolic activities towards LA fermentation (Liu et al., 2014; Sewsynker-Sukai and Gueguim Kana, 2018). Furthermore, the cellulase-based enzyme, Cellic CTec2 is recognized for its high performance and robustness, demonstrating efficient saccharification capabilities. This may lead to a cellulose conversion rate that is faster than microbial consumption, causing sugar accumulation in the medium (Aguilar-Reynosa et al., 2017)

To investigate the combinatory effect of Tween 80 and MnO nanoparticle supplementation on the sugar recovery in the prehydrolysis stage, sDWW-PSSF$_{\text{PH with MnO+Tween 80, CaCO3}}$ process observed a reducing sugar release of 117.31 g/L, translating to a more than 11.98% increase in comparison to the sDWW-PSSF$_{\text{CaCO3}}$, sDWW-PSSF$_{\text{CaCO3+MnO nanoparticle}}$, sDWW-PSSF$_{\text{PH with Tween 80, CaCO3}}$ and sDWW-PSSF$_{\text{PH with MnO +Tween 80, CaCO3}}$ bioprocesses. Tween 80 is a non-ionic surfactant that can improve the solubility and dispersion of hydrophobic lignin and cellulose (Taoka et al., 2011). It forms micelles around hydrophobic areas of the substrate, preventing enzyme inhibition caused by lignin adsorption and facilitating enzyme-substrate interactions. Moreover, the presence of Tween 80 can stabilize enzymes by forming a protective layer around them, shielding them from denaturation and proteolytic degradation (Zhang et al., 2018). This stabilizing effect ensures prolonged enzymatic activity, leading to enhanced LCB

conversion, thus higher sugar recovery. Furthermore, MnO nanoparticles can serve as enzyme mimetics and provide catalytic sites for enzymatic reactions (Sanusi et al., 2020). They act as a support structure for the enzymes, allowing for increased surface area and better access to the substrate, thus promoting higher enzyme loading and activity. The combination of both the Tween 80 and MnO nanoparticle inclusion was shown to improve the hydrolytic stage of the PSSF reaction. This may be attributed to the synergistic effect of the singular characteristics of these components. Overall, the action of MnO nanoparticles and Tween 80 in the standard enzymatic hydrolysis of the CCW offers a promising approach to enhance the efficiency and sustainability of bioconversion processes. The fermentation process (25 g/L CSL, 10% *L. plantarum* and 30 g/L $CaCO_3$) produced a LA concentration of 29.35 g/L and sugar utilization up to 46.77%.

Based on the outcomes of the present study, the SSF bioprocess (sDWW-$SSF_{CaCO3(30)+MnO,\ pH\ 5.5}$) achieved the highest LA concentration of 31.12 g/L, while the PSSF system (sDWW-$PSSF_{PH\ with\ MnO+Tween\ 80,\ CaCO3}$) produced 29.35 g/L of LA. The sDWW-$SSF_{CaCO3(30)+MnO,\ pH\ 5.5}$ bioprocess translates a 5.69 % higher LA concentration using the SSF process when compared to the PSSF method. Furthermore, the PSSF hydrolysis stage displayed no significant variations when compared with the SSF process. More importantly, the SSF system has demonstrated its attractiveness by eradicating costs associated with separate reactors, reducing time consumption by 33% and minimizing energy intensive enzymatic hydrolysis steps. This finding was consistent with our previous study that comparatively evaluated three different bioprocess types (SSF, SHF and PSSF) for bioethanol production from corn cobs (David et al., 2020). In response to these findings, the sDWW-$SSF_{CaCO3+MnO,\ pH\ 5.5}$ process was further subjected to scale-up criteria and kinetic studies. Although a pH of 5.5 was initially utilized within this system (sDWW-$SSF_{CaCO3(30)+MnO,\ pH\ 5.5}$), pH 4.8 will be carried forward throughout scale-up studies to maintain Cellic CTec 2 enzyme stability.

Henceforth, the optimized process will be denoted as the MDWW-SSF process during scale up and kinetic studies.

7.3.4. Bioprocess scale-up

7.3.4.1. Effects of varying scale up parameters on the lactic acid process performance at 0.5 L

The MDWW-SSF process was subjected to scale-up evaluation in a 2 L bioreactor with a working volume of 0.5 L over a period of 48 h. The experimental profiles for LA concentration and reducing sugar consumption in the scale-up fermentation reaction are illustrated in Fig. 7.2A and 2B, respectively, with constant impeller tip speed (V_{tip}) and power input per unit volume (P/V) as scale-up criteria. As shown in Fig. 7.2A, the LA concentration increased from 1.82 g/L to 27.49 g/L after 30 h when exposed to 58 rpm based on the constant V_{tip} parameter. Thus showing a decrease in LA concentration of 11.66% in comparison to the flask scale reaction ($sDWW\text{-}SSF_{CaCO3(30)+MnO,\ pH\ 5.5}$=31.12 g/L). The impeller tip speed of 58 rpm suggests a low agitation rate that potentially compromises the mixing efficiency, resulting in inadequate distribution of nutrients, fermentable sugars and gases to the *L. plantarum* microorganisms throughout the fermentation medium (Pérez et al., 2018). This can lead to localized concentration gradients with limited mass and heat transfer rates, negatively impacting microbial growth and LA production. The reduced mixing efficiency may also result in incomplete substrate utilization as shown by the reducing sugar utilization ranging between 19.39% to 42.15%, leading to suboptimal yields compared to the flask scale process. Conversely, the LA concentration increased from 1.10 g/L to 33.64 g/L after 18 h when exposed to an agitation speed of 74 rpm, determined according to the constant P/V criterion. This indicates a 7.49% increase in LA concentration compared to the corresponding flask scale reaction ($sDWW\text{-}SSF_{CaCO3(30)+MnO,\ pH\ 5.5}$=31.12 g/L). The enhanced

agitation system of the bioreactor can promote higher mixing efficiency, enhanced mass transfer, and improved nutrient availability, thereby positively influencing microbial growth and LA production. The higher power consumption may also lead to better substrate utilization and increased productivity, resulting in higher LA yields. More so, the optimum LA yield was reached in a 40% faster time for the constant P/V criterion process (corresponding to 18 hr) in comparison to the constant V_{tip} system (30 hr). According to Table S7.5, the hydrodynamic parameter, scale of turbulence (λ) was computed as 1.96 x 10^{-4} m and 1.64 x 10^{-4} m for the constant V_{tip} and constant P/V, respectively. The λ values show the size of Kolmogorov eddy that represents the smallest scale of turbulent motion in a fluid flow and plays a crucial role in the energy cascade of turbulence (Trush et al., 2020). The λ of 1.64 x 10^{-4} m for the constant P/V is lower in comparison to the constant V_{tip} (1.96 x 10^{-4} m) and is an indirectly proportional indicator of the fluid dynamics and turbulence of the reaction process. More specifically, the smaller the eddy size, the higher the turbulence intensity and the faster the energy dissipation (Cimarelli and De Angelis, 2011). The presence of Kolmogorov eddies is essential for efficient mixing and mass transfer. The small eddy sizes lead to intense turbulence, which ensures uniform distribution of nutrients, gases and microorganisms in the bioreactor, promoting higher LA production rates (Bujalski et al., 2002). However, it is crucial to note that while turbulence and Kolmogorov eddies are beneficial for mixing and mass transfer, excessive turbulence leads to shear stress which can be detrimental to the viability of the microbial cells. This entails physical damage to the cell membrane, compromised cell integrity and intracellular leakage, resulting in reduced metabolic activity and impaired growth rates (Esperanca et al., 2020). It also elicits stress responses that shift cellular resources away from metabolic pathways essential for LA production. In fact, when the Kolmogorov eddy size is equal to or smaller than the cell diameter, the flow lines pattern could shear microbial cells (Deniz et al., 2015). Although,

excess shear stress can greatly impact cell viability, a certain degree of shear rate is necessary to achieve sufficient transfer of materials and energy within the bioreactor. Therefore, it is essential to strike a balance between achieving efficient mixing through turbulence and avoiding high shear stresses that may impact cell viability and LA productivity. Furthermore, the pumping capacity (V_p) was established as 1.11 x 10^{-4} m^3/s and 1.40 x 10^{-4} m^3/s for the constant V_{tip} and constant P/V, respectively. The V_p refers to the ability of the pump system to circulate and mix the fermentation medium effectively within the bioreactor, directly influencing its homogeneity. The higher V_p (1.40 x 10^{-4} m^3/s) for the constant P/V exhibits increased circulation and mixing efficiency within the fermentation medium. This ensures adequate nutrient, oxygen, and temperature distribution to the *L. plantarum* cells to promote uniform growth and metabolism, ultimately contributing to higher yields and product quality in LA bioreactor processes. Another indication of enhanced mixing efficiency is the circulation time, which refers to the time it takes for the fermentation medium to complete one full cycle of circulation within the bioreactor. Based on the results, t_c was lower for the constant P/V (3.57 s) when compared to the constant V_{tip} (4.51 s) criteria. The shorter t_c led to more frequent and intense mixing, minimizing concentration gradients and maintaining uniform conditions throughout the fermentation medium for optimal LAB function. It is also worth noting that the MnO nanoparticle has the potential to positively bond with the carbon source to form a nano-sugar composite that enhances cell to sugar interactions for efficient sugar utilization, cellular growth, and metabolic activities (Sanusi et al., 2020). The constant P/V mixing regime promotes uniform dispersion of the nano-sugar composite within the bioreactor that ensures higher conversion rates and more efficient use of substrates for improved productivity and yield. Both these outcomes can be validated by the higher LA yield (33.64 g/L) and shorter production time (18 hr) when subjected to the constant P/V parameter (27.49 g/L LA, 30 hr). As a result of these aforementioned parameters, significant

insight pertaining to the mixing efficiency and process performance has informed the decisions to further evaluate constant P/V regime at a larger scale.

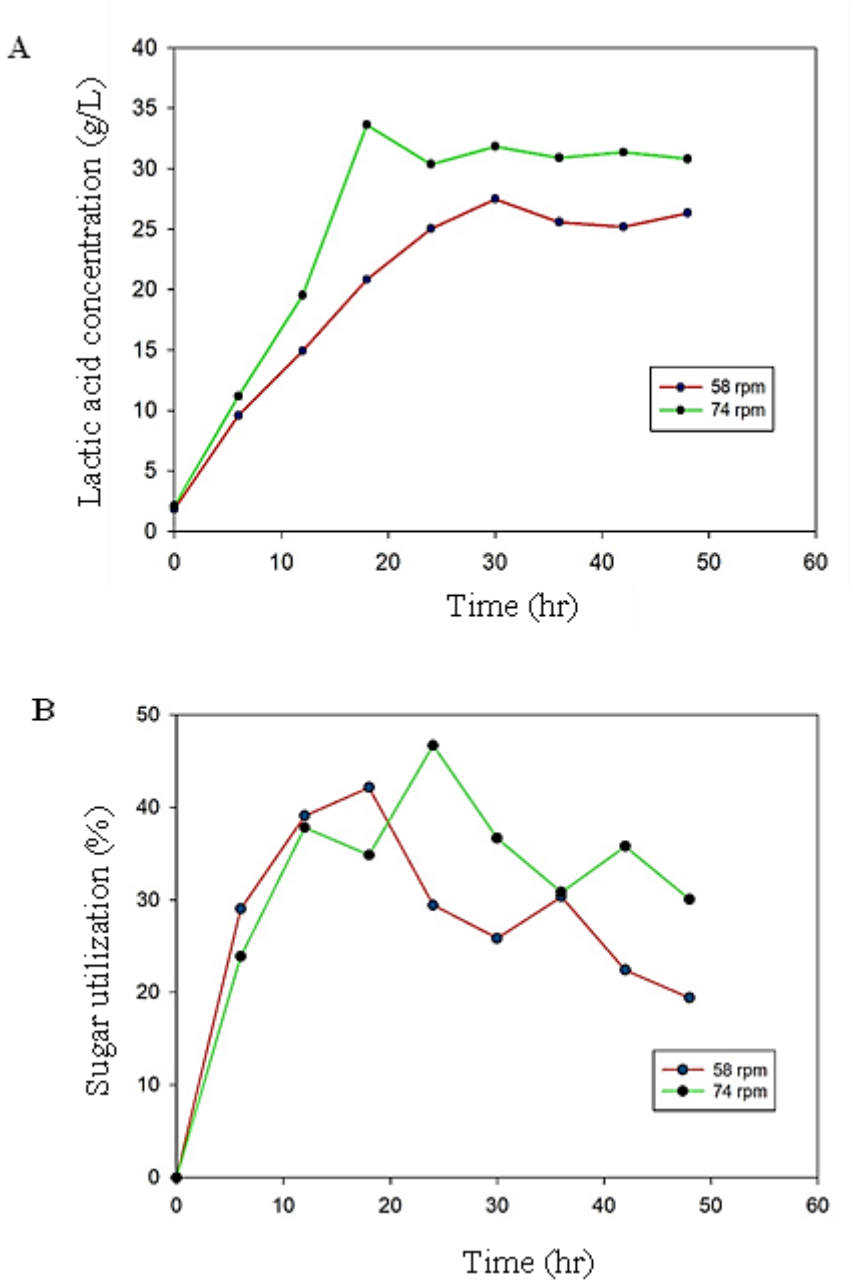

Fig. 7.2. Lactic acid production (A) and sugar utilization (B) of the 0.5 L MDWW-SSF bioprocess scale-up.

7.3.4.2. The effects of constant P/V on the lactic acid process performance at 5 L

The optimized bioprocess was carried out in a 13L bioreaction with a working volume of 5L at 54 rpm over a period of 24 h. The experimental LA concentration and sugar utilization of 31.43 g/L and 40.98%, respectively, were observed after 18 h under the constant P/V scale-up criteria (Fig. 7.3A and 3C). The LA concentration and sugar utilization obtained for the MDWW-SSF process at the 5 L scale slightly decreased by 6.57% and 12.23%, respectively, in comparison to it corresponding 0.5 L reaction process in the same time period (18 h). The λ values were recorded as 1.53 x 10^{-4} m for the constant P/V at 5 L and 1.64 x 10^{-4} m for the constant P/V at 0.5 L. The lower λ value for the constant P/V at 5 L (1.53 x 10^{-4} m) indicates a smaller eddy size with a higher turbulence intensity and faster energy dissipation (Cimarelli and De Angelis, 2011). In addition, the V_p for the constant P/V at 5 L was depicted as 2.23 x 10^{-4} m^3/s in comparison to the constant P/V at 0.5 L (1.40 x 10^{-4} m^3/s). The higher V_p demonstrates high circulation and mixing within the fermentation medium. Despite the low λ and high V_p for enhanced mixing efficiency, the slight decrease of 6.57% in the LA concentration may potentially be attributed to the partial cell shearing that reduced metabolic activity in the bioprocess due to the turbulence. In another instance, there was an 84.06% higher t_c when subjecting constant P/V (22.4 s) criterion at 5 L compared to the constant P/V (3.57 s) at 0.5 L. This implies that the MDWW-SSF process at 5 L completed one full cycle of circulation within the bioreactor after 22.4 s. The higher t_c may lead to reduced frequency and mixing, thus decreasing conversion rates and LA yield. This parameter value corresponds directly to the revolutions per second (n), where the n value is 0.90 rps for the constant P/V at 5 L while the constant P/V at 0.5 L shows an n value of 1.23 rps. The lower n value denotes a slower rotational speed of the impeller resulting in reduced mixing.

7.3.5. Kinetic modelling

7.3.5.1. Kinetic modelling of L. plantarum cell growth for the MDWW-SSF process at 5 L

The kinetics of *L. plantarum* cell growth was evaluated using the logistic model (Table 7.1). The evolution of the microbial cell growth was assessed over a period of 24 h using the optimized process conditions for the MDWW-SSF system at 5 L scale under the constant P/V mixing criterion (Fig. 7.3B). A maximum biomass concentration of 7.29 g/L was observed. The exponential phase of the microorganism was represented between 2 h-24 h. This increment in the *L. plantarum* cell growth corresponds to reducing sugar utilization up to 43.55% (Fig. 7.3C).

The *L. plantarum* cell biomass concentration over a 24-h period (Fig. 7.3B) was used to fit the logistic model with a high correlation coefficient (R^2) of 0.98. The maximum specific growth rate (μ_{max}) of 0.26 h^{-1} was obtained for the MDWW-SSF process at 5 L scale. Our previous study gave a μ_{max} of 0.35 h^{-1} for the sDWW-SSF process under microaerophilic conditions (David et al., 2022). The MDWW-SSF process exhibited a 28.57% decrease in the microbial growth rate when compared to the sDWW-SSF bioprocess. During the LA bioreaction in at 5 L scale, the process was subjected to batch fermentation, where no constituents were added or removed once the reaction was initiated. The closed batch fermentation process may have induced limited oxygen availability (anaerobic conditions) with a high concentration of the substrate and accumulation of glycolytic intermediates such as glucose-6-phosphate, glyceraldehyde-3-phosphate, and pyruvate amongst others. The absence of oxygen causes the *L. plantarum* to follow the glycolytic pathway that shifts metabolism towards the production of specific end products, such as LA instead of microbial cell growth. The microbial cells cannot rely on oxidative phosphorylation for ATP synthesis due to the absence of oxygen, thus, the glycolytic pathway becomes a primary route for ATP

production. This metabolic adaptation helps regenerate nicotinamide adenine dinucleotide (NAD^+) from NADH, allowing glycolysis to continue, and maintaining the redox balance under anaerobic conditions (Sano et al., 2020). Interestingly, the lower μ_{max} of 0.26 h^{-1} may be attributed to the rheology and hydrodynamic parameters of the bioreactor, demonstrating increased turbulence, high circulation and mixing that could result in partial cell shearing that reduces metabolic activity in the bioprocess.

In another instance, the maximum cell concentration (X_{max}) of 7.11 g/L was obtained for the MDWW-SSF fermentation process. The high X_{max} value may be attributed to the synergistic effect of additives, $CaCO_3$ and MnO nanoparticles in the established MDWW-SSF medium. $CaCO_3$ plays a key role in neutralizing the fermentation medium and maintaining its pH stability. During LA production, the pH of the medium decreases as a result of excessive acidification, thus, initiating an inhibitory impact on *L. plantarum* cellular metabolism (Othman et al., 2017). Usually, the optimal pH range for most LABs is between 4.5 to 6.5 and the addition of $CaCO_3$ acts as a buffer to maintain the pH within this favourable range (Wang et al., 2014; Anagnostopoulou et al., 2022). As LA accumulates in the medium, $CaCO_3$ reacts to form a basic calcium lactate salt and carbon dioxide gas (Wang et al., 2014). The alkaline nature of the medium supported robust *L. plantarum* microbial growth, promoting higher cell concentrations. Moreover, introducing the alkaline $CaCO_3$ created an environment less favourable for the growth of contaminating microorganisms. This is particularly important during the early stages of LA fermentation when the LABs are establishing purity and dominance in the culture. In addition, MnO nanoparticles provide unique physicochemical properties that enhance nutrient adsorption and activation by the LAB. Due to their nanoscale size, MnO nanoparticles have a significantly high surface area-to-volume ratio which provides more active sites for enhanced nutrient adsorption (Sanusi et al., 2020). The nano-nutrient composite improves microbial cell affinity for the fermentable

sugars and stimulates activation of nutrients. More so, MnO nanoparticles contain redox-active properties for electron transfer during metabolic processes in order to regulate intracellular redox balance (Archibald and Fridovich, 1981; Watanabe et al., 2012). Furthermore, it has the potential to mitigate increased stress tolerance due to its antioxidant properties and biocompatibility to LABs (Archibald and Fridovich, 1981; Watanabe et al., 2012). This reduces significant toxicity and oxidation risks on account of reactive oxygen species (ROS). Noting the abovementioned properties, MnO nanoparticles facilitate increased cell viability, efficient cellular growth, and metabolic activities (Ban and Paul, 2014, Sanusi et al., 2020). Liu et al. (2010) studied the effects of five alternative nitrogen sources when replacing yeast extract, peptone, and beef extract in the MRS medium for LA production by thermophile *L. plantarum* As.1.3. The results indicated that the malt sprout (MS) and corn steep liquor (CSL) optimized MRS medium showed significant effects on the μ_{max} (1.09 h^{-1}) when compared to standard MRS medium (0.64 h^{-1}) (Liu et al., 2010). Interestingly, the X_{max} for the malt sprout (MS) and corn steep liquor (CSL) optimized MRS (10.14 g/L) was slightly lower in comparison to the standard MRS media (14.19 g/L) (Liu et al., 2010). The optimized medium offered a high μ_{max}, ascribed to easily assimilation of the CSL and MS, whereas the MRS medium generated a high X_{max}, due to a significant concentration of nitrogen present in the MRS derived constituents.

7.3.5.2. Kinetic modelling of lactic acid production for the MDWW-SSF process at 5 L

The LA production represented over a duration of 24 h for the MDWW-SSF process at 5 L using the constant P/V regime is illustrated in Fig. 7.3A. A rapid progression in LA concentration from 1.90 g/L to 31.43 g/L occurred within 0 h to 18 h for the MDWW-SSF bioprocess. This was in line with the short lag phase and subsequent rapid exponential phase of the *L. plantarum* cell growth, suggesting that LA was being produced almost

instantaneously. The increase in LA concentration took place during the exponential growth phase of microbial cells and thereafter plateaued after 18 h.

The resultant experimental data from the production of LA (Fig. 7.3A) was used to fit the modified Gompertz model with a high R^2 value of 0.99 for the MDWW-SSF fermentation process. A short lag time (t_L) of 0.62 h was obtained for the MDWW-SSF process. The short lag times may be accounted for by the addition of CSL in the medium formulation since it contains nitrogen, amino acids, vitamins, minerals, and water-soluble carbohydrates, sufficient for *L. plantarum* metabolism. The rich source of nutrients available at the outset of the process is able to sustain the metabolic activity of the *L. plantarum*, while the enzyme hydrolyses the cellulose to fermentable sugars for microbial utilization. The cellulase-based enzyme, Cellic CTec2 used in the present study, is a high performing and robust enzyme that displays high saccharification efficiencies in comparison to traditional cellulase enzymes (Sewsynker-Sukai and Gueguim Kana, 2018). The rapid saccharification efficiency leads to high cellulose conversion within a short period of time, resulting in high sugar accumulation in the medium. This was evident by the reducing sugar utilization of 18.77% at the 2 h sampling point. Apart from nutrient availability, the introduction of $CaCO_3$ into the buffered system increased the pH within its optimal microbial function (pH 5.5-6.6), thereby establishing a conducive environment for the *L. plantarum* growth and product formation. The short lag time implied that microbial metabolism and product formation were highly dependent on the availability of fermentable sugars, nutrients, and pH in the initial fermentation medium.

The modified Gompertz model showed a high maximum potential LA concentration (P_m) of 35.11 g/L. During the SSF bioprocess, the high performing Cellic CTec2 enzyme rapidly converted cellulose to fermentable sugar at a rate that was faster than microbial consumption, thus causing sugar accumulation in the medium. This is substantiated by the increase in

reducing sugar of 58.82% (increase from 7.73 g/L to 18.77 g/L) after only 2 hours of saccharification in the control (uninoculated sample). It is noted that high reducing sugar levels, with glucose as its main contributing sugar in the medium promotes increased glycolytic rates that encourage the lactate dehydrogenase reaction, resulting in enhanced lactate production (Sano et al., 2020). Moreover, the rapid fermentation rates can cause the accumulation of NADH in the cells. In such conditions, the Embden-Meyerhof Parnas (EMB) glycolytic pathway may be favoured as it provides an opportunity for the regeneration of NAD^+, which is critical for maintaining redox balance and allowing glycolysis to continue (Sano et al., 2020). In addition, the presence of elements such as iron, zinc and magnesium in the fermentation medium can stimulate the EMB pathway since these are co-factors for glycolytic enzymes and contribute to the regulation of glycolysis. The sufficient availability of these components allows LABs to adapt in a changing environment and optimize their metabolic pathways for efficient LA production. The mechanism of the EMB reactions aims to produce ATP for the phosphorylation steps in the glycolytic pathway to drive microbial metabolism forward. During this process oxidized NAD^+ is consumed in the glyceraldehyde triphosphate dehydrogenase (GAPDH) reaction to produce pyruvate (Sano et al., 2020). Subsequently, the microorganism converts pyruvate to lactate by the lactate dehydrogenase (LDH) reaction, while simultaneously regenerating NAD^+ (Sano et al., 2020). The regeneration of the NAD^+ is imperative in maintaining the glycolytic flux.

The observed maximum LA production rates ($r_{p.m}$) reached 1.79 g/L/h in the MDWW-SSF process. This enhanced $r_{p.m}$ is likely attributable to the incorporation of MnO nanoparticles in the reaction medium, which could enhance enzymatic activity, regulate redox balance, improve substrate adsorption, and exhibit antioxidant properties alongside targeted nutrient delivery (Powell et al., 2010; Abdelsalam et al., 2016). Such improvements may lead to heightened substrate conversion, fostering improved microbial cell growth and metabolism,

and thereby yielding higher production rates. Furthermore, the interaction between nanoparticles and microbes may alter microbial behaviour and gene expression, potentially inducing metabolic shifts that favour LA production. Additionally, the presence of growth-promoting Mn^{2+} and Mg^{2+} ions, derived from CSL and DWW contributes to the increased production rate within the MDWW-SSF process. These ions serve as essential cofactors in enzymatic reactions crucial for cell division, nucleic acid stabilization (DNA and RNA), peptide hydrolysis, synthesis of the Gram-complex, and enhancing the enzyme-substrate complex's binding affinity, thereby facilitating metabolic processes vital for LA production (Yu et al., 2008; Lew et al., 2013). Beyond growth factors, the presence of high reducing sugar and CSL in the medium provides abundant nutrients, including carbon and nitrogen sources, as well as other essential nutrients required for the growth and metabolism of the LABs for LA production. This nutrient-rich environment supports the glycolytic pathway towards LA production, indicating that the combined effect of enhanced enzymatic activity, beneficial micronutrient availability, and optimal nutrient conditions significantly contributes to the observed increase in LA production rates.

Moreover, surfactant-assisted fermentation using Tween 80 enhances nutrient uptake, medium emulsification, and enzyme stability while $CaCO_3$ ensures pH regulation in the preferred pH range, integral for the optimal functioning of the enzymes. An increase in the $r_{p.m}$ may be ascribed to several factors working synergistically, where the singular benefits of each component are amplified to create an environment that promotes efficient microbial growth and metabolic activity, leading to higher LA production rates. The high $r_{p.m}$ of 1.79 g/L/h coincides with the high maximum potential LA concentration (P_m=35.11 g/L). Recently, Germec et al. (2018) assessed the kinetics of LA production in a modified MRS media with carob extract as the carbon source for *L. casei* ATCC 11443. Findings depicted a P_m (33.62 g/L) and $r_{p,m}$ (2.21 g/L/h). Notably, while Germec et al. (2018) achieved slightly

higher kinetic values in comparison to the present study, the study's experimental setup involved the use of costly commercial MRS media, highlighting a reduced economic and sustainability approach.

Table 7.1. Kinetic parameters from the logistic and Modified Gompertz models for the MDWW process at 5 L scale-up

Kinetic model type	Kinetic parameter	Kinetic value
Logistic model	μ_{max} (h^{-1})	0.26
	X_o (g/L)	0.50
	X_{max} (g/L)	7.11
Modified Gompertz model	P_m (g/L)	35.11
	$R_{p.m}$ (g/L/h)	1.79
	t_L (h)	0.62

Footnote: μ_{max}=maximum specific growth rate, X_0=initial cell concentration, X_{max}=maximum cell concentration, P_m=maximum potential lactic acid concentration, $r_{p,m}$ =maximum lactic acid production rate, t_L=lag time.

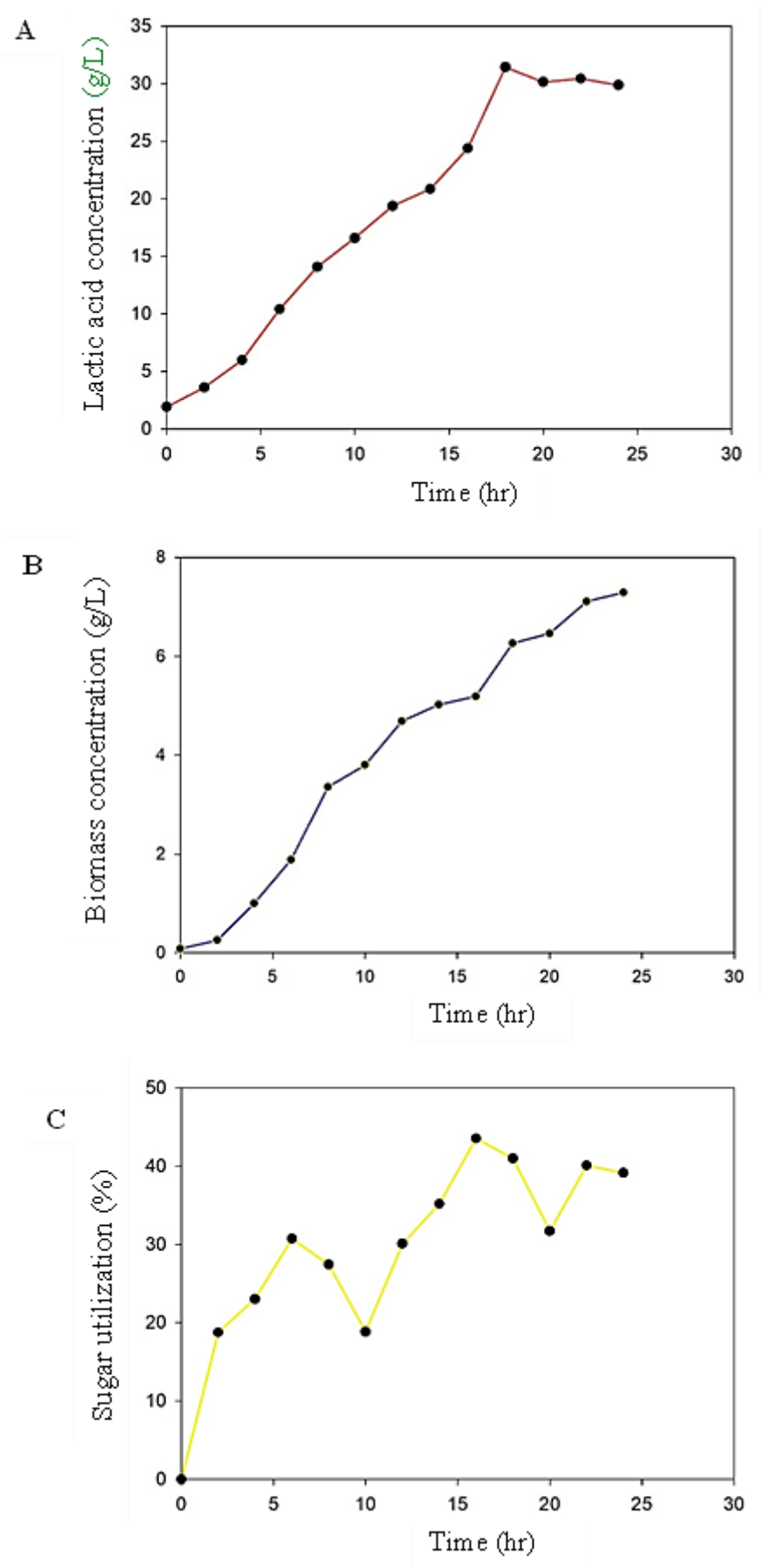

Fig. 7.3. Lactic acid production (A), *Lactobacillus plantarum* ATCC 14917 cell growth (B) and sugar utilization (C) for the 5 L scale-up of the MDWW-SSF process.

7.3.6. Comparison of the MDWW-SFF process with previous studies for lactic acid producing using lignocellulosic and dairy wastes

Results from the present study at bioreactor scale level (5 L) were compared with previous reports on lignocellulosic and dairy waste-derived LA production using various microbial species (Table S7.6). Based on the optimized results, the MDWW-SSF at 5 L scale exhibited a 64.52% and 65.48% substantially higher LA concentration in comparison to our previous study with the sDWW-$SSF_{microaerophilic}$ and sDWW-$SSF_{anaerobic}$ bioprocesses, respectively, that were devoid of $CaCO_3$ and MnO nanoparticle supplementation (David et al., 2022). This increase in LA can be attributed to the synergistic effects of the buffering agent ($CaCO_3$) and MnO nanoparticle addition, which eliminates excessive acidification as well as enhances substrate availability, microbial metabolism, and fermentation efficiency, accordingly (Yanga et al., 2015). Moreover, the MDWW-SSF process obtained a 56.47% increment in LA in comparison to our previous study that included a modified MRS medium by replacing the pure glucose with SGLD-PWW pretreated CCW substrate (David et al., 2022). In the same vein, Oonkhanond et al. (2017) used an adapted MRS media containing sugarcane bagasse hydrolysate for *L. casei* TISTR 390 microbial growth and LA production, resulting in a 32.23% reduction in the product yield when compared to the MDWW-SSF process. On the other hand, Karnouri et al. (2020) investigated two different lignocellulosic wastes, namely beechwood (49.31% higher) and pine (13.65% higher) in a modified MRS medium, respectively, and reported higher LA concentrations during the SSF process using *L. delbrueckii* subsp. *bulgaricus* ATCC® 11842 when compared to the MDWW-SSF process. Conversely, a study by Liu et al. (2018) using MRS medium modified with cheese whey powder as a carbon source for *L. bulgaricus* metabolism observed a 13.01% reduction in LA concentration in comparison to the MDWW-SSF system. Interestingly, Alexandri et al. (2018) conducted a SSF process consisting of sugar beet pulp in a minimally supplemented

yeast extract and mineral salt solution for the metabolism of a co-culture of *L. coryniformis* subsp. *torquens* DSM 20005 and *Leuconostoc pseudomesenteroide* A250. The study recorded a 30.96% decline in the LA concentration when compared to the present MDWW-SSF method (Alexandri et al., 2018). In line with the valorization of dairy waste, Verma et al. (2023) established a modified paneer whey medium that generated a LA concentration that was 53.87% lower than the yield using the MDWW-SSF process medium formulated in the present study. These reports underscore the significance of process optimization and innovative media formulations for enhancing LA production from waste and low-cost resources.

The variation in nutrients and fermentable sugars, microbial strain, physiological parameters (temperature, pH, agitation etc.), enzyme type as well as oxygen availability amongst others, can significantly impact the metabolic pathways of LABs, leading to shifts in their overall metabolism (David et al., 2022). LABs are known to adapt according to varying nutrient conditions and changes in nutrient availability which influence their growth rate, redox balance, product formation and metabolic preferences (Sano et al., 2020). For the most part, LA concentrations produced by previous studies implemented a modified MRS media with different lignocellulosic wastes as its carbon source, in the fermentation system. This primarily consists of raw materials of an expensive and intricate nature, accounting for over 30% of the bioprocess costs, as highlighted by Tang et al. (2013). Moreover, the significant water footprint associated with these lignocellulosic bioprocessing systems poses additional challenges that impact both capital costs, the conservation of finite resources, along with environmental concerns. In contrast, the present study offers a sustainable alternative by substituting fresh water with DWW and supplementing this medium with low-cost and waste materials. This innovative approach not only addresses the environmental impact and cost contributions but maintains comparable LA yields to earlier studies. By embracing such

strategies, it can pave the way for more efficient and economically beneficial LA production, while aligning with sustainable practices on both an industrial and environmental level.

7.3.7. Evaluation of lactic acid effluent for potential animal feed and biofertilizer use

The MDWW-SSF process effluent and solid biomass obtained at 5 L under the constant P/V criterion were exposed to a compositional and nutritional analysis to assess its potential as animal feed and biofertilizer (Table S7.7 and Table S7.8, respectively). The solid biomass from the LA bioprocessing effluent contains 5.81 % crude protein (CP), 0.91% fat and 10.59% fibre. According to the National research council (NRC, 2001), protein content between 1.6 and 26% have been commonly reported in feedstock compositions, indicating that the protein content of 5.8% was within range. Further knowledge by the NRC (2001) depicts the average corn cob CP to be 3%. The higher protein content from the MDWW-SSF process can be accounted for from both the CCW residues and *L. plantarum* microbial biomass that remains in the effluent after fermentation. Other common LCB for animal feed are cotton seed hulls (6.2% CP) and wheat straw (4.8% CP) (NRC, 2001). The reported fat content of 0.93 % was within the reported range of 0.1 to 19.3% using various LCB (NRC, 2001). Usually, corn cob fat content averages 0.6% (NRC, 2001). The increased fat content in the MDWW-SSF solid biomass may be ascribed to the contribution by the DWW that contains residual fats during dairy processing. Since fat is highly concentrated sources of energy, it provides higher caloric density compared to carbohydrates and proteins. Therefore, it is an essential component in animal feed to carry out physiological functions (Gurr, 1984). Cotton seed hulls and wheat straw have previously been reported to contain a similar fat content of 2.5 and 1.9 % respectively. Animal feeds also require high digestibility with fibre requirements between 1.3 to 76.9% (NRC, 2001), which is in line of the MDWW-SSF effluent containing CCW (10.59%), thereby enhancing the digestibility. Furthermore, it also comprises of Ca (7.99%), Mg (0.16%), K (0.58%), Na (1.69%), P (0.40%), Zn (39 mg/L), Cu

(35 mg/L), Mn (152 mg/L), Fe (133 mg/L). Apart from the composition of the dried solid biomass, the liquid effluent portion of the effluent underwent analysis and results showed concentrations of Ca (8447 mg/L), Mg (0187 mg/L), K(729 mg/L), Na (3393 mg/L), P (253 mg/L), Zn (0.24 mg/L), Cu (<0.17 mg/L), Mn (3.39 mg/L), Fe (3.37 mg/L), Total N (36 g/L) and Total organic C (79110 mg/L). Ca is a key element for bone formation, muscle function, nerve transmission, and blood clotting in animals. It is especially important for animal feed to contain P, Mg, Mn and Zn for enzyme activation, protein synthesis, energy, and lipid metabolism while Mg, K and Na is required for nerve function, muscle contraction and fluid balance. More especially, according to Nguyen et al. (2021), Zn is a pivotal growth hormone mediator and acts as a supplement in poultry diet because it can positively affect bone formation and osteoblasts production. On the other hand, Mn also plays an important role in bone mineralization, regulation of protein and energy metabolism (EFSA, 2016a). In addition, Cu is involved in the formation of connective tissues and the absorption of Fe. It is important to note that the level of Zn requires a comparable concentration of Cu in animal feed to ensure the best synergic action of these metals (López-Alonso, 2012). Consequently, Fe is imperative for oxygen transport in the blood and participates in various enzyme systems in animals. Based on the European Food Safety Authority (EFSA), it has been shown that a suitable supply of Fe could avoid anaemia caused by iron deficiency and Zn could bypass the growth and immune system problems caused by zinc deficiency (EFSA, 2014; EFSA, 2016b).

Apart from serving as animal feed, the constituents found in the MDWW-SSF effluent offer valuable resources for biofertilizer production. Essential elements such as Ca, K, and P play critical roles in various plant developmental processes, including cell wall formation, root growth, and the progression through flowering, fruiting, and seed formation. According to Malhotra et al. (2018), these elements are foundational for overall plant growth and vitality.

Additionally, Ca is known for its role in preserving soil structure and neutralizing soil acidity, while K is key to bolstering plant resistance against diseases and environmental stresses. Mg, Fe, and Cu further contribute to the synthesis of chlorophyll, the facilitation of photosynthetic pathways, energy transfer, and nutrient uptake (Clemens, 2019). In addition, Zn and S are crucial in activating enzymes, synthesizing amino acids and proteins, and producing growth hormones, which are all vital for the healthy development of plants. Beyond these specific nutrients, the lignocellulosic fermentation waste effluent rich in organic matter content (total organic C of 79,110 mg/L), serves as an essential carbon and energy source for plant-beneficial microorganisms. This contributes to the structure, growth, and reproductive needs of plants. N is an indispensable element for the synthesis of proteins, enzymes, and chlorophyll, as well as for micronutrient assimilation and enhancing plant health and productivity, as highlighted by Zhou et al. (2023).

In biofertilizers, these elements are often present in suitable amounts to provide the necessary nutrients to plants and enhance soil fertility. Properly balanced biofertilizers can improve soil health, nutrient availability, and plant growth, leading to higher crop yields and better overall plant performance. It is important to note that the composition and application rate of the resultant waste effluents on the potential animal feed or biofertilizer can be adjusted accordingly, based on the specific needs of the animal or crop and soil conditions in order to achieve the desired results. Therefore, developing a suitable methodology for the use of this waste-stream for animal feeding and biofertilizer could enhance the environmental and economic payout of this process since no waste treatment and disposal of effluents will be required.

7.3.8. Economic considerations for lignocellulosic biorefineries using industrial waste residues

The design and optimization of a comprehensive bioprocess, along with a detailed techno-economic assessment, are pivotal for the successful commercialization of bioprocesses. These steps are instrumental in defining economic feasibility thresholds and identifying cost-reduction opportunities without compromising on LA yield and purity. In this study, the focus is pivoted towards the innovative and complete valorization of lignocellulosic (CCW) and industrial wastes (GLD, PWW, and DWW) for LA production. This approach utilizes a minimally supplemented medium, focuses on bioprocess scale-up, and explores the potential of LA effluent as animal feed and biofertilizer. Consequently, it becomes imperative to acquire broad spectrum data on capital and operational expenses, credit from by-products, and the aggregate cost of LA production. This holistic approach not only emphasizes the economic aspects but also underscores the environmental benefits of waste valorization in LA production.

The capital investment of a lignocellulosic LA production relies on the feedstock type, pretreatment strategy, fermentation media formulation, scale-up parameters and effluent disposal technologies that will be used. A major concern of the LA production processes is the standard media components that contribute approximately 30% to the production costs (Tang et al., 2013). In response to these challenges, recent research has turned towards developing minimally supplemented fermentation media for LA production. This strategic approach provides an avenue for more sustainable, economically viable, and scalable LA production processes with reduced environmental impacts. Minimally supplemented media are often made from low-cost or waste materials that makes the process economically viable and more attractive for commercial applications. In respect of feedstock choice, LCB such as CCW is a renewable and sustainable source of carbon in LA production processes. Notably, it

has a global output of approximately 500 million tons per annum that is usually discarded (David et al., 2020). Despite its abundance, LCB contains complex carbohydrates like cellulose and hemicellulose, which cannot be directly accessed due to recalcitrant lignin crosslinks. Therefore, pretreatment is essential to break down the complex structure of lignocellulosic waste for enzymatic saccharification and subsequent conversion to fermentable sugars (Kim et al., 2015). Interestingly, lignocellulosic biorefinery processes revealed that pretreatment steps contribute to ~40% of the process costs in LA production plants due to specialized equipment, expensive chemicals, and problematic disposal (Saravanan et al., 2023). For this reason, to mitigate these costs on the operation, a combinatory pretreatment of Kraft wastes, namely, green liquor dregs (GLD) and paper wastewater (PWW) discarded during the kraft pulping process, were used on CCW under steam-assisted heating (David et al., 2021). This pretreatment technology highlighted the proposed concept to eliminate chemical and feedstock costs, reduce water consumption, energy, and time for effective fermentable sugar recovery. Furthermore, the global pulp and paper industry disposes approximately 4-11 kg of GLD per ton of pulp (Kinnarinen et al., 2016) and 40% of the global industrial wastewater (Toczylowska-Maminska, 2017), thus making it an attractive renewable resource. On that note, a nitrogen source such as yeast extract accounts for a majority of the media cost allocation, thus impairing the economics of LA production (Tang et al., 2013). CSL has piqued interest in terms of its ability to fulfill the nutritional requirements of LABs during LA production, at a cost that is approximately one fifth of conventionally used yeast extract (Tan et al., 2016). The incorporation of Tween 80, a non-ionic surfactant embodies a multi-fold action including nutrient uptake enhancement, medium emulsification, and enzyme stability during substrate saccharification in a single optimized dosage (Taoka et al., 2011; Zhang et al., 2018). This leads to improved process efficiency, higher LA production and optimal resource utilization. In addition, pH regulation

with buffer agent, $CaCO_3$, prevents excessive acidification and provides a conducive environment for LA-producing microorganisms (Yanga et a., 2015). To further enhance LA production, the utilization of MnO nanoparticles offers unique physicochemical properties at the nanoscale, enhancing nutrient adsorption, enzyme activation and potentially increase in LA production rates. More especially, in terms of economics, this study introduces a nanoparticle concentration of 0.025 g/L as its Mn^{2+} ion contribution in comparison to the 0.05 g/L $MnSO_4$ that is present in the commercially used MRS media. This translates to a 50% reduction is the resource utilization when using MnO nanoparticles. Furthermore, the recyclability of nanoparticles is a unique advantage that is crucial for cost-effectiveness. Nano-sized MnO particles exhibit obvious ferromagnetic behaviour (Chang et al., 2005). After catalysing the conversion process, the nanoparticles can be recovered and reused in subsequent batch processes using separation methods like centrifugation, filtration, or magnetic separation (Sanusi et al., 2020).

Apart from the meticulous design of the medium's nutritive components, the freshwater footprint of a lignocellulosic LA bioprocessing system has placed major constraints on sustainability, capital costs and the environment. The dairy industry generates approximately 0.2-10 L of wastewater/L of processed milk production with the volume of processed milk reaching 930 million tons in 2022 (FAO, 2023). This indicates that DWW is a reliable medium solvent to provide better nutrient performance, reduce the cost associated with water acquisition and effluent treatment. Hence, recycling and repurposing the DWW in the LA bioprocess align with sustainable practices, reducing overall water usage and waste disposal while initiating a process that is more economical.

The scale-up criteria such as constant P/V and constant V_{tip} used in the present study can significantly impact the technoeconomic outcome of LA production. Scaling up a bioprocess requires efficient mixing, reduced energy consumption, enhanced cell growth and

productivity while maintaining product quality. Proper management and optimization of these factors are essential to achieve a cost-effective and efficient LA bioproduction process on a larger scale. For large scale production, vast volumes of waste effluent are produced that can notably influence the economic viability of LA production. Several key aspects may necessitate additional investments such as waste treatment methods, specialized equipment, disposal facilities, long-term sustainability, and environmental regulatory compliance. To optimize the technoeconomic response, it is imperative to implement effective waste management strategies and explore options for waste valorization of the LA waste effluent. Depending on the effluent composition, recycling the residual nutrients after the LA fermentation process may offer opportunities of resource utilization for other processes or industries, such as the biofertilizer production or animal feed formulation. At this stage, the resultant resource recovery can offset the waste treatment and disposal associated costs while enhancing the environmental and economic payout. Furthermore. industries that prioritize environmentally friendly waste management may gain a competitive edge and positive reputation, affecting long-term profitability margins.

All the components selected for a sustainable LA plant have the ability to withstand the demands of production due to its high global output, reduced capital investment and repurposing nature. However, the infrastructure in which the process takes place must be able to accommodate vast raw material intake and LA production volumes. The Kraft paper and pulp industry generate large quantities of pulp, and its milling infrastructure capacity far exceeds its production volumes. To this end, the Kraft paper and pulp industry can store high volumes of LCB and are equipped with an array of technologies for fractionation and conversion of these substrates (Stoklosa and Hodge, 2014; Koutinas et al., 2014). Consequently, biorefinery plants have the potential to integrate seamlessly with existing pulp and paper mills globally, aiming to produce LA and other value-added products from GLD

and PWW pretreated LCB. This strategy involves redirecting the waste GLD and PWW from the Kraft pulping sector towards the pretreatment of LCB and subsequent LA production within the same facility. Cohabitating the Kraft paper and pulping process with the LA production process can diversify the industry's product portfolio, create new revenue streams to enhance its overall profitability and reduce net energy consumption of the operating facility. More importantly, it further exemplifies the waste-to-wealth approach and promotes a circular bioeconomy model in an ecologically benign, cost-effective manner. Aghbashlo et al. (2018) and Soltanian et al. (2019) conducted an evaluation of the economic viability of LA production and proposed that a biorefinery annexed with a sugar mill, producing LA, steam, and electricity using sugarcane bagasse and harvesting residues; demonstrated economic feasibility. To establish the circular economy model, research and innovation is required to optimize the process, develop suitable technologies, and ensure the feasibility of the venture. Overall, using the kraft pulping industry's infrastructure for LA production represents a promising avenue for sustainable biorefinery development. It aligns with the principles of circular bioeconomy and can contribute to a more environmentally friendly and economically viable future for these industries.

7.4. Conclusion

This study investigated the impact of various bioprocess development technologies such as buffer agents, pH adjustments, micronutrient supplementation and bioprocess types for improved LA production and sugar utilization in a minimally supplemented DWW medium. The strategy containing $CaCO_3$ and MnO nanoparticles contributed to a more than 2.7-fold increase in LA concentration and up to 46.27% reducing sugar utilization when compared to the base experiment. The exploration of mixing criteria such constant V_{tip}, and constant P/V

at different scales (0.5 L and 5 L) and kinetic modelling (5 L), revealed significant improvements in LA concentration, reduced production time and bioprocess scalability potential. The waste effluent exhibited a rich composition of essential elements to produce excellent grade animal feed and biofertilizer. These findings affirm the potential of a low-cost waste-based LA production system by eliminating media chemical costs, reducing freshwater consumption, energy, and reaction time. It also aligns with the principles of circular bioeconomy, achieving efficient waste valorization and providing valuable insights for the development of sustainable bioprocesses towards value-added product commercialization.

7.5. References

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Chapter 7: Supplementary Material

Table S7.1. Preliminary screening of various buffering agents within the sDWW-SSF process and its resultant lactic acid outputs

Experiment	Supplementation	Fermentation Input	Lactic acid concentration (g/L)	Reducing sugar utilization (%)
sDWW-SSF$_{NaHCO3}$	$NaHCO_3$	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* inoculum, 15 g/L $NaHCO_3$, 48 h, pH 4.8	10.19	14.48
sDWW-SSF$_{NaHCO3+MnO\ nanoparticle}$	$NaHCO_3$+MnO nanoparticle	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum inoculum*, 15 g/L $NaHCO_3$, 0.025 g/L MnO nanoparticle, 48 h, pH 4.8	13.23	16.70
sDWW-SSF$_{KH2PO4}$	KH_2PO_4	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* inoculum, 2 g/L KH_2PO_4, 48 h, pH 4.8	11.90	16.49
sDWW-SSF$_{KH2PO4+MnO\ nanoparticle}$	KH_2PO_4+MnO nanoparticle	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* inoculum, 2 g/L KH_2PO_4, 0.025 g/L MnO nanoparticle, 48 h, pH 4.8	11.05	15.36

Footnote: SL=Solid loading, EL= Enzyme loading

Table S7.1. Continued…

Experiment	Supplementation	Fermentation Input	Lactic acid concentration (g/L)	Reducing sugar utilization (%)
sDWW-SSF$_{Na2HPO4.12H2O}$	$Na_2HPO_4.12H_2O$	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* inoculum, 15 g/L $Na_2HPO_4.12H_2$, 48 h, pH 4.8	15.01	19.34
sDWW-SSF$_{Na2HPO4.12H2O+MnO}$	$Na_2HPO_4.12H_2O$+MnO nanoparticle	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* inoculum, 15 g/L $Na_2HPO_4.12H_2O$, 0.025 g/L MnO nanoparticle, 48 h, pH 4.8	14.22	17.91
sDWW-SSF$_{CaCO3}$	$CaCO_3$	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* inoculum, 15 g/L $CaCO_3$, 84 h, pH 4.8	18.61	27.88
sDWW-SSF$_{CaCO3+MnO}$	$CaCO_3$+MnO nanoparticle	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* inoculum, 15 g/L $CaCO_3$, 0.025 g/L MnO nanoparticle, 48 h, pH 4.8	19.25	28.92

Footnote: SL=Solid loading, EL= Enzyme loading

Table S7.2. Supplementation of various enhancement agents to the sDWW-SSF process and its resultant lactic acid outputs

Run	Experiment	Physicochemical supplementation	Fermentation Input	Lactic acid concentration (g/L)	Reducing sugar utilization (%)
1	sDWW-SSF$_{control}$	No supplementation	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* suspension, 84 h, pH 4.8	13.85	25.13
2	sDWW-SSF $_{Mn2+ and Mg2+}$	Mn^{2+} and Mg^{2+} ions supplementation	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* suspension, 0.05 g/L $MnSO_4$ and 0.1 g/L $MgSO_4$ ions, 84 h, pH 4.8	14.76	22.21
3	sDWW-SSF$_{MnO}$	MnO Nanoparticle supplementation	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* suspension, 0.025 g/L MnO nanoparticle, 84 h, pH 4.8	15.43	25.02
4	sDWW-SSF$_{pH 5.5}$	pH change	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* suspension, 84 h, pH 5.5	17.50	22.61

Footnote: SL=Solid loading, EL= Enzyme loading

Table S7.2. Continued…

Run	Experiment	Physicochemical supplementation	Fermentation Input	Lactic acid concentration (g/L)	Reducing sugar utilization (%)
5	sDWW-SSF$_{CaCO3}$	$CaCO_3$ buffering	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* suspension, 30 g/L $CaCO_3$, 84 h, pH 4.8	30.76	45.48
6	sDWW-SSF$_{CaCO3, Mn2+ and Mg2+, pH 5.5}$	Mn^{2+} and Mg^{2+} ions supplementation $CaCO_3$ buffering pH change	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* suspension, 0.05 g/L $MnSO_4$ and 0.1 g/L $MgSO_4$ ions, 30 g/L $CaCO_3$, 84 h, pH 5.5	26.94	43.27
7	sDWW-SSF$_{CaCO3, MnO, pH 5.5}$	$CaCO_3$ buffering MnO Nanoparticle pH change	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* suspension, 0.025 g/L MnO nanoparticle, 30 g/L $CaCO_3$, 84 h, pH 5.5	31.12	46.27
8	sDWW-SSF$_{CaCO3, pH 5.5}$	$CaCO_3$ buffering pH change	10% SL, 10% EL, 25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* suspension, 30 g/L $CaCO_3$, 84 h, pH 5.5	28.69	43.05

Footnote: SL=Solid loading, EL= Enzyme loading

Table S7.3. Supplementation of various enhancement agents to a separate prehydrolysis and fermentation stage within sDWW-PSSF process and its resultant lactic acid outputs

Run	Experiment	Prehydrolysis input	Fermentation input	Lactic acid concentration (g/L)	Reducing sugar utilization (%)
1	sDWW-PSSF$_{CaCO3}$	10% SL, 10% EL, DWW citrate buffer, pH 4.8, 24 h	25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* suspension, 30 g/L CaCO3, 84 h	24.38	43.00
2	sDWW-PSSF$_{CaCO3+MnO}$	10% SL, 10% EL, DWW citrate buffer, pH 4.8, 24 h	25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* suspension, 30 g/L CaCO3, 0.025 g/L MnO nanoparticle, 84 h	25.99	44.43
3	sDWW-PSSF$_{PH\ with\ Tween\ 80, CaCO3}$	10% SL, 10% EL, DWW citrate buffer, pH 4.8, 2mL/L Tween 80, 24 h	25 g/L CSL, 10% *L. plantarum* suspension, 30 g/L CaCO3	25.55	45.79
4	sDWW-PSSF$_{PH\ with\ MnO, CaCO3}$	10% SL, 10% EL, DWW citrate buffer, pH 4.8, 0.025 g/L MnO nanoparticle, 24 h	25 g/L CSL, 2 mL/L Tween 80, 10% *L. plantarum* suspension, 30 g/L CaCO3	23.86	41.31
5	sDWW-PSSF$_{PH\ with\ MnO+Tween\ 80, CaCO3}$	10% SL, 10% EL, DWW citrate buffer, pH 4.8, 0.025 g/L MnO nanoparticle, 2 mL/L Tween 80, 24 h	25 g/L CSL, 10% *L. plantarum* suspension, 30 g/L CaCO3	29.35	46.77

Footnote: SL=Solid loading, EL= Enzyme loading

Table S7.4: Bioreactor geometry of the lactic acid scale-up bioprocesses

Parameters	2 L bioreactor	13 L bioreactor
Total bioreactor volume (m^3)	0.002	0.013
Working volume (m^3)	0.0005	0.005
Bioreactor height [h] (m)	0.237	0.427
Bioreactor diameter [D] (m)	0.125	0.200
Number of impellers (N)	1	2
Impeller diameter [d_i] (m)	0.054	0.070
Impeller thickness (m)	0.001	0.002
Power number (N_p)	5.20	10.40
Broth density [ρ] (kg/m^3)	1050.53	1050.53
Broth viscosity [η] (Pa s)	2.87×10^{-3}	2.87×10^{-3}
Impeller type	Rushton turbine	Rushton turbine

Table S7.5: Rheology and hydrodynamic parameters of scale up fermentation criteria

Parameters	2 L bioreactor		13 L bioreactor
	Constant υ tip	Constant P/V	Constant P/V
n (rps)	0.97	1.23	0.90
V_{tip} (m/s)	0.16	0.21	0.20
P (W)	0.012	0.012	0.012
P/V_L (W/m^3)	24	24	2.4
V_p (m^3/s)	1.11×10^{-4}	1.40×10^{-4}	2.23×10^{-4}
t_c (s)	4.51	3.57	22.4
λ (m)	1.96×10^{-4}	1.64×10^{-4}	1.53×10^{-4}
γ (1/s)	580	740	540

Footnote: rps =Revolutions per second, V_{tip} =impeller tip speed, V_p =pumping capacity, t_c =circulation time, λ =scale of turbulence

Table S7.6. Comparisons of the lactic acid production from lignocellulosic and dairy wastes using various microorganisms

Microorganism	Substrate/carbon source	Medium type	Process conditions	Lactic acid concentration (g/L)	Reference
Lactobacillus plantarum ATCC 14917	Corn cob waste	Supplemented dairy wastewater	10% (w/v)[a], 10% (w/v)[b], Cellic CTec2[c], 10 FPU/g[d], 25 g/L CSL, 2mL/L Tween 80, 30% $CaCO_3$, 0,025 g/L MnO nanoparticle, 37°C[e], 54 rpm[f],	31.43	This study
Lactobacillus plantarum ATCC 14917	Corn cob waste	Supplemented dairy wastewater	10% (w/v)[a], 10% (w/v)[b], Cellic CTec2[c], 10 FPU/g[d], 25 g/L CSL, 2mL/L Tween 80, 30% $CaCO_3$, 0,025 g/L MnO nanoparticle, 37°C[e], 120 rpm[f]	11.15	David et al. (2022)
Lactobacillus plantarum ATCC 14917	Corn cob waste	Supplemented dairy wastewater	10% (w/v)[a], 10% (w/v)[b], Cellic CTec2[c], 10 FPU/g[d], 25 g/L CSL, 2mL/L Tween 80, 30% $CaCO_3$, 0,025 g/L MnO nanoparticle, 37°C[e], 120 rpm[f]	10.85	David et al. (2022)
Lactobacillus plantarum ATCC 14917	Corn cob waste	Modified MRS	10% (w/v)[a], 10% (w/v)[b], Cellic CTec2[c], 10 FPU/g[d], 25 g/L CSL, 2mL/L Tween 80, 30% $CaCO_3$, 0,025 g/L MnO nanoparticle, 37°C[e], 120 rpm[f]	13.68	David et al. (2022)
Lactobacillus casei TISTR 390	Sugarcane bagasse hydrolysate	Modified MRS	10 % v/v[a], 10% (w/v)[b], Accellerase 1500[c], 200 FPU/g[d], 37°Ce, 30-35 rpm[f].	21.3	Oonkhanond et al. (2017)

Footnote: [a]=microorganism concentration, [b]=solid loading, [c]=enzyme type, [d]=enzyme concentration, [e]=temperature, [f]=agitation speed, MRS= De Man, Rogosa and Sharpe.

Table S7.6: Continued…

Microorganism	Substrate/carbon source	Medium type	Process conditions	Lactic acid concentration (g/L)	Reference
Lactobacillus delbrueckii subsp. *bulgaricus* ATCC® 11842.	Beechwood	Modified MRS	10% (v/v)[a], 9% (w/v)[b], Cellic CTec2[c], 9.6FPU/g[d], 44°C[e], 160 rpm[f].	62	Karnaouri et al. (2020)
Lactobacillus delbrueckii subsp. *bulgaricus* ATCC® 11842.	Pine	Modified MRS	10% (v/v)[a], 9% (w/v)[b], Cellic CTec2[c], 9.6FPU/g[d], 44°Ce, 160 rpm[f].	36.4	Karnaouri et al. (2020)
Lactobacillus bulgaricus CGMCC 1.6970	Cheese whey powder	Modified MRS	10% (v/v)[a], 59.25 g/L[b], 42°C[e], 200 rpm[f]	27.34	Liu et al. (2018)
Lactobacillus coryniformis subsp. torquens DSM 20005 and *Leuconostoc pseudomesenteroide* A250	Sugar beet pulp	Yeast extract in mineral salt solution	6 % v/v[a], 10% (w/v)[b], Accellerase 1500[c], 0.5 mL/g cellulose[d], 30°C[e], 200 rpm[f].	21.7	Alexandri et al. (2022)
Pediococcus pentosaceus NCDC 273	Paneer whey (lactose)	Modified paneer whey medium	2% (v/v)[a], 37°C[e]	14.5	Verma et al. (2023)

Footnote: [a]=microorganism concentration, [b]=solid loading, [c]=enzyme type, [d]=enzyme concentration, [e]=temperature, [f]=agitation speed, MRS= De Man, Rogosa and Sharpe.

Table S7.7. Elemental analysis of dairy wastewater and MDWW-SSF medium

Element	Dairy wastewater	MDWW-SSF medium (before fermentation)	MDWW-SSF medium (after fermentation)
Dissolved calcium	12.9 mg/L	7396 mg/L	8447 mg/L
Potassium	7.74 mg/L	482 mg/L	729 mg/L
Dissolved magnesium	3.59 mg/L	215 mg/L	187 mg/L
Sodium	932 mg/L	2259 mg/L	3393 mg/L
Dissolved copper	<0.17 mg/L	<17 mg/L	<0.17 mg/L
Dissolved iron	0.28 mg/L	52 mg/L	3.37 mg/L
Dissolved manganese	<0.17 mg/L	<17 mg/L	3.90 mg/L
Dissolved sulfur	1.38 mg/L	150 mg/L	77 mg/L
Dissolved zinc	<0.02 mg/L	<1.79 mg/L	0.24 mg/L
Total phosphorus	3.74 mg/L	261 mg/L	253 mg/L
Total nitrogen	27 mg/L	20 mg/L	36 mg/L
Total organic carbon	202 mg/L	34696 mg/L	79110 mg/L
COD	643 mg O_2/ℓ	115152 mg O_2/ℓ	522613 mg O_2/ℓ
BOD	<2 mg O_2/ℓ	2138 mg O_2/ℓ	2173 mg O_2/ℓ

Table S7.8. Compositional feed analysis for the MDWW-SSF solid residues

Component	MDWW-SSF solid biomass
Ash	28.26 %
Fat	0,91%
Crude protein	5.81%
Ca	7.99%
Mg	0.16%
K	0.58%
Na	1.69%
P	0.40%
Zn	39 mg/kg
Cu	35 mg/kg
Mn	152 mg/kg
Fe	133 mg/kg

CHAPTER 8

Conclusions and Recommendations

8.1. Conclusions

Bioconversion of lignocellulosic biomass to platform chemicals and biofuels will contribute significantly to driving a sustainable, carbon-neutral bioeconomy. However, these bioprocesses are currently plagued with high capital costs, energy and water demands coupled with low product yields, expensive downstream procedures and environmental effects. This study addressed key challenges in lignocellulosic pretreatment and microbial fermentation bioprocesses for lactic acid (LA) production towards viable industrial production approaches. Major findings and their significance are summarized as follows:

8.1.1. Two waste-based pretreatment strategies consisting of a (1) steam-assisted combined green liquor dregs and paper wastewater (SGLD-PWW), and (2) microwave-assisted combined green liquor dregs and paper wastewater (MGLD-PWW) method were modelled and optimized for enhanced sugar release from corn cob waste (CCW). The optimized SGLD-PWW pretreatment (49.89% GLD, 118°C, 5 min) gave higher reducing sugar yield (rsy) (1.53±0.36 g/g) and glucose yield (gy) (0.85±0.16 g/g) when compared to the MGLD-PWW strategy (48.70% GLD, 800 W, 9 min, rsy=1.04±0.01 g/g, gy=0.51±0.06 g/g). The CCW substrate composition showed slightly higher cellulose improvement (25.86%) and hemicellulose solubilization (38.13%) for the SGLD-PWW pretreatment in comparison to the MGLD-PPW method (cellulose improvement=25.48%, hemicellulose solubilization= 35.89%). Scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) analysis further confirmed the structural modifications in the CCW, resulting in enhanced sugar release. The SGLD-PWW pretreatment conferred a 32% and 40% higher reducing sugar and glucose yield, respectively, with a 44.4% lower pretreatment time than the MGLD-

PWW regime. As a result, the SGLD-PWW method was selected for simultaneous saccharification and fermentation (SSF) process development.

8.1.2. Two Artificial Neural Network (ANN) models were developed as predictive tools for glucose responses from existing steam- and microwave-assisted Kraft waste-based pretreatments. The steam- and microwave-assisted Kraft waste-based models achieved high R^2 scores of 0.95 and 0.97, respectively for the observed and predicted glucose responses, indicating good model fit. A sensitivity analysis revealed that the glucose responses for the steam model were highly susceptible to the stepwise variation in GLD concentration from 0% to 50% (>3.3-fold increase) in relation to its baseline concentration, owing to the alkaline Kraft waste interactions with the CCW. For the microwave model, an increase in power intensity from 100 W to 900 W resulted in a >2.6-fold increase from the baseline value, on account of the dielectric polarization of microwave irradiation, enhancing lignocellulosic breakdown and altering chemical covalent bonding in the CCW. A comparative evaluation on the capability of the Generative Artificial Intelligence model, ChatGPT, to provide innovative and factually accurate insights from the process data was conducted. The novel process insights deduced by ChatGPT aligned with authors contextual interpretation, enabling more nuanced understanding and interpretation of data trends related to parameter impacts on glucose yields in steam- and microwave-assisted Kraft waste-based pretreatment.

8.1.3. The supplemented dairy wastewater-based simultaneous saccharification and fermentation (sDWW-SSF) process parameters of corn steep liquor (CSL), Tween 80 and CCW solid loading (SL) concentrations were modelled and optimized for LA production using *Lactobacillus plantarum* ATCC 14917. The sDWW-SSF process resulted in an experimental LA concentration and LA conversion of 11.15±0.42 g/L and 18.90±0.75%, respectively, under the optimized conditions (25g/L CSL, 2 mL/L Tween 80, 10% SL). These results showed that increased CSL and Tween 80 concentration while the substrate SL

remained at its base level had a significant impact on the production of LA. Kinetic studies on *L. plantarum* cell growth and LA formation demonstrated the highest maximum specific growth rate (μ_{max}) (0.64 h^{-1}) and maximum potential LA concentration (P_m) (14.01 g/L) for the standard De Man, Rogosa and Sharpe (MRS) medium modified with pretreated CCW (mMRS-$SSF_{microaerophilic}$) process compared to the DWW-based microaerophilic (sDWW-$SSF_{microaerophilic}$: μ_{max}= 0.35 h^{-1}, P_m= 13.01 g/L) and DWW-based anaerobic (sDWW-$SSF_{anaerobic}$: μ_{max}= 0.34 h^{-1}, P_m= 12.01 g/L) systems. These observations suggest that microaerophilic environments are preferred over anaerobic systems based on the *L. plantarum* respiro-pathway profile that shifts to a high energy metabolism sufficient for growth and product formation. Considering that the microaerophilic conditions facilitate product formation, the sDWW-$SSF_{microaerophilic}$ process highlights the application of a waste-based media formulation to produce a high value product (lactic acid). This process substantially reduces resource costs and water usage by utilizing cheaper nutrient media components and substituting dairy wastewater in place of fresh water. Hence, a waste-based media approach for LA production is a vital contribution to the industrialization of this bioprocess.

8.1.4. Based on the sDWW-SSF bioprocess for LA production, findings demonstrated a residual glucose content at the end of the fermentation process, indicating the potential for further improvement in the LA concentration and sugar utilization using various physicochemical strategies. Data indicated that the sDWW-SSF process supplemented with $CaCO_3$ (30 g/L) and MnO nanoparticle (0.025 g/L) at pH 5.5 (sDWW-$SSF_{CaCO3(30)+MnO, pH5.5}$) produced a high LA concentration (31.12 g/L) and sugar utilization (up to 46.27%) at flask scale. These results were attributed to the pH regulating characteristics of the $CaCO_3$ that prevent product inhibition and enhanced activation reactions by the MnO nanoparticles. The optimized process was scaled from flask to bioreactor (0.5 L) and revealed an 18.25% higher

LA concentration and 40% reduced production time for constant P/V (33.64 g/L, 18 h, 78 rpm) compared to the constant V_{tip} (27.49 g/L, 30 h, 58 rpm). The enhanced mixing efficiency and process performance of the constant P/V mixing regime prompted scale up at 5 L. Under the constant P/V criteria, the LA concentration peaked at 31.43 g/L after 18 h at 54 rpm while achieving up to 43.55% sugar utilization. Interestingly, the slight decrease of 6.57% in the LA concentration at 5 L scale compared to 0.5 L scale may be ascribed to the partial cell shearing that reduced metabolic activity in the bioprocess due to turbulence. Kinetic assessment at 5 L scale showed a μ_{max} and P_m of 0.26 h^{-1} and 35.11 g/L, respectively. Lastly, the waste effluent rich in essential elements (Ca, Mg, K, Na, P, Zn, Cu, Mn and Fe) demonstrated a suitable nutritive profile for animal feed and biofertilizer application.

8.1.5. The present study developed the SGLD-PWW and MGLD-PWW pretreatment strategies that provide a cost-effective system with high fermentable sugar recovery from CCW. In that respect, the optimized pretreatments gave a glucose yield >0.50 g/g, which was higher than widely used chemical-based methods such as NaOH. The effective degradation of the CCW was further validated by the compositional modifications, SEM and FTIR analysis. Based on these findings, the complete waste-based pretreatment regimes have set the stage towards the conceptualization of the “waste to wealth” initiative and will pave the way for microbial conversion of lignocellulosic-derived feedstocks into commodity bioproducts such as LA. Moreover, utilizing an Artificial Intelligence modelling approach enhances comprehension of underlying mechanisms, aiding improved predictive technology in future research and substantially contributing to the public domain's knowledge on Kraft waste-based pretreatment of lignocellulosic residues. With regards to LA production, the optimized SSF process revealed the utilization of a Kraft waste pretreated CCW and minimally supplemented DWW medium for microbial cell growth and LA formation. It is noticeable that the mMRS-$SSF_{microaerophilic}$ bioprocess displayed comparable μ_{max} and P_m values to the

wastewater-based processes (sDWW-SSF$_{microaerophilic}$ and sDWW-SSF$_{anaerobic}$), which offsets the extensive resource costs in LA production. More so, the sDWW-SSF$_{microaerophilic}$ system negates costly complex media, fresh water and anaerobiosis within lignocellulosic processes. Interestingly, the $CaCO_3$ and MnO nanoparticle assisted strategy (sDWW-SSF$_{CaCO3(30)+MnO, pH5.5}$) contributed significantly (>2.7-fold increase) to the LA concentration when compared to the sDWW-SSF system, offering enhanced pH regulation and production rates towards scale up. The results elucidated a marked improvement in the efficiency of the optimized bioprocess under a constant P/V regime, as evidenced by a 18.25% increase in LA concentration and a 40% reduction in production times, when compared with the constant V_{tip} approach. This may be attributed to enhanced mixing efficiency that is pivotal for successful bioprocess scale-up, improving the kinetics of the process, and consequently, the overall productivity. Such insights play a crucial role in decision-making prior to industrial bioprocesses for the valorization of lignocellulosic, Kraft and dairy wastes towards valuable bioproducts, fostering technological, economic, and environmental advancements. This underscores the food-energy-water nexus, emphasizing the integration and optimization of resources to promote sustainable development.

8.2. Recommendations for future studies

Building upon the insights from this study, the following directions for future research in the area of lignocellulosic pretreatment systems and the development of LA production processes are proposed:

8.2.1. Recycling and Reuse of Hydrolysate: Future investigations should focus on evaluating the feasibility of recycling the hydrolysate produced after the Kraft waste-based lignocellulosic pretreatment. This approach aims to reduce environmental remediation and

disposal costs while promoting the circular use of waste-derived chemicals within the pretreatment unit of lignocellulosic biorefineries.

8.2.2. Valorization of Waste Residues: The potential for extracting and purifying valuable compounds such as hemicellulosic oligomers, lignin, and carboxylic acids from spent liquid post-pretreatment should be explored. Such efforts would enable the complete valorization of the waste residues, transforming them into high-value products in addition to diversifying revenue streams.

8.2.3. Utilization of Alternative Sugars: Given the varied sugar composition in lignocellulosic biomass, research should extend to the use of alternative sugars, including xylose and arabinose, alongside glucose for microbial product generation. This strategy would optimize resource use and increase the yield of value-added microbial products.

8.2.4. Enhancement of Microbial Strains: The exploration of metabolically engineered strains capable of producing LA more efficiently is critical. These microbial strains should exhibit enhanced capability in utilizing a broader spectrum of carbohydrates, thus improving the bioconversion efficiency of lignocellulosic waste into LA.

8.2.5. Integrated Biorefinery Approaches: The development of integrated biorefinery concepts that leverage fermentation effluents for the production of additional valuable commodities, such as bioethanol, biohydrogen, and microalgal biomass, should be pursued. This strategy offers new revenue opportunities, enhances substrate conversion efficiency and the overall economic viability of the processes.

8.2.6. Technoeconomic Analysis: A comprehensive technoeconomic analysis (TEA) is essential to assess the economic viability of producing LA from lignocellulosic materials through waste-based methodologies. This analysis offers significant insights for strategic research and development, investment outlooks and knowledge generation.

By addressing these recommendations, future research can significantly contribute to the advancement of lignocellulosic bioprocessing technologies, leading to more sustainable and economically viable production of LA and other bio-based products.

Appendix A

Fuel 274 (2020) 117797

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel

Full Length Article

Development of a green liquor dregs pretreatment for enhanced glucose recovery from corn cobs and kinetic assessment on various bioethanol fermentation types

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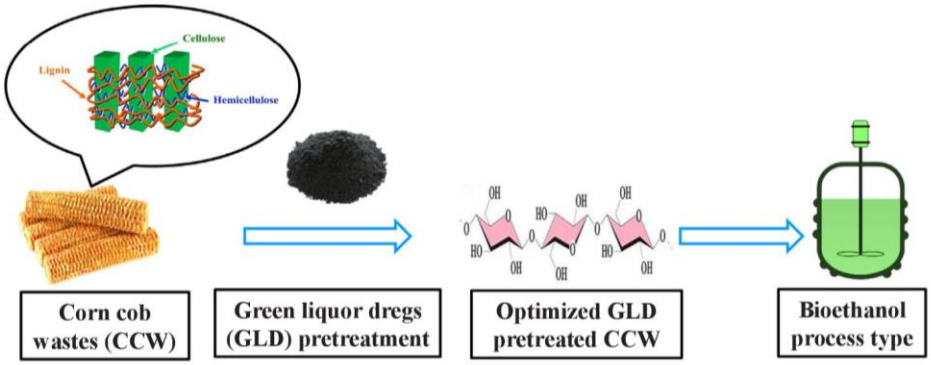
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Corn cob waste
Green liquor dregs
Lignocellulosic pretreatment
Bioethanol process type
Waste beneficiation

ABSTRACT

This study optimized a novel pretreatment of corn cob wastes (CCW) using green liquor dregs (GLD), a waste product from the chemical kraft pulping industry. Subsequently, the microbial growth and ethanol production kinetics of different bioprocess types were comparatively evaluated using the logistic and modified Gompertz models respectively. The optimized GLD pretreatment conditions released a maximum glucose yield of 0.42 g/g. Separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and SSF with pre hydrolysis (PSSF) processes were assessed and gave comparable kinetic data. The SHF and PSSF processes displayed slightly higher microbial and bioethanol kinetic coefficients compared to the SSF system. Implications from this study provide major insights for reducing energy, time and costs incurred with lignocellulosic substrate pretreatment, bioethanol process design and scale up. Additionally, the study demonstrates a possible route for beneficiation of waste material such as GLD.

1. Introduction

The rapid exhaustion of fossil fuels has raised concerns on its long-term supply and severe environmental implications [1]. This has led to the development of renewable, sustainable and affordable fuel carriers for future energy requirements. Lignocellulosic bioethanol production

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https://doi.org/10.1016/j.fuel.2020.117797
Received 8 November 2019; Received in revised form 1 April 2020; Accepted 4 April 2020
Available online 18 April 2020
0016-2361/

has emerged as a promising replacement for fossil fuels [2]. Agricultural food sources such as corn, sugarcane and wheat are used to produce first generation bioethanol, but these do not comply with food sustainability [3].

Lignocellulosic wastes are considered as promising feedstocks that are renewable, abundant, cheap, and a food security-wise alternative that can be used for second generation biofuel production [4–6]. Examples of feedstocks that have previously been assessed include sugarcane bagasse [7], sorghum leaves [3], corn stover [8], wheat straw [9] and corn cobs [10]. The compositional structure of lignocellulosic material comprises of 38–50% cellulose, 23–32% hemicellulose and 15–25% lignin [11]. Corn is a staple crop in many countries and its agricultural yield on a global scale surpasses 1.03 billion metric tons annually. Almost 50% of the corn produced consists of the leaves, cobs and husks and are classified as wastes that are often disposed [12]. The corn cob wastes (CCW) particularly have displayed several benefits for biofuel production processes with approximately 500 million tons (CCW) generated annually. For instance, its energy density is within the range of 4960–5210 MJ/Kg, approximately two times higher than switchgrass *(Panicum virgatum)* (2500 MJ/Kg) and corn stover (2550 MJ/Kg) [13]. Additionally, corn cobs are easily available, possess a low market value and are considered a more consistent feedstock compared to corn stover which is a mixture of the stalks, husks and cobs. This improves the efficiency of bioethanol production by eliminating the need for compositional adjustments [14]. However, substrates such as CCW contain resistant lignin structures that prevent enzymatic hydrolysis of cellulose to monomeric sugars. This presents a major limitation since several bioethanol-producing microbes require simple sugars such as glucose for metabolic processes [15]. Overcoming this barrier necessitates the application of pretreatment regimes for the breakdown of the recalcitrant lignocellulosic structures [6,16]. Pretreatment techniques reduce cellulose crystallinity, thus exposing the lignocellulosic structures to hydrolytic enzymes for the release of fermentable sugars [15,17].

Various pretreatment methods have been explored in the last few years and include hydrothermolysis, ball-milling, acid, alkaline, inorganic salt, organosolvents, microwave and steam-assisted thermal pretreatments [3,15,18]. The main objective of an efficient pretreatment is to produce high sugar yields that can be utilised for the production of biofuels and value-added products but should be cost and energy efficient. Therefore, the development of a pretreatment technique that meets these criteria is crucial to improve its large-scale feasibility.

Currently, the most common chemical pretreatment that is industrially employed is alkaline (NaOH) and has proven to be effective due to its low energy consumption and effective lignin removal [15,19]. Other alkaline catalysts include sodium sulfide (Na_2S), sodium carbonate (Na_2CO_3) and calcium carbonate ($CaCO_3$) among others. The alkaline pretreatment mechanism of action causes (1) cleavage of acetyl groups from xylan polymers, (2) alteration and removal of lignin and its compounds, and (3) reduction in cellulose crystallinity and increases the substrate porosity [20]. Cellulase enzyme accessibility is thereby enhanced to ensure efficient cellulose hydrolysis for the release of glucose monomers [21]. Despite the effectiveness of alkaline pretreatments, they have shown to be costly for industrial processes, thus rendering it economically non-viable.

Recently, green liquor dregs (GLD) have surfaced as a potential low-cost and abundant pretreatment agent. While green liquor (GL) is a well-known chemical pretreatment agent [22,23], green liquor dregs (GLD) has not yet been assessed for this purpose. GLD is a paper mill chemical waste material generated from the kraft pulping industry when wood is treated with alkaline chemicals such as NaOH and Na_2S to release cellulose [24]. The resulting GLD is then directed towards various downstream treatment processes. This waste has shown to be problematic to filtration pipes present within the operational equipment. Landfill systems are then used to address this problem but in turn present additional costs for the pulp and paper mill industry [24]. To avoid the aforementioned expensive treatment systems, the GLD that is recovered from this industry may be channelled towards alternative processes in an effort to convert waste to wealth. Interestingly, the presence of alkaline species such as $CaCO_3$, Na_2S and Na_2CO_3 in the GLD contribute to its strong alkalinity (pH $>$ 10) and excellent buffering capacity, which are generally viewed as advantageous characteristics in pretreatment systems [8,25]. This is due to the effective mimicry of expensive alkaline pretreatment methods such as NaOH (pH 13), while it is considered a “dead load” in the pulping process [24,25]. The application of GLD for lignocellulosic pretreatment processes will provide an economical, green method of disposal for the paper industry and simultaneously result in high value products such as glucose and biofuels.

Another challenge for lignocellulosic pretreatment is the lack of information on the factors that affect the pretreatment efficiency. Some of these include temperature, time, chemical concentration and solid loading [12,26]. The optimization of these pretreatment parameters will lead to the development of an economically viable and efficient system, which enables increased sugar yields for microbial biofuel processes such as ethanol production.

Bioethanol, a renewable fuel with simple storage, high oxygen content (34.73%) and high octane number has demonstrated its potential to serve as an alternative renewable energy resource. The production of ethanol may occur via three different bioprocess types: (1) separate hydrolysis and fermentation (SHF), (2) simultaneous saccharification and fermentation (SSF), and (3) SSF combined with pre hydrolysis (PSSF). SHF is a conventional method that consists of two separate biochemical reactions with different temperatures and includes an enzymatic hydrolysis stage (50 °C for 72 h) followed by the ethanol fermentation process (30–37 °C for 24–48 h). On the other hand, the SSF process is performed in a single bioreactor and finds a common temperature (35–40 °C) that can accommodate both the cellulase enzyme and the microbial culture. The PSSF process type has been investigated as an intermediate system for the SHF and SSF methods. This process type (PSSF) consists of a short pre hydrolysis or enzymatic saccharification step (6–24 h) and is carried out at optimal temperature (50–60 °C) followed by inoculation of the microbial culture. Once inoculation occurs, the fermentation process is induced and may occur for 24–48 h at temperatures between 30 and 37 °C.

Although all three process types have previously demonstrated benefits and limitations, selection at an industrial scale remains contentious. For example, the SHF system ensures that a high sugar is released for the ethanol fermentation [27]. Nevertheless, the SHF process type is challenged by the use of separate bioreactors, which proves to be time and energy consuming, thus increasing process costs at large scale. The implementation of separate bioreactors also present a higher risk of contamination when transferring the enzymatic hydrolysate to the ethanol fermentation reactor. One striking characteristic of the PSSF process compared to the SHF system is that the hydrolysed lignocellulosic biomass is not removed prior to ethanol fermentation. This leverages the advantages of both the SHF and SSF process types. The short hydrolysis step prior to the SSF process releases a relatively high sugar yield at a reduced time, which provides a balance between the inhibitory and rate-controlling factors. However, an additional pre hydrolysis stage would incur higher costs due to energy consumption and longer process times, therefore reducing its feasibility at large scale. Compared to the SHF and PSSF process types, the SSF system boasts several benefits for large scale application owing to the reduced process time and energy by completely omitting additional hydrolysis steps. The elimination of separate enzymatic saccharification steps has shown to reduce the bioprocess cost by 20% [28].

Apart from the development and selection of a suitable lignocellulosic pretreatment regime, assessing the kinetics of microbial growth and bioethanol production to determine the most efficient ethanol bioprocess type is imperative for scale up studies. Kinetic

Table 1
Combined mixture and factorial experimental design for the GLD pretreatment.

Run	Mixture components		Solid loading (%)	Heating time (min)	Glucose yield (g/g)	
	CCW concentration (%)	GLD concentration (%)			Experimentally observed	Model predicted
1	50	50	12.5	45	0.26	0.27
2	50	50	12.5	45	0.28	0.27
3	35	65	20	30	0.33	0.33
4	80	20	5	50	0.22	0.19
5	50	50	12.5	60	0.28	0.28
6	50	50	5	30	0.14	0.14
7	50	50	20	40	0.26	0.20
8	50	50	15	30	0.26	0.18
9	20	80	20	60	0.38	0.40
10	20	80	12.5	52.5	0.39	0.40
11	80	20	20	30	0.08	0.11
12	80	20	5	30	0.08	0.09
13	20	80	20	40	0.35	0.38
14	40	60	5	60	0.31	0.34
15	65	35	20	50	0.21	0.22
16	20	80	5	40	0.37	0.37
17	50	50	20	60	0.34	0.29
18	20	80	15	30	0.02	0.06
19	80	20	12.5	37.5	0.11	0.15
20	80	20	5	45	0.11	0.10
21	20	80	12.5	52.5	0.41	0.40
22	35	65	10	30	0.37	0.37
23	80	20	20	60	0.16	0.16
24	20	80	5	60	0.41	0.38
25	80	20	10	60	0.12	0.19
26	40	60	5	60	0.32	0.34
27	50	50	12.5	45	0.31	0.27
28	50	50	12.5	45	0.25	0.27

Footnote: g/g = g glucose/ g pretreated dry weight corn cobs.

models play an important role in defining the parameters for bioprocess control when considering industrial scale applications [29]. These process models assist during scale up by increasing the product yield and productivity, while it minimises undesired by-product formation in order to produce a cost efficient, high quality product [30]. Moreover, knowledge on the kinetics of microbial cell growth, substrate consumption and product formation provide in depth information on the development of process design and yield [31,32]. The logistic model evaluates the microbial growth pattern and relates it to the growth rate, initial and maximum concentrations over time. Additionally, the modified Gompertz model assesses the bioethanol formation by determining (1) the production lag time, (2) maximum production rate and (3) maximum production concentration on a given substrate [31]. To date, a comparative assessment of microbial and bioethanol kinetic parameters on all three process types has not yet been reported. Kinetic data on SHF, PSSF and SSF bioprocess types will provide extensive knowledge on the most effective one, thus enhancing ethanol fermentation design and economic feasibility for industrial scale.

Therefore, the specific objectives of the present study were to: (1) model and optimize the green liquor dregs (GLD) pretreatment for improved glucose recovery from corn cob wastes (CCW) using a combined RSM mixture and factorial design, (2) determine the individual and interactive effects of GLD concentration, corn cob concentration, solid loading and heating time on the glucose yield, and (3) assess and compare the different bioethanol process types (SHF, SSF and PSSF) based on the kinetic coefficients for microbial cell growth and bioethanol formation using the logistic and modified Gompertz models respectively.

2. Materials and methods

2.1. Materials

The CCW substrate was acquired from the Ukulinga research farm (Pietermaritzburg, South Africa) (29° 67′ E, 30° 40′ S). It was oven dried at 60 °C for 24 h and thereafter milled to a particle size of less than 1–2 mm (Retsch ZM-1, South Africa). The powdered substrate was stored at room temperature. Compositional analysis of the treated and untreated (native) substrate was carried out according to the National Renewable Energy Laboratory method [33]. The green liquor dregs (GLD) were obtained from a local paper mill industry (Durban, South Africa). The Cellic® CTec 2 enzyme was generously donated by Novozyme (Novozymes A/S, Denmark). Its enzyme activity was determined to be 160 FPU/ml according to the Laboratory Analytical Procedure (LAP) of the National Renewable Energy Laboratory (NREL, 2008). The *Saccharomyces cerevisiae* BY4743 yeast strain (ATCC® 201390™) was kindly provided by the Department of Genetics, University of KwaZulu-Natal (Pietermaritzburg, South Africa). All chemical components were purchased from Merck, South Africa.

2.2. Pretreatment of corn cobs

2.2.1. Primary screening

The primary screening pretreatments were carried out according to the Box-Behnken experimental design and consisted of seventeen runs (Table S1). The aforementioned preliminary experiments were performed to determine the input parameter ranges for an extensive optimization thereafter. The initial experiments were carried out in 100 mL Erlenmeyer flasks with GLD concentration, substrate solid loading and temperature ranging from 5–15% (w/v), 10–20% (w/v) and 105–115 °C respectively. All seventeen pretreatments were carried out in a laboratory autoclave at a fixed time of 60 min. The resulting pretreated CCW was thereafter filtered using a domestic sieve (< 1mm) and washed several times with deionized water until it was free of any residual GLD. The washed biomass was oven dried at 70 °C overnight and thereafter stored at room temperature for the enzymatic hydrolysis stage. The glucose recovered after the enzymatic hydrolysis stage was used as an index of pretreatment efficiency for all seventeen

experiments.

2.2.2. Combined mixture and factorial optimization

Following the primary screening stage, a combined mixture and factorial design was implemented for an extensive optimization of the key pretreatment input parameters. This was performed to examine the threshold level that yields maximum glucose when a mixture ratio of GLD and CCW was employed. Pretreatment input parameters comprised of the mixture components: CCW concentration (20–80%, w/v) and GLD concentration (20–80%, w/v) while the factorial design incorporated the solid loading (5–20%) and heating time (30–60 min) parameters (Table 1). The addition of the mixture components consisting of CCW and GLD concentration gave a total of 100% when combined and was dependent on the solid loading (SL) parameter. For example, a 20% CCW, 80% GLD and 10% SL corresponded to a 10 g SL that comprised of 2 g CCW and 8 g GLD immersed in a 100 mL constant reaction volume. The combined optimization generated twenty-eight experimental runs as shown in Table 1. The pretreatments were carried out in a laboratory autoclave at a fixed temperature of 121 °C. The pretreated CCW was filtered, washed and dried as previously stated above.

2.3. Enzymatic saccharification

The GLD pretreated biomass was submerged in sodium citrate buffer (pH 4.8, 0.05 M) with a working volume of 10 mL. Standard enzyme loading (10 FPU/g) and solid loading (10%, w/v) were used. The enzymatic hydrolysis stage was performed at a temperature of 50 °C and an agitation of 120 rpm for 72 h. After the 72 h incubation period, the samples were centrifuged at 9000 rpm for 5 min to remove any unhydrolyzed biomass. The glucose that was released after enzymatic saccharification was analysed using Megazyme glucose kits (©Megazyme, Wicklow, Ireland). The sugar yields obtained were used to fit the polynomial model equation that relates the input parameters to the glucose yields using Design Expert software, V11 (Stat-Ease Inc., USA).

2.4. Yeast inoculum development

Yeast peptone dextrose (YPD) growth medium was used and contained bacteriological peptone (20 g/L) (catalogue no. 91249), glucose (20 g/L) (catalogue no.108337) and yeast extract (10 g/L) (catalogue no. 111926). The YPD medium was inoculated with *Saccharomyces cerevisiae* BY4743 and the cells were grown at 30 °C and 120 rpm for 16 h. The initial yeast culture contained 7.25×10^6 cells/mL and was counted using a Neubauer counting chamber (Neubauer, Germany).

2.5. Bioethanol process type

Three different bioprocess types were performed with a final working volume of 25 mL and the specified conditions detailed below (Table 2).

2.5.1. Separate hydrolysis and fermentation (SHF) process

A separate enzymatic hydrolysis stage was carried out for all SHF experiments. The enzymatic reaction solution (30 mL) contained pretreated CCW with solid loading of 10% (w/v), sodium citrate buffer (pH 4.8, 0.05 M) and enzyme loading of 10 FPU/g in 100 mL Erlenmeyer flasks. The reaction flasks were incubated at 50 °C and 120 rpm for 72 h. The hydrolysate solution was centrifuged at 9000 rpm for 5 min to remove the biomass and 20 mL of the supernatant (containing glucose) was added to 2.5 mL of nutrients (10 g/L peptone and 5 g/L yeast extract) and 2.5 mL of the *S. cerevisiae* cells. The fermentation stage (25 mL) was carried out at 35 °C and 120 rpm for 24 h.

2.5.2. SSF with pre hydrolysis (PSSF) process

A 24 h pre hydrolysis stage was performed for the PSSF experiments. The reaction solution (20 mL) contained pretreated CCW with a 10% (w/v) solid loading, sodium citrate buffer (pH 4.8, 0.05 M) and 10 FPU/g enzyme loading and were incubated at 50 °C and 120 rpm for a 24 h pre hydrolysis stage. After the short pre hydrolysis stage (24 h), nutrients (2.5 mL made up of 10 g/L peptone and 5 g/L yeast extract) and *S. cerevisiae* cells (2.5 mL) were added directly to the 20 mL enzymatic hydrolysate (uncentrifuged) and incubated at 35 °C and 120 rpm for 24 h.

2.5.3. Simultaneous saccharification and fermentation (SSF) process

The SSF reaction solution (25 mL) contained the pretreated CCW with a 10% (w/v) solid loading, 10 FPU/g enzyme loading, sodium citrate buffer (pH 4.8, 0.05 M), 2.5 mL nutrients and 2.5 mL *S. cerevisiae* cells. The SSF flasks were incubated at 35 °C and 120 rpm for 24 h.

For the sample analysis, 0.5 mL aliquots were extracted in 2 h intervals from all three process types (SHF, PSSF and SSF). All three fermentation experiments were performed with corresponding controls (uninoculated) for estimation of the initial glucose concentration.

2.6. Analytical methods

2.6.1. Scanning electron microscopy (SEM)

The CCW (untreated and optimally pretreated) were visualised using SEM. CCW samples were first fixed onto aluminium specimen mounts, then gold sputter coated (Q150R ϵS) and viewed at a magnification of 500× (ZEISS EVO LS 15) [12].

2.6.2. Fourier Transform Infrared (FTIR) analysis

To detect functional group changes, the untreated and optimally pretreated CCW samples were examined by Fourier Transform Infrared (FTIR) spectroscopy using a Perkin Elmer 100 (Waltham, MA, USA). The CCW samples were milled with spectroscopic grade KBr and compressed to form diameter pellets. The resulting FTIR spectra were recorded between 400 and 4000 cm^{-1} [12].

2.6.3. Ethanol, biomass and glucose concentration

The ethanol content (g/L) was analysed using Megazyme ethanol assay kits, product code (K-ETOH) (©Megazyme, Wicklow, Ireland)

Table 2
Methodology of the different process types (SHF, PSSF and SSF) for bioethanol production from corn cob waste.

Bioethanol process type	Enzymatic hydrolysis conditions	Fermentation parameters
SHF	10% SL, 30 mL buffer[a], 10 FPU/g Cellic CTec2[b] and incubated at 50 °C and 120 rpm for 72 h	20 mL hydrolysate[c], 2.5 mL nutrients[e], 2.5 mL inoculum[f] and incubated at 35 °C and 120 rpm for 24 h
PSSF	10% SL, 20 mL buffer[a], 10 FPU/g Cellic CTec2[b] and incubated at 50 °C and 120 rpm for 24 h	20 mL hydrolysate[d], 2.5 mL nutrients[e], 2.5 mL inoculum[f] and incubated at 35 °C and 120 rpm for 24 h
SSF	No separate enzymatic hydrolysis stage	10% SL, 20 mL buffer[a], 10 FPU/g Cellic CTec2[b], 2.5 mL nutrients[e], 2.5 mL inoculum[f] and incubated at 35 °C and 120 rpm for 24 h

Note: SHF = Separate hydrolysis and fermentation, PSSF = Simultaneous saccharification and fermentation with pre hydrolysis, SSF = Simultaneous saccharification and fermentation, SL = solid loading, [a] = sodium citrate buffer, [b] = enzyme loading, [c] = enzyme hydrolysate (obtained and centrifuged from enzymatic stage), d = enzyme hydrolysate (uncentrifuged), [e] = nutrients (10 g/L peptone and 5 g/L yeast extract), [f] = *S. cerevisiae* cell suspension volume.

Table 3
Analysis of variance (ANOVA) of the developed GLD pretreatment model.

Factor	Sum of Squares	Degrees of freedom (*df*)	Mean Square	F-value	p-value (probability > F)	
Intercept or Model	0.3043	19	0.0160	5.49	0.0094	Significant
Linear Mixture	0.1536	1	0.1536	52.61	< 0.0001	
AC	0.0002	1	0.0002	0.0714	0.7961	
AD	0.0028	1	0.0028	0.9533	0.3575	
BC	0.0380	1	0.0380	13.03	0.0069	
BD	0.0018	1	0.0018	0.6181	0.4544	
CD	0.0046	1	0.0046	1.56	0.2468	
ACD	0.0008	1	0.0008	0.2872	0.6066	
BCD	0.0046	1	0.0046	1.56	0.2468	
AC^2	0.0070	1	0.0070	2.40	0.1598	
AD^2	0.0055	1	0.0055	1.89	0.2067	
BC^2	0.0009	1	0.0009	0.3123	0.5916	
BD^2	0.0024	1	0.0024	0.8244	0.3904	
AC^2D	0.0026	1	0.0026	0.8983	0.3710	
ACD^2	0.0000	1	0.0000	0.0054	0.9430	
BC^2D	0.0001	1	0.0001	0.0439	0.8393	
BCD^2	0.0039	1	0.0039	1.35	0.2785	
AC^3	0.0003	1	0.0003	0.0870	0.7756	
AD^3	0.0021	1	0.0021	0.7297	0.4178	
BC^3	0.0410	1	0.0410	14.03	0.0057	
BD^3	0.0001	1	0.0001	0.0382	0.8498	
Residual error	0.0234	8	0.0029	–	–	
Cor Total	0.3277	27				

Footnote: A = CCW (%), B = GLD (%), C = SL (%), D = heating time (min).

with its specified protocols and VERSAmax tuneable microplate reader (Molecular Devices, California, USA). Glucose concentrations in the three different ethanol fermentation process types and their corresponding control samples were detected by using Megazyme glucose kits product code (K-GLUC), (©Megazyme, Wicklow, Ireland). The *S. cerevisiae* biomass concentration (g/L) was quantified by relating the yeast cell count as a function of the dry cell weight. An exponentially growing *S. cerevisiae* culture cultivated in YPD broth was diluted (1, 1/2, 1/4, 1/8 and 1/16). A total volume of 10 mL of each of the aforementioned dilutions was centrifuged at 5000 rpm for 10 min. The resulting supernatant was discarded, and the biomass pellet was dried in an oven at 90 °C until a constant mass was obtained. Subsequently, a standard curve relating the cell dry weights (g/L) with its corresponding cell counts (cells/mL) was plotted and an equation generated. The experimental biomass concentration from the kinetic experiments was thereafter extrapolated by substituting the yeast cell count in the equation from the standard curve.

2.7. Kinetic assessment

2.7.1. The logistic model

The logistic model in the differential form of Eq. (1) was integrated to form Eq. (2) that represents the exponential and stationary phases of growth. This logistic model illustrates the relationship of biomass (X) to initial cell concentration (X_0), maximum cell concentration (X_{max}) and maximum specific growth rate (μ_{max}) at specific times (t) during the exponential and stationary phases of yeast growth. Nevertheless, it does not predict the death phase of microorganisms after the stationary phase [34].

$$\frac{dX}{dt} = \mu_{max}\left(1 - \frac{X}{X_{max}}\right)X \tag{1}$$

$$X = \frac{X_0 \exp(\mu_{max} t)}{1 - \left[\left(\frac{X_0}{X_{max}}\right)(1 - exp(\mu_{max} t))\right]} \tag{2}$$

2.7.2. The modified Gompertz model

The bioethanol production data derived from the different fermentation process types were used to fit the modified Gompertz model by using the least squares method (CurveExpert V1.5.5, MyBiosource, Inc., USA). This model revealed the lag time (t_L), maximum bioethanol production rate ($r_{p,m}$), and the maximum potential bioethanol concentration (P_m) as shown in Equation (3).

$$P = P_m . exp\left\{-exp\left[\frac{r_{p,m} . exp(1)}{P_m}\right] . (t_L - t) + 1\right\} \tag{3}$$

where P is bioethanol concentration (g/L), P_m is potential maximum bioethanol concentration (g/L), $r_{p,m}$ is maximum bioethanol production rate (g/L/h) and t_L is the time from the beginning of fermentation to exponential bioethanol production (h).

3. Results and discussion

3.1. Primary screening stage

The initial primary GLD pretreatment screening stage (Box-Behnken design) was used to briefly assess the pretreatment input ranges for maximum glucose recovery from CCW. A high coefficient of determination (R^2) value of 0.958 was achieved for the primary screening RSM model. Additionally, analysis of variance (ANOVA) further confirmed the model fitness and reliability (Table S2). Glucose yields of 0.07 g/g and 0.34 g/g were obtained for the untreated (native) and optimally pretreated (10% substrate solid loading, 14.28% GLD at 115 °C) samples respectively, resulting in a 79% increase in glucose after pretreatment (Table S1). Following the primary screening using the Box-Behnken design, the combined mixture and factorial design was used for an extensive pretreatment optimization of key process parameters. The pretreatment parameter ranges selected for the mixture components (20–80% GLD concentration and 20–80% CCW concentration) and SL (5–20%) corresponded to 1–16% (w/v) GLD concentration and 1–16% (w/v) CCW concentration. These parameter ranges were previously determined from the optimum pretreatment conditions obtained for the primary screening RSM model (10% substrate solid loading, 14.28% GLD at 115 °C). Furthermore, the effect of heating time (30–60 min) was investigated for the combined mixture and factorial design.

3.2. Combined RSM mixture and factorial model development

The combined RSM mixture and factorial model suitability was tested using the Analysis of Variance (ANOVA) and these results are presented in Table 3. RSM model significance is denoted by having p-values < 0.05 [35]. A high F-value (5.49) and low p-value (0.0094) were noted and illustrated model significance. Additionally, parameter significance is determined using the p-values (< 0.05). The linear mixture that comprised of the CCW and GLD concentration (p-value < 0.0001) was shown to have the most significant effect followed by the SL and GLD interaction (p-value = 0.0069). The mixture components of CCW and GLD concentration were expressed as a combined entity and comprises of a total of 100%. For instance, the CCW concentration represented by factor A (Table 3) is considered as both the CCW concentration + GLD concentration since these parameters consist of the mixture components in the present RSM model and always occur together. Similarly, the GLD concentration (factor B) denotes both the GLD concentration + CCW concentration. The significant effect of the linear and combined interactions of the CCW and GLD concentration parameters may be attributed to changes in the substrate quantity and alkalinity of the pretreatment. While the CCW concentration impacts the substrate surface area accessibility for lignocellulosic degradation, the GLD concentration influences the alkalinity of the solution that directly affects the delignification process [36].

The coefficient of determination (R^2) has been used as a statistical index to evaluate RSM models. A high R^2 value of 0.9287 was obtained and was indicative of a high correlation between the predicted and observed results. Therefore, the combined RSM mixture model could account for 92.87% of variation in the observed data. The proposed model proved to have a good data representation and relationship between the parameters and the responses. The polynomial model Equation (4) below was generated for the glucose yield response and describes the relationship between the parameters and glucose output.

$$\begin{aligned}\text{Glucose yield}\left(\tfrac{g}{g}\right) = {} & 0.177A + 0.368B + 0.0739AC + 0.136AD \\ & - 0.735BC + 0.110BD - 0.0222ACD \\ & - 0.0483BCD - 0.0927AC^2 + 0.0738AD^2 \\ & + 0.0444BC^2 - 0.0699BD^2 + 0.115AC^2D \\ & - 0.00508ACD^2 - 0.0223BC^2D + 0.0802BCD^2 \\ & - 0.0813AC^3 - 0.189AD^3 + 0.715BC^3 \\ & - 0.0397BD^3 \end{aligned} \quad (4)$$

where, A, B, C and D are CCW (%), GLD (%), SL (%) and heating time (min), respectively.

3.3. Interactive effects of the pretreatment inputs on the glucose recovery

The operational process parameters with corresponding glucose yields are depicted in Table 1. The glucose yields ranged from 0.02 to 0.41 g/g. Pretreatment experimental run 21 (20% CCW, 80% GLD, 12.5% SL and 52.5 min) and run 24 (20% CCW, 80% GLD, 5% SL and 60 min) both gave the highest glucose yield of 0.41 g/g. On the other hand, the lowest glucose yield (0.02 g/g) was observed for run 18 (20% CCW, 80% GLD, 15% SL ratio and 30 min). Additionally, when all the input process parameters were held at their median values (run 1, 2, 27 and 28), the glucose yields ranged between 0.25 and 0.31 g/g.

Interactions between the various process inputs and glucose yields are illustrated in Fig. 1A-C. The interactive effect of heating time and SL while the GLD and CCW concentration was maintained at their midpoint values is depicted in Fig. 1A. Simultaneous increases in the heating time and SL from 30 to 55 min and 5 to 8% respectively, raised the glucose yield from 0.14 to 0.46 g/g. A longer heating time along with the pretreatment chemical agent increases the degradation process of the lignin recalcitrant structures [35]. The higher glucose yield at longer heating times may be attributed to the increased contact time for

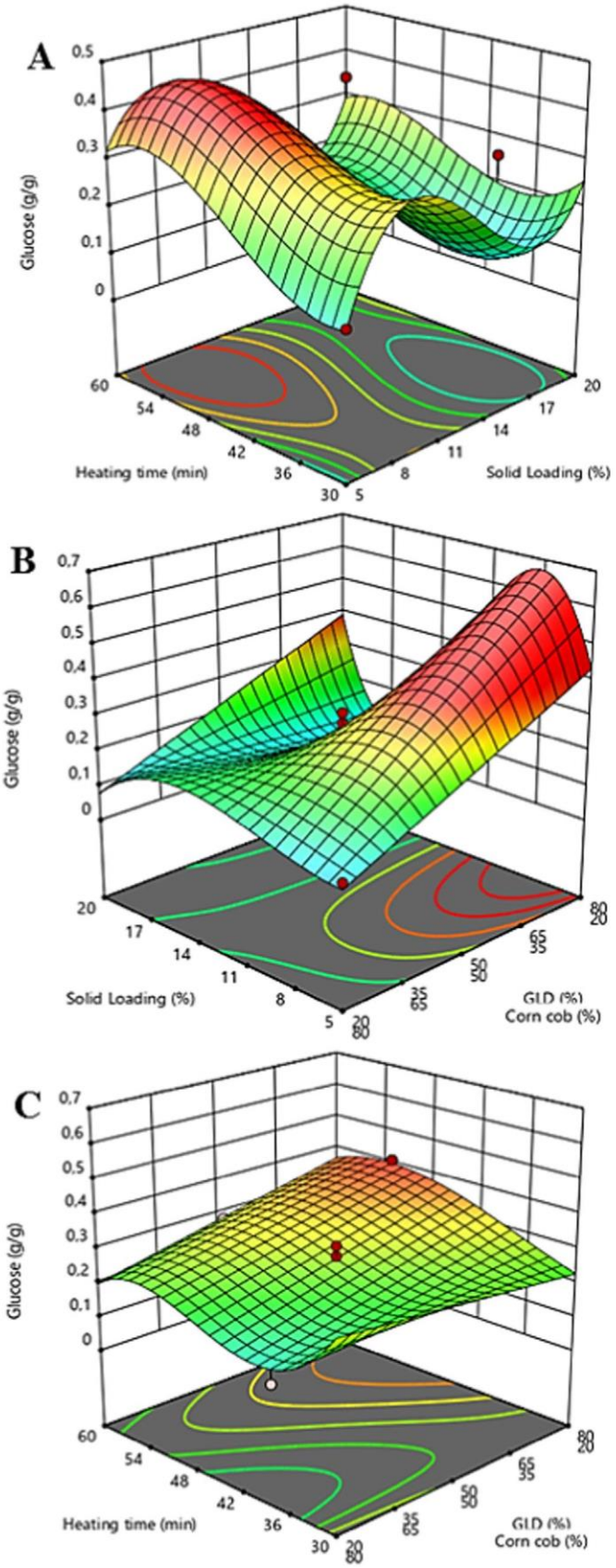

Fig. 1. Response surface graphs demonstrating the effect of the various input parameter interactions that affect the glucose recovery from corn cobs (A) heating time (min) and solid loading (%), (B) solid loading (%) and GLD to CCW (%), (C) heating time (min) and GLD (%) to CCW (%).

polymeric sugar degradation into monomeric sugars [37]. Moodley and Gueguim Kana [17] reported similar results, where increases in the pretreatment time from 2 to 8 min resulted in a reducing sugar increment from 0.10 to 0.185 g/g. Moreover, the low SL (5–8%) provides easy accessibility of the pretreatment chemical to penetrate the biomass due to the larger surface area present [38].

Further increases in the heating time (55–60 min) and SL (8–17%), negatively influenced the glucose yield (0.46–0.16 g/g). Longer pretreatment heating times accelerate the evaporation process and this may reduce the pretreatment efficiency [39]. Water plays an essential role in pretreatment reactions for mass transfer by diffusion and aids with chemical processes that occur within the pretreatment reactor. Water molecules bind differently to varying fractions of lignocellulosic material. For example, hemicellulose has a high water-holding capacity, whereas lignin and cellulose display the opposite [40]. Therefore, an increase in the viscosity of the pretreatment solution by decreasing the lubricity of the particles (high SL $>$ 8%), provides a poor medium for solubilization of sugars [41]. The study by Raghavi et al. [38] observed that the sugar yield decreased from 0.70 to $<$ 0.50 g/g when the SL was increased from 10 to 15% using a NaOH-based pretreatment.

The combined effects of SL, GLD and CCW concentration on the glucose yield are shown in Fig. 1B while the heating time was kept at its median value. Increments in the SL (5–8%) and GLD (20–80%) with decreasing CCW (80–20%) concentration led to an increase in the glucose yield from 0.10 to 0.68 g/g. The powder-like GLD chemical does not substantially affect the viscosity of the solution when its concentration was increased beyond 20% (20–80%). Saturated GLD solutions lead to an enhancement in the surface area accessibility to the lignocellulosic biomass. This provides a higher permeation efficiency of the resistant structures, which make it vulnerable to enzymatic attack and can account for the higher glucose yields observed [35]. Moreover, the higher SL (5–8%) increases both the GLD and CCW concentrations, accordingly. The proportional increment in both these parameters ensures that sufficient GLD is available to penetrate the increased CCW concentration. A similar observation was recorded by Sewsynker-Sukai and Gueguim Kana [12], where a low SL ($<$ 7.5%) and high alkalic salt concentration (5–15% $Na_3PO_4{\cdot}12H_2O$) increased the sugar yield from 0.73 to 1.1 g/g. Alkaline pretreatments cause swelling of the lignocellulosic biomass, thereby increasing the surface area for improved penetration [18,42]. This results in hydrolytic cleavage of intermolecular ester bonds and the dissolution of lignin and hemicellulose [43]. Pretreatment catalysts such as alkaline chemicals modify the physical properties (porosity, crystallinity and weight reduction) of the biomass [43]. On the other hand, further increments in the SL from 8 to 17% with a fixed GLD (80%) and CCW (20%) decreased the glucose yield from 0.66 to 0.1 g/g. Despite the high SL ($>$ 8%) that induces saturated GLD solutions (80%) and low CCW concentrations (20%), the pretreatment process is limited because insufficient biomass is available for lignocellulosic degradation and the pretreatment threshold is reached.

Fig. 1C illustrates the relationship between heating time, GLD and CCW concentration when the SL was maintained at its centre point. A simultaneous increase in the heating time (30–60 min) while the GLD and CCW was maintained at 20 and 80%, respectively, resulted in a slight decrease in the glucose recovery (0.26–0.21 g/g). This illustrated that the heating time did not significantly impact on the glucose yields at GLD (20%) and CCW (80%). The low glucose yields may be ascribed to the low GLD concentration (20%), thus preventing sufficient diffusion of the high CCW concentration (80%) that reduces the water availability. The resulting slurry (20% GLD and 80% CCW concentration) is highly viscous and restricts adequate GLD penetration and mixing. Water provides a platform for temperature increases when the heating time is increased, however, regardless of the longer heating times, thermal transfer is restricted within dense slurries [35] and may have led to the lower glucose yield (0.21 g/g). Incremental variations in the heating time (30–52 min) and GLD (20–80%) with a decrease in CCW (80–20%) concentration, increased the glucose yield from 0.21 to 0.40 g/g. The heating time affects the substrate penetration efficiency whereas the CCW and GLD concentration influences the viscosity and delignification respectively [36] and can account for the improvements in the glucose yield.

3.4. Validation of the developed GLD pretreatment model

Experimental validation of the developed model was performed, and these data were compared to the model predictions. Optimum input parameters of 20% CCW, 80% GLD, 19.833% SL and a heating time of 50.33 min resulted in an experimental glucose yield of 0.42 g/g compared to 0.43 g/g predicted by the model. The slight variation (2.33%) between the experimentally observed glucose yield and the model predicted value was considered negligible.

3.5. Impact of the GLD pretreatment on the lignocellulosic structure

The native and optimized CCW samples comprised of cellulose (40.49% and 57.71%), hemicellulose (42.95% and 22.62%) and lignin (7.18% and 12.12%), respectively (Table S3). GLD pretreatment (pH 11–13) has been shown to mimic effective alkaline catalysts such as NaOH (pH 13). Strong alkaline agents result in the hydrolytic cleavage of the ester bond between the lignin and hemicellulose polymers causing structural damage [43]. The alkaline pretreatment characteristics of GLD can account for the 42.53% improvement in cellulose content coupled with a 47.33% hemicellulose solubilisation for the optimized CCW sample. The increase in lignin (68.80%) may be due to the synthesis of pseudo-lignin that results from the degradation of sugar compounds providing “false” lignin detection [44]. Sahare et al. [36] and Xie et al. [10] reported similar cellulose improvements (38.46% and 86.46%) and hemicellulose solubilisation (8.65% and 46.61%) with a 50% and 6.86% lignin removal respectively when treating CCW with NaOH.

Morphological changes in the CCW biomass due to GLD pretreatment were visualised using scanning electron microscopy (SEM) (Fig. S1). The native (untreated) CCW exhibited a smooth, rigid and compressed surface due to the tightly packed lignin fibre networks. Conversely, the optimized GLD pretreated sample demonstrated perforations, fractures and indentations on the surface of the biomass indicating unwinding of the resistant lignocellulosic structures. GLD pretreatments like alkaline techniques disrupt ester bonds between lignin causing these components to be solubilized and appear as fragile and distorted structures [41]. Similar visual observations were previously noted by Sahare et al. [36], Moodley and Gueguim Kana [19] and Zheng et al. [45] using NaOH-based pretreatments.

In addition to SEM analysis, FTIR spectra compared the chemical changes in the untreated and optimized GLD pretreated CCW (Fig. S2). Absorption peaks with reference to the cellulose, hemicellulose and lignin moieties displayed a stronger banding pattern for the optimized GLD pretreatment sample compared to the native (untreated) biomass. The band formation at 3435 cm^{-1} and 2928 cm^{-1} wavelengths were attributed to O—H stretching vibration and C—H stretching vibration of intramolecular hydrogen within the cellulose structure respectively [35,45]. The peaks showing the C—O—C function group associated with the pyranose ring skeletal vibration (1044 cm^{-1}), asymmetric stretching (1165 cm^{-1}) and β-glycosidic linkage stretching (900 cm^{-1}) displays effective liberation of cellulose as a result of the GLD pretreatment [12]. The aromatic skeleton stretching vibration of the C=C bond of lignin was observed at 1457 cm^{-1} and 1651 cm^{-1} [46]. In addition, the band at 1382 cm^{-1} may be ascribed to the C—H bending vibrations in the cellulose and hemicellulose moieties [47]. These results were in accordance with previous lignocellulosic studies on alkaline-based pretreatment strategies [12,19,45].

3.6. Comparison of the developed GLD pretreatment with other studies on CCW

Sugar yields from various previous pretreatment methods on CCW were compared with the present study (Table S4). The optimized GLD pretreatment resulted in a higher sugar yield when compared to previous studies using CCW. For instance, the present optimized GLD pretreatment gave a 43% higher sugar yield compared to Sewsynker-Sukai and Gueguim Kana [39] using a microwave-assisted alkalic salt pretreatment. In addition, the current study observed a 1.83-fold increment in sugar yield compared to the previous report by Amenaghawon et al. [48] when CCW was treated with a dilute acid method (1.72% H_2SO_4). Likewise, a previous report by Potumarthi et al. [13] on CCW pretreatment under alkaline conditions (1 M NaOH) noted a 73.81% lower sugar yield when compared to the present study. GLD is a paper mill chemical waste material generated from the kraft pulping industry and is made up of $CaCO_3$, Na_2CO_3 and Na_2S [24]. Interestingly, the nature of these species ($CaCO_3$, Na_2CO_3 and Na_2S) present within GLD contributes to its strong alkalinity (pH > 10) and excellent buffering capacity, which are generally viewed as advantageous characteristics in pretreatment systems [8,25]. The chemical reaction mechanisms of GLD mimic strong alkaline-catalysts such as NaOH. For instance, the effectiveness of Na_2CO_3 is due to the cleavage of the ester and glycosidic bonds in the cell wall matrix, resulting in the alteration of lignin structure, cellulose swelling, and the partial decrystallization of cellulose [20]. Additionally, the strong nucleophilic attack of the HS^- species of the Na_2S promotes the cleavage of phenolic β-aryl ether bonds of lignin, while removing lignin moieties with limited attack on carbohydrates [23]. On the other hand, the pretreatment mechanism of NaOH results in the hydrolysis of ester bonds between the ferulic acid and hemicellulose causing lignin solubilisation and lignocellulose particle swelling [41]. The higher sugar yield observed in the present study may be attributed to the combined effect of the alkaline species within GLD since it targets various sites in cellulose, hemicellulose and lignin while NaOH mainly attacks the lignin matrix. Moreover, the parameters selected for pretreatment optimization in the present work significantly impacted on the glucose yield. Conversely, Gao and Rehmann [49] and Boonsombuti et al. [50] investigated NaOH pretreatments using corn cobs and observed a reducing sugar concentration of 0.92 g/g and 0.68 g/g respectively. Although the aforementioned studies displayed a higher sugar yield compared to the optimized GLD pretreatment, the reducing sugar yield incorporates all monosaccharides (glucose, fructose, galactose), along with some disaccharides (sucrose, lactose, maltose), oligosaccharides (raffinose), and polysaccharides (starch, glycogen, cellulose). Therefore, some sugars that make up the reducing sugars are not indicative of a high glucose concentration and are not a true representation of the fermentable sugars. The metabolic pathways within standard ethanol fermenting microbes such as *S. cerevisiae* harness glucose only and are unable to utilise complex sugars [51,52]. The GLD pretreatment system demonstrates advantageous characteristics such as low cost, paper waste recycling and abundance of chemical for the treatment of lignocellulosic biomass. This drives industry to engage in such methods as opposed to expensive alkaline pretreatments such as NaOH which provide similar or less treatment effects.

3.7. Kinetic modelling of:

3.7.1. Cell growth for the SHF, SSF and PSSF processes

The *S. cerevisiae* cell growth under SHF, SSF and PSSF processes is shown in Fig. 2A. Yeast cells have three main growth phases that encompass the lag phase, exponential phase and stationary phase. During the lag phase, cells are biochemically active and synthesise the necessary intermediate metabolites and enzymes in preparation for rapid cell growth. The cell number remains relatively constant during their acclimatisation stage, which is usually approximately 2 h, depending on the yeast species [53]. A short lag phase of the *S. cerevisiae* BY4743 lasted for a period of 2 h for all three bioprocess types and is in accordance with previous studies on *S. cerevisiae* [30,31,52]. Once a specific cell concentration is reached, metabolic activity increases and DNA replication occurs, which in turn causes cell doubling and is known as the exponential phase [53]. Similar exponential phase times were displayed by the SHF (2.5–18 h), SSF (2.5–17 h) and PSSF (2.5–20 h) process types in the present study. The sharp increments in *S. cerevisiae* cell growth during the exponential phase corresponded to high glucose utilisation of > 12%, > 46% and > 59% for the SHF, SSF and PSSF bioprocesses respectively (Fig. S3). The exponential phase was directly followed by the stationary phase, in which, cell growth and thus metabolic processes cease. Complete utilisation of glucose (100%) occurred after 8 h (SSF and PSSF process) and 20 h (SHF system) which initiated the stationary phase. The sugar utilisation data observed for the SSF and PSSF systems (100% after 8 h) is attributed to the simultaneous consumption and production of glucose that is a major characteristic of these process types.

The *S. cerevisiae* cell biomass concentration over the 24 h period (Fig. 2A) was used to fit the logistic models with high correlation coefficients (R^2) of 0.93 (SHF), 0.97 (SSF) and 0.98 (PSSF). The highest maximum specific growth rate (μ_{max}) of 0.61 h^{-1} was observed for the PSSF process, followed by SHF (0.28 h^{-1}) and the SSF (0.23 h^{-1}) process (Table 4). The high μ_{max} value recorded for the PSSF process (0.61 h^{-1}) may be attributed to the readily available glucose that was present in the enzyme pre hydrolysate. The presence of glucose in the PSSF experiment has shown to speed the yeast cell growth compared to the SSF process (0.23 h^{-1}) that does not contain sugar initially for consumption. On the other hand, the relatively low μ_{max} value recorded for the SHF (0.28 h^{-1}) system could be due to the high glucose concentration (34.65 g/L) present at the beginning of the fermentation. This increases the glycolytic rates and drives the cells metabolic processes toward ethanol production instead of cell growth [54]. In addition to this, high glucose levels may cause sugar osmotic shock on the yeast cells and is accompanied by water outflow, cell membrane damage and the shrinking of microbial cells [55].

The maximum cell concentration (X_{max}) obtained for the SSF process (1.63 g/L) was higher than the PSSF (0.87 g/L) and SHF (0.70 g/L) processes. The lower X_{max} values obtained for the PSSF and SHF processes compared to the SSF system may be ascribed to the Crabtree effect. Yeast cells such as *S. cerevisiae* follow the ethanol fermentation pathway when excess glucose is available in the presence of oxygen (aerobic or microaerophilic environments). This is commonly known as the Crabtree effect and has shown to be the major cause of low yeast cell concentrations by SHF and PSSF systems because these processes contain a high initial glucose concentration that drive the microbial metabolism towards ethanol production instead of cell growth [54]. Previous ethanol kinetic reports on bioethanol production gave comparable μ_{max} and X_{max} in the range of 0.19–0.28 h^{-1} and 2.53–8.38 g/L respectively [3,31,52]. Differences observed in the kinetic coefficients between the present work and previous studies may be as a result of substrate type and concentration, yeast strain and process operating conditions.

3.7.2. Bioethanol production for the SHF, SSF and PSSF processes

Bioethanol production under SHF, SSF and PSSF processes are depicted in Fig. 2B. Short lag phases (4 h) for all three process types were noted during bioethanol evolution. The ethanol concentration rapidly increased from 3.54 to 23.69 g/L between 4 and 18 h for the SHF process. Similarly, the PSSF process displayed sharp inclinations in the ethanol concentration from 1.99 to 20.12 g/L between 4 and 20 h. The SSF system, however, required a longer time (4–24 h) to reach peak ethanol production (1.84–17.49 g/L). Peak ethanol production in all three processes coincided with maximum glucose utilisation. The maximum bioethanol concentration occurred during the exponential phases for all three bioprocesses due to high metabolic rates and glucose consumption, which results in the conversion of the readily

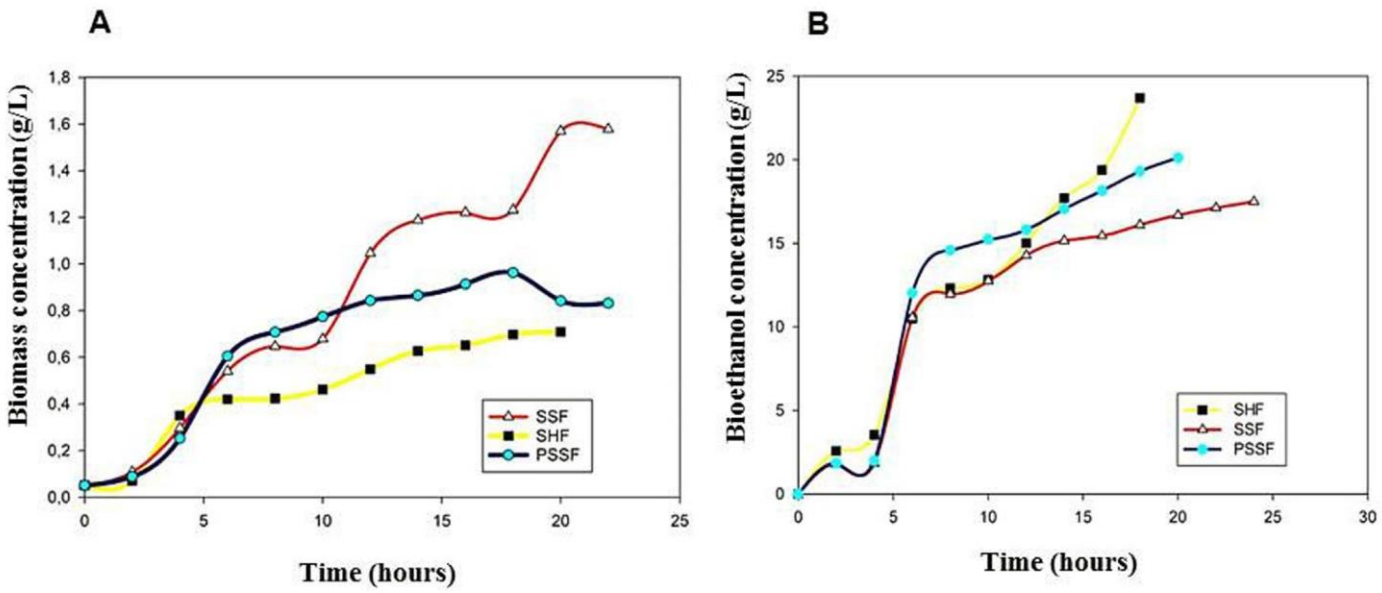

Fig. 2. *S. cerevisiae* cell growth (A) and bioethanol production (B) for the SHF, SSF and PSSF processes.

Table 4
Cell growth kinetic parameters obtained from the logistic model for the SHF, SSF and PSSF processes.

Kinetic parameter	Bioethanol process type		
	SHF	SSF	PSSF
μ_{max} (h^{-1})	0.28	0.23	0.61
X_o (g/L)	0.11	0.13	0.03
X_{max} (g/L)	0.70	1.63	0.87

Footnote: μ_{max} = maximum specific growth rate, X_o = initial cell concentration, X_{max} = maximum cell concentration.

available sugars to ethanol via fermentative metabolism. *S. cerevisiae* follows the glycolytic pathway to obtain energy for cell growth by catabolism of glucose into two carbon dioxide molecules and produces ethanol as a by-product in the presence of oxygen [53].

Higher bioethanol concentrations observed for the SHF process (23.69 g/L) was due to a 72 h separate hydrolysis stage while the PSSF system (20.12 g/L) only involved a 24 h pre hydrolysis stage. Nevertheless, both these systems produced a high initial glucose for utilisation. Likewise, the lower ethanol concentration (17.49 g/L) obtained for the SSF process was attributed to slow release rates of glucose since the enzyme (Cellic CTec 2) was functioning below its optimum temperature [56]. Additionally, the SHF and PSSF process types achieve higher saccharification rates and lower initial viscosity at the start of the fermentation process compared to the SSF system [52,57].

The glucose utilisation displayed a directly proportional relationship with ethanol formation. The initial low ethanol concentration in the PSSF process may be ascribed to the high metabolic activity and low cell number during the lag phase. Upon depletion of the initial fermentable sugar, the bioprocess is assumed to follow the trend of the SSF process by simultaneously producing and consuming the glucose. The similar bioethanol concentration values observed for the SSF (17.49 g/L) and PSSF (20.12 g/L) processes implies that the pre hydrolysis stage in the PSSF system did not significantly influence ethanol formation. However, the pre hydrolysis stage in the PSSF process caused a sharper initial increment in the ethanol production over a shorter fermentation time (20 h) compared to the SSF system (24 h). The ethanol production for all three fermentation process types halted after the exponential phase due to glucose depletion and this causes diauxic shifts that lead to the generation of CO_2 [53]. The previous study by Saha et al. [9] comparatively evaluated SHF and SSF processes and achieved similar ethanol concentrations (SHF = 21.90 g/L and SSF = 17.40 g/L) to the present study. Dahnum et al. [27] and Szambelan et al. [58] deduced that SSF processes resulted in high ethanol production with a lower economic investment and energy efficiency over the SHF process.

The bioethanol concentration data (Fig. 2B) were used to fit the modified Gompertz model with high R^2 values for the SHF (0.96), SSF (0.97) and PSSF (0.96) processes. The model obtained short lag times (t_L) of 0.71, 2.02 and 2.73 h for SHF, SSF and PSSF processes respectively (Table 5). Longer lag times obtained for the SSF and PSSF processes may be accounted for by the diversion of yeast metabolism toward cell development, replication and maintenance [52]. Maximum ethanol production rates ($r_{p.m}$) for the SHF, SSF and PSSF bioethanol processes were 1.48, 2.06 and 3.08 g/L/h respectively. The higher glucose accumulation in SHF processes may cause inhibition of the yeast culture and result in low ethanol productivity. This is because sugar osmotic effects on the yeast cells halt metabolic activities towards ethanol fermentation [52,59]. The high maximum cell concentration achieved by the SSF (1.63 g/L) and PSSF (0.87 g/L) processes further elucidates the high ethanol production rates and longer lag times. Similarly, Yeh et al. [60] revealed that a higher enzyme loading resulted in rapid glucose accumulation and lower ethanol production rate due to sugar inhibition of yeast fermentation.

The SHF process generated a high maximum potential bioethanol concentration (P_m) of 26.82 g/L while SSF process and PSSF showed slightly lower values of 16.49 and 18.28 g/L respectively. The main advantage of the SHF and PSSF processes is the initial glucose available for the yeast culture at the beginning of the fermentation process [57]. Unlike the SHF and PSSF process types, SSF systems experience several difficulties due to the high viscosity of solutions because of the absence of liquefaction of the solid biomass during the enzymatic step [9]. The

Table 5
Bioethanol kinetic parameters achieved for the SHF, SSF and PSSF processes using the modified Gompertz model.

Kinetic parameter	Bioethanol process type		
	SHF	SSF	PSSF
P_m (g/L)	26.82	16.49	18.28
$r_{p.m}$ (g/l/h)	1.48	2.06	3.08
t_L (h)	0.71	2.02	2.73

Footnote: P_m = maximum potential bioethanol concentration, $R_{p.m}$ = maximum bioethanol production rate, t_L = lag time.

difference in optimum temperature for both the cellulase-based enzyme (50 °C) and the ethanol producing *S. cerevisiae* (30–37 °C) reduces the efficiency of the SSF system [56]. Moreover, the lack of initial sugar available for microbial consumption poses additional problems as a result of longer lag times. Nevertheless, cellulase-based high performing Cellic CTec2 enzymes display high saccharification efficiencies compared to traditional cellulase enzymes [52]. The present work utilises this enzyme, thus decreasing viscosity limitations that are commonly observed in SSF processes. Dahnum et al. [27] reported that SSF processes generated a higher ethanol yield and productivity due to simultaneous glucose production and consumption. This is because SSF systems prevent cellulase inhibition as a result of elevated glucose yields that accumulate during the enzymatic hydrolysis step. Nevertheless, PSSF process also assists with reducing enzyme inhibition by performing short hydrolysis steps (6–24 h). PSSF offers a hybrid system of both the SHF and SSF processes and have proven to be more efficient with regards to time and energy consumption, high ethanol yields and reduced contamination.

3.8. Comparison of bioethanol production with previous reports

Bioethanol production using different lignocellulosic substrates indicated that the experimental conditions used in this study for all three process types favoured higher bioethanol concentrations compared to previous reports (Table S5). For instance, the use of a 72 h separate hydrolysis step improved bioethanol concentrations by 7.66% compared to the SHF process using wheat straw by Saha et al. [9]. The study by Koppram et al. [61] investigated the SSF process using corn cobs and recorded a 7.13% lower bioethanol concentration compared to the current SHF process. Similarly, Saha et al. [9] assessed the SSF process type and noted a 1.01-fold lower bioethanol concentration when paralleled to the present SSF system. In the same vein, He et al. [62] evaluated an SSF process with a 4 h pre hydrolysis step and observed a 1.04-fold lower bioethanol concentration compared to this study (PSSF = 20.12 g/L). Moreover, the present work gave a 14.51% higher bioethanol concentration for the PSSF experiment compared to the study by Xie et al. [10]. Higher bioethanol concentrations were achieved with the SHF and PSSF bioprocesses in the present study and may be attributed to the readily available glucose that allow for increased metabolic rates of the microbial culture. However, the 72 h hydrolysis stage for the SHF process displayed no significant variations in comparison with the PSSF process. Over and above, despite the slightly higher bioethanol concentrations obtained for the SHF and PSSF process types, the SSF system has proven to be more attractive. This is because SSF systems eradicate cost and energy intensive enzymatic hydrolysis steps and will assist process design and economic feasibility for industrial scale lignocellulosic bioethanol production.

3.9. Economic considerations for lignocellulosic biorefineries using GLD

A suitable technoeconomic analysis is required in bioethanol production plants with the aim of providing information on the capital cost, operating expenses, by-product credit and ethanol production cost [63]. The capital investment of a lignocellulosic bioethanol production is dependent on the location and size of the process plant, as well as the type of feedstock and pretreatment strategy that will be used. Kang et al. [63] reported that the largest portion of a biorefineries' operating costs are attributed to feedstock expenses. Second generation bioethanol production utilises lignocellulosic biomass, which is the waste portion of feed harvests that are abundant and cheap, therefore eliminating the costs attached to expensive feedstocks. Regardless of the abovementioned advantages, lignocellulosic substrates face major difficulty with regards to its recalcitrant nature, that hinder its conversion to simple sugars for bioethanol production. Therefore, pretreatment is a necessary step for enhancing enzymatic hydrolysis of complex carbohydrates to fermentable sugars. Criteria that characterize an effective pretreatment system include economic feasibility, low energy usage and high efficiency in terms of sugar release. According to Kucharska et al. [64], lignocellulosic biorefinery processes have shown that the pretreatment step accounts for ~40% of the process costs in bioethanol production plants. Additionally, pretreatment strategies harbour many drawbacks such as toxicity, corrosion of equipment, expensive chemicals, release of inhibitor compounds and problematic disposal [64,65]. Consequently, a cost-effective pretreatment strategy is imperative to reduce these operation costs. The application of green liquor dregs (GLD), an alkaline chemical waste, that is discarded by the kraft pulping industry, eliminates the chemical costs and highlights numerous benefits with regards to an efficient pretreatment system. For instance, the strong alkalinity (pH > 10) of GLD minimises the use of harsh reaction conditions (high temperature, pressure and residence time), while generating little to no problematic enzyme inhibitor compounds during pretreatment [66]. Moreover, due to its low causticity, alkaline pretreatments avoid corrosion and the need for special reactor designs that often utilise expensive materials [65]. The characteristics proposed by the GLD pretreatment relieves the system of expensive equipment costs and reduces the energy input required for effective release of fermentable sugar.

Another aspect of consideration is the need for a sustainable bioethanol plant that can withstand the demands of production, while reducing capital investment. The global pulp and paper industry is one of the largest industries in the world, with approximately 187.2 billion tons of pulp (~0.74 to 2.10 million tons of GLD) being produced in 2018 [67]. The chemical milling infrastructure within the kraft industry can accommodate high volumes of lignocellulosic biomass and has employed technology that can fraction and convert these substrates [68,69]. As a result, biorefinery plants could merge with the existing pulp and paper mills globally, with the intention of producing bioethanol and other value-added products from GLD and lignocellulosic waste. The concept of using the existing infrastructure and equipment could improve both the economics of the bioethanol process and the overall profitability of the kraft pulp and paper industry. This can be achieved by diverting the waste GLD from the kraft pulping industry towards the pretreatment of lignocellulosic biomass and subsequent bioethanol production within the same plant. Furthermore, GLD poses several challenges in the kraft pulping process, since it leads to the blockage of filtration pipes, which has shown to be detrimental to the fibre line. The kraft pulping industry therefore extracts the GLD components from the process and landfills it. Interestingly, landfilling of GLD negatively impacts on the environment and presents added process costs for the kraft pulping industry [24,70]. Therefore, the application of GLD will alleviate environmental issues and reduce landfill costs by diverting this waste from the pulping industry towards the pretreatment of lignocellulosic biomass and subsequent bioethanol production within the same plant. The abovementioned characteristics of GLD pretreatment not only maximises lignocellulosic biomass valorisation by reducing these wastes to renewable resources but also decreases the capital investment and net energy consumption of the operating facility.

4. Conclusion

The present work optimized a novel green liquor dregs (GLD) pretreatment and assessed the kinetics of bioethanol production using corn cobs. Optimized conditions gave a high glucose yield of 0.42 g/g. Different ethanol process types such as separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and SSF with pre hydrolysis (PSSF) gave maximum bioethanol concentrations of 23.69, 17.49 and 20.12 g/L respectively. This study developed an efficient GLD pretreatment regime for enhanced glucose yields and ethanol production from low cost and easily available lignocellulosic wastes such as corn cobs, while reducing the energy input and bioprocess cost.

CRediT authorship contribution statement

Anthea Naomi David: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Y. Sewsynker-Sukai:** Conceptualization, Methodology, Software, Validation, Investigation, Data curation, Writing - review & editing, Visualization, Project administration, Resources, Supervision, Funding acquisition. **B. Sithole:** Writing - review & editing, Resources. **E.B. Gueguim Kana:** Software, Validation, Data curation, Writing - review & editing, Resources, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thankfully acknowledge the National Research Foundation (NRF) of South Africa (Grant number 115465) for financially supporting this research. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily attributed to the NRF.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fuel.2020.117797.

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Appendix A: Supplementary material

Table S1. Box-Behnken primary screening experimental runs for the GLD pretreatment.

Run	GLD concentration (%)	Substrate solid loading (%)	Heating temperature (°C)	Glucose (g/g)
1	5	15	115	0.17
2	5	10	110	0.18
3	10	10	105	0.26
4	15	10	110	0.34
5	15	15	115	0.30
6	15	15	105	0.25
7	5	15	105	0.13
8	15	20	110	0.29
9	5	20	110	0.13
10	10	15	110	0.28
11	10	15	110	0.24
12	10	15	110	0.23
13	10	20	115	0.27
14	10	15	110	0.23
15	10	15	110	0.24
16	10	20	105	0.22
17	10	10	115	0.31

Table S2. Analysis of Variance (ANOVA) of the developed RSM model obtained during the primary screening.

Source	Sum of Squares	df	Mean Square	F-value	p-value (Prob > F)
Model	0.06	9	6.10×10^{-3}	17.83	5×10^{-4} significant
A-GLD concn	0.04	1	0.04	118.70	$< 1 \times 10^{-4}$
B-SL	4.05×10^{-3}	1	4.05×10^{-3}	11.84	0.011
C-Heating temperature	4.51×10^{-3}	1	4.51×10^{-3}	13.19	8.40×10^{-3}
AB	0.00	1	0.00	0.00	1.00
AC	2.50×10^{-5}	1	2.50×10^{-5}	7.30×10^{-2}	0.79
BC	0.00	1	0.000	0.00	1.00
A^2	3.98×10^{-3}	1	3.98×10^{-3}	11.64	1.13×10^{-2}
B^2	1.99×10^{-3}	1	1.99×10^{-3}	5.82	4.66×10^{-2}
C^2	2.37×10^{-6}	1	2.37×10^{-6}	6.92×10^{-3}	0.94
Residual	2.40×10^{-3}	7	3.42×10^{-4}		
Cor Total	0.06	16			

Table S3. Composition analysis of the control and optimized CCW samples.

Sample	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Native	40.49	42.95	7.18
Optimized	57.71	22.62	12.12

Table S4. Comparison of the different pretreatment methods used on corn cob waste.

Pretreatment conditions	Reducing sugar or glucose yield (g/g)	Reference
20% CCW, 80% GLD, 20% SL, 50 min, 121°C	0.42[a]	This study
14.86% CH_3COONa, 10% SL, 800W for 8 min	0.24[a]	Sewsynker-Sukai and Gueguim Kana [39]
1.72% H_2SO_4 (w/w), 169.26°C, 60 min	0.23[b]	Amenaghawon et al. [48]
1M NaOH, 20% SL, 121°C, 20 min	0.11[b]	Potumarthi et al. [13]
0.5M NaOH, 12.5% SL, 121°C, 30 min	0.92[b]	Gao and Rehmann [49]
2% NaOH, 6.7% SL, 100°C for 30 min	0.68[b]	Boonsombuti et al. [50]

Note: [a] = Glucose, [b] = reducing sugar, CCW= Corn cob waste, GLD= Green liquor dregs, SL= Solid loading.

Table S5. Comparisons of the different process types for bioethanol production from corn cob waste.

Microorganism	Substrate	Process type	Process conditions	Ethanol concentration (g/L)	Reference
Saccharomyces cerevisiae BY4743	Corn cobs	SHF	0.051 g/L[a], 10%[b]. 10 FPU/g[c]	23.69	This study
Saccharomyces cerevisiae BY4743	Corn cobs	SSF	0.051 g/L[a], 10%[b], 10 FPU/g[c].	17.49	This study
Saccharomyces cerevisiae BY4743	Corn cobs	PSSF	0.051 g/L[a], 10%[b], 10 FPU/g[c].	20.12	This study
Saccharomyces cerevisiae KE6-12	Corn cobs	SSF	5 g/L[a], 7.5%[b], 5 FPU/g[c]	22	Koppram et al. [61]
Saccharomyces cerevisiae	Corn cobs	PSSF	5 g/L[a], 10%[b], 15 FPU/g[c]	19.30	He et al. [62]
Escherichia coli FBR5	Wheat straw	SHF	5% (v/v)[a], 8.6%[b], 150 µl/g[c]	21.90	Saha et al. [9]
Escherichia coli FBR5	Wheat straw	SSF	5% (v/v)[a], 8.6%[b], 150 µl/g[c]	17.40	Saha et al. [9]
Saccharomyces cerevisiae	Corn cobs	PSSF	2 g/L[a], 10%[b], 30 FPU/g[c]	17.20	Xie et al. [10]

Note: SHF= Separate hydrolysis and fermentation, SSF= Simultaneous saccharification and fermentation, PSSF= Simultaneous saccharification and ferementation with prehydrolysis, [a] = yeast concentration, [b] = solid loading, [c] = enzyme loading.

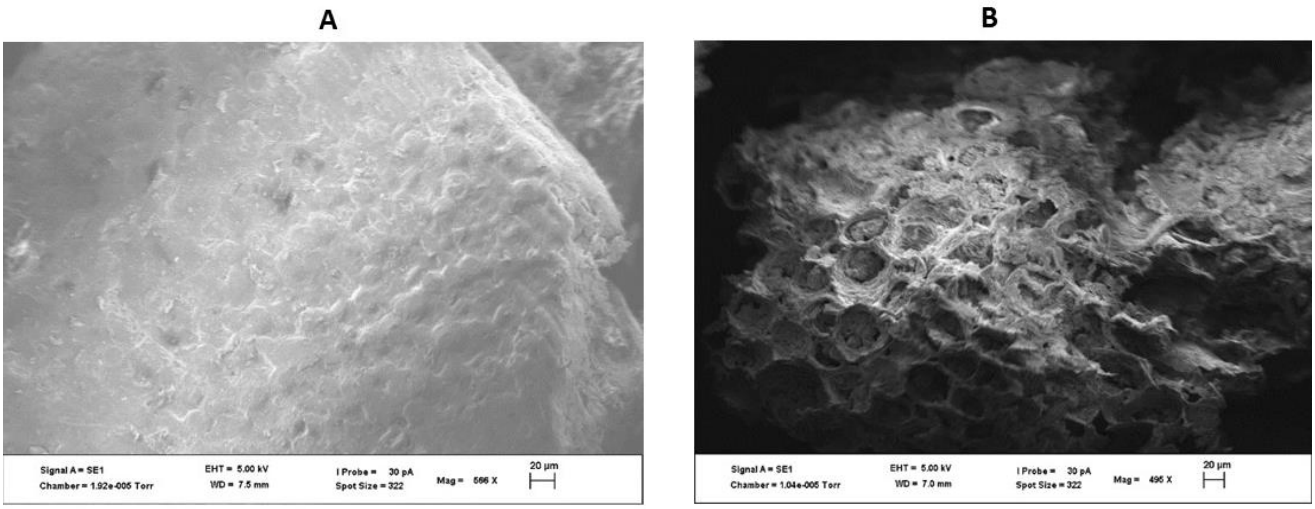

Fig. S1. Scanning electron microscopy images of native (A) and optimized pretreated (B) CCW samples.

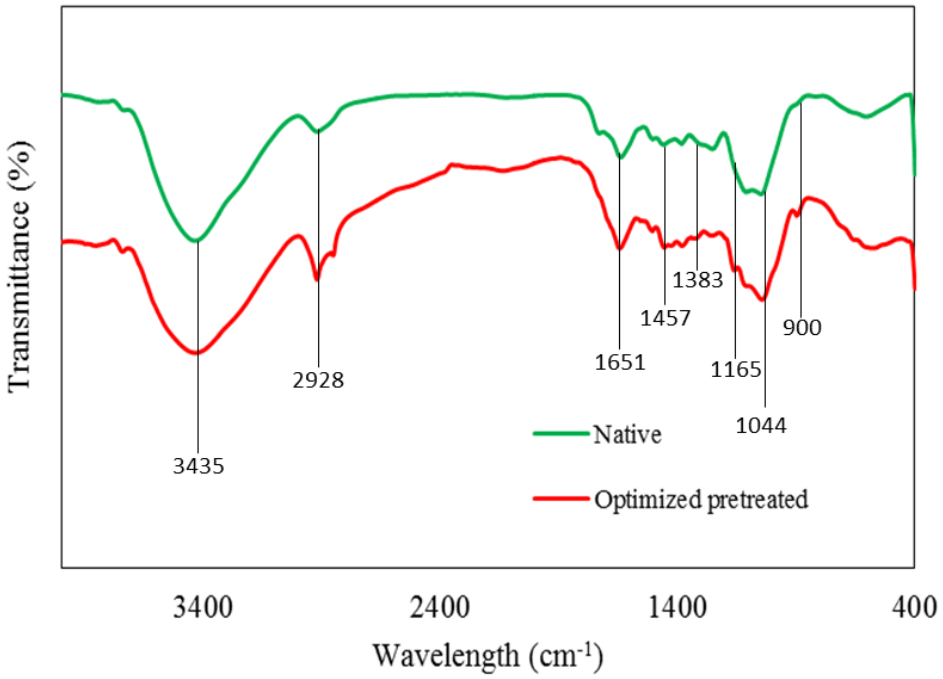

Fig. S2. Fourier transform infrared spectroscopy of the native and optimized GLD-pretreatment CCW samples.

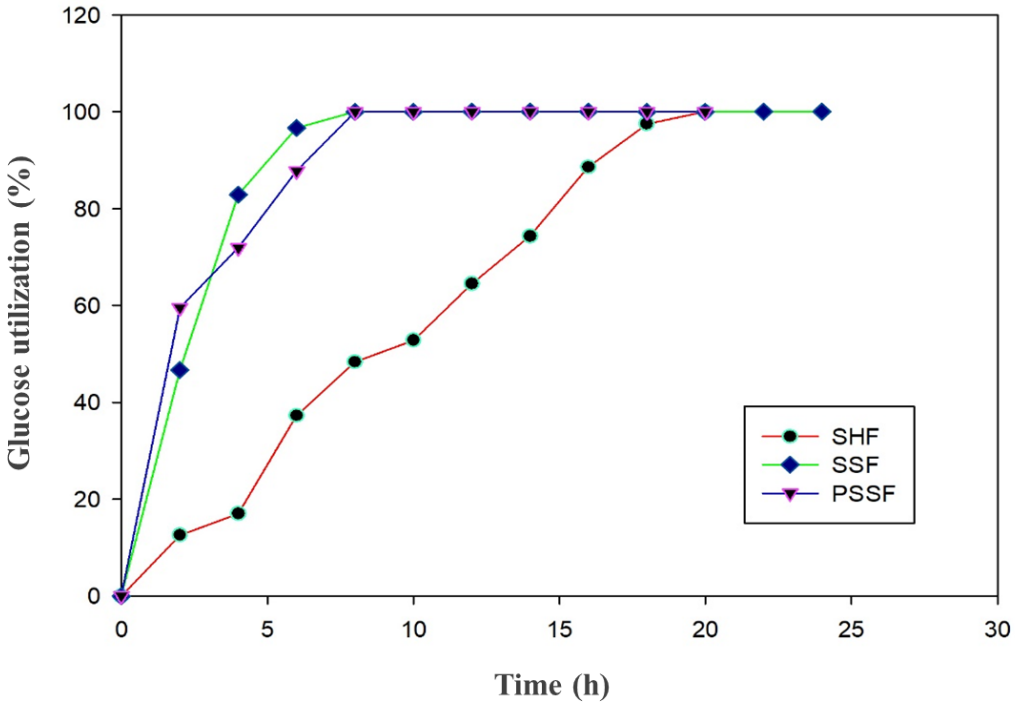

Fig. S3. Glucose utilization of *S. cerevisiae* for the SHF, SSF and PSSF processes during bioethanol production.

Appendix B

Lignocellulosic biofuel production: Insight into microbial factories

10

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10.1 Introduction

Microbial factories produce an array of bioproducts that include biofuels and biochemicals with the potential to revolutionize the global bioeconomy and address key challenges (Banu et al., 2021). Currently, one major challenge is the dependence on nonrenewable petroleum-based energy sources with their subsequent depletion due to the ever-changing growth in the human population coupled with social development and industrialization (Patel and Shah, 2021). Therefore the development of alternative energy sources and efficiently harnessing energy from them is imperative (Sewsynker-Sukai et al., 2020). In part, biofuels have emerged as potential alternative energy sources to replace conventional fossil fuels. They have shown to be promising since they are renewable, sustainable, and minimize the carbon footprint (Sewsynker-Sukai et al., 2018). However, biofuels have been traditionally produced using food crops such as sugarcane, corn, and wheat, which has questioned their application due to their negative impact on global food security (Mohapatra et al., 2018; Ray and Ramachandran, 2018; Sarawan et al., 2019).

Over the past decade, lignocellulosic biofuel production has increasingly gained widespread attention to curb the use of food crops since these substrates are considered wastes, abundant, and renewable (Moodley et al., 2020; Moodley, 2021). However, the presence of resistant lignin structures within the lignocellulosic biomass (LCB) prevents its direct utilization by microorganisms. Therefore, the use of LCB for microbial bioprocesses requires pretreatment to release fermentable sugars that microorganisms can harness for their growth, replication, and metabolic functioning (Jugwanth et al., 2020). Examples of biofuels that can be generated using LCB include bioethanol, biobutanol, biohydrogen, and biogas. These fuels represent a green energy perspective, each with its merits and limitations. Biofuels have previously been produced using wastes from sugarcane, corn, wheat, or rice, including the bagasse, leaves, stalks, and husks. However, various key technological issues have challenged economic lignocellulosic biofuel production (Sewsynker-Sukai et al., 2018). Identifying and addressing the challenges of lignocellulosic biofuel processes pave the way toward industrial-scale application. The major bottleneck is the high cost that renders these processes unfeasible for large-scale production.

Advances in Lignocellulosic Biofuel Production Systems. DOI: https://doi.org/10.1016/B978-0-323-91192-4.00013-4

The lignocellulosic biofuel production process entails pretreatment, enzymatic hydrolysis, and microbial fermentation. The lignocellulosic biofuel production system may be carried out using different bioprocess types that include:

1. Separate hydrolysis and fermentation (SHF).
2. Simultaneous saccharification and fermentation (SSF).
3. SSF with a prehydrolysis step (PSSF) (Moodley et al., 2020; David et al., 2020).

Several strategies are currently being undertaken globally to enhance the lignocellulosic biofuel production process toward reducing the cost and achieving an industrial-scale system that can be commercialized. These approaches are tailored in line with developing and enhancing: (1) cost-effective pretreatment methods that produce high sugar yields from LCB, (2) microbial fermentation process types that result in high biofuel yields and shorter fermentation times, and (3) kinetic knowledge of bioprocesses with regards to microbial growth and product formation for a translatable outcome when replicated at industrial scale (David et al., 2020).

Kinetic modeling of cell growth and product formation has recently been tagged as a practical approach to determining the efficiency of the fermentation process with regard to specific parameters (David et al., 2020). Some examples of kinetic parameters include the maximum specific growth of the cells, lag time, and yield productivity since these parameters can be used as translation indices during scale-up toward industrialization (Sewsynker-Sukai and Gueguim Kana, 2018). Despite the abundance of knowledge that is conferred from these kinetic parameters, the majority of reports have been concentrated on bioethanol (David et al., 2020; Sewsynker-Sukai and Gueguim Kana, 2018; Moodley and Gueguim Kana, 2019; Jugwanth et al., 2020; Sarawan et al., 2019) and to a lesser extent biobutanol (Chen et al., 2013; Rochón et al., 2018, 2020). Nonetheless, biofuel production and the microbial metabolic pathways involved are influenced by factors such as temperature, pH, and nutrient concentration, revealing key information for maximizing the process yield.

This chapter provides insight into the advancement of microbial biofuel production from LCB. Biofuels such as bioethanol, biobutanol, biohydrogen, and biogas are highlighted with the current progress on these processes pertaining to the kinetics, microbial metabolic pathways, and key process parameters briefly discussed. Furthermore, the current challenges and progress in lignocellulosic biofuel production are outlined.

10.2 Lignocellulosic biomass and pretreatment

LCB substrates are plant waste materials presently being sought as potential feedstocks for biofuel production without negative consequences on food security (Jugwanth et al., 2020). The annual global production of LCB is approximately 120 billion tons (Abraham et al., 2020). LCB substrates include corn stover, cobs, sugarcane bagasse, banana pseudostem, switchgrass, *Miscanthus*, and wheat straw.

Nevertheless, LCB substrates consist of recalcitrant structures such as cellulose, hemicellulose, and lignin that cannot be directly utilized by microorganisms (Muthuvelu et al., 2019). The composition of LCB ranges between 30% and 50% cellulose, 10% and 45% hemicellulose, and 2% and 40% lignin, depending on the type of substrate (McKendry, 2002). Cellulose, hemicellulose, and lignin structures are connected by hydrogen and covalent bonds that influence the recalcitrance of lignocellulosic substrates (Rebello et al., 2020). Consequently, a pretreatment step is crucial for the degradation of the recalcitrant structures within LCB (Kumar et al., 2020).

Pretreatment of LCB is a prerequisite step for its conversion to biofuels and other biobased products (Kim, 2013). A major obstacle that hinders the use of LCB as an energy source in biorefinery systems is the presence of recalcitrant structures within these materials (Himmel et al., 2007). Pretreatment methods are crucial for disrupting resistant lignin structures and enhancing subsequent cellulose conversion to glucose during enzymatic hydrolysis reactions. Lignocellulosic pretreatment consists of physical (mechanical, microwave, and ultrasound), chemical (acids, bases, inorganic salts, ionic liquids, and organosolvents), physicochemical (steam explosion, ammonia fiber explosion, CO_2 explosion, and liquid hot water), and biological methods (enzymes and whole cells) (Fig. 10.1) (Jędrzejczyk et al., 2019). Due to the higher efficiency, hybrid strategies that combine some of the abovementioned methods are typically used. However, present pretreatment methods are expensive, release high concentrations of enzymatic and fermentation inhibitory compounds, produce too low sugar yields, and are resource-demanding and energy exhaustive, thus posing a significant hindrance to the use of lignocellulosic substrates. The development of high-performance pretreatments is ongoing and remains a crucial aspect for use of lignocellulosic bioprocesses. Criteria for an ideal pretreatment method include using cost-effective chemicals or wastes with low energy that release high sugar yields and low inhibitor concentrations and do not negatively impact the environment.

10.3 Microbial fermentation and process types

Fermentation is a metabolic process by which various microorganisms metabolize sugars for the production of valuable products such as biofuels and biochemicals (Kang et al., 2014). The fermentation of lignocellulosic wastes into useful bio-based products or renewable biofuels can be achieved through various strategies, including SHF, SSF, and PSSF (Fig. 10.2) (Morales-Martínez et al., 2017, Dahnum et al., 2015). The SHF process is a conventional method performed in two separate biochemical steps with different temperatures. It includes an enzymatic hydrolysis stage followed by the fermentation process. On the other hand, the SSF process allows hydrolysis and fermentation to be carried out in a single bioreactor at a median temperature that can accommodate the cellulase enzyme and the microbial culture, respectively (David et al., 2020). A variation of SSF and SHF processes is the PSSF process. In the PSSF process type, pretreated biomass is prehydrolyzed at the optimal temperature for cellulolytic enzymes for a short period of time (6–24 h), which is followed by a change in temperature for

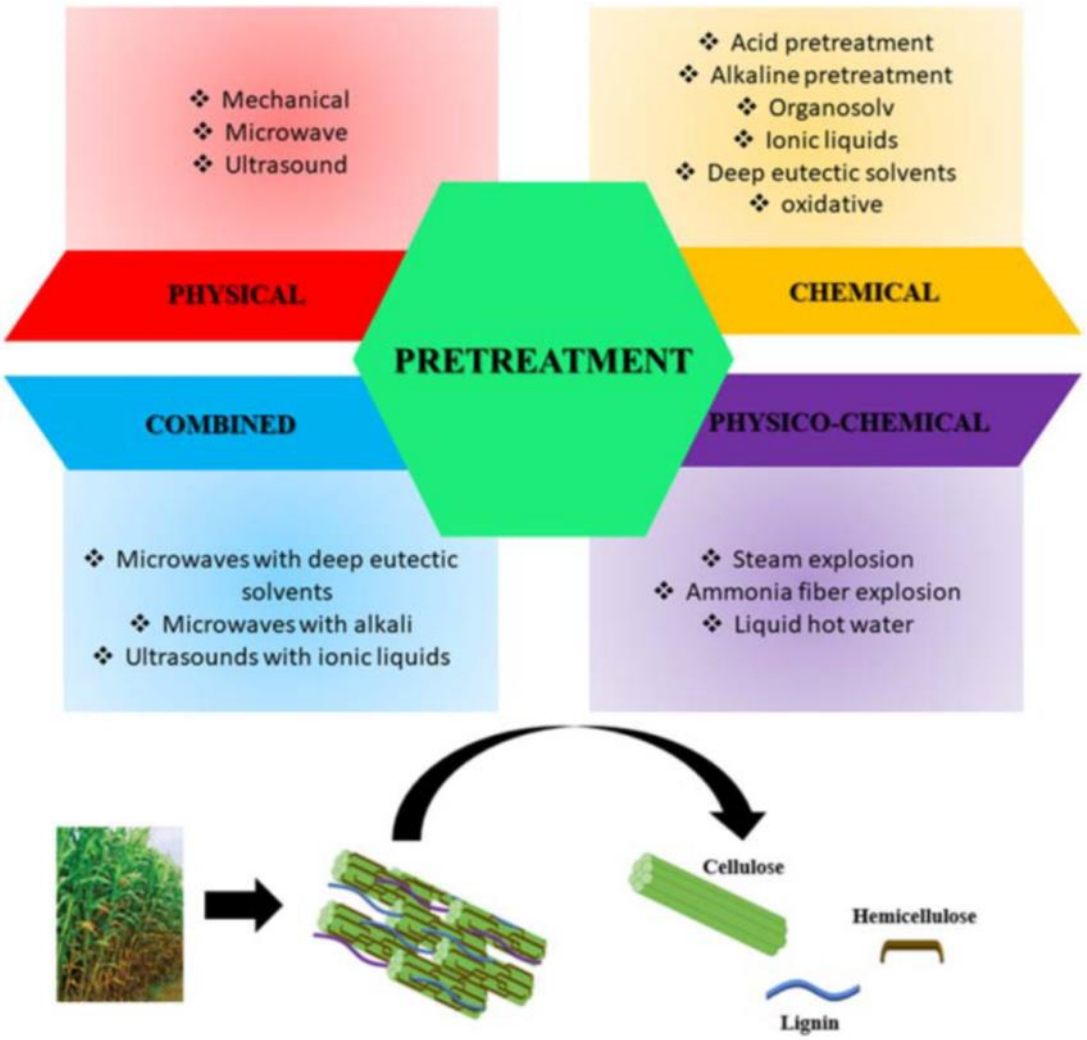

Figure 10.1 Different methods of lignocellulosic biomass pretreatment.

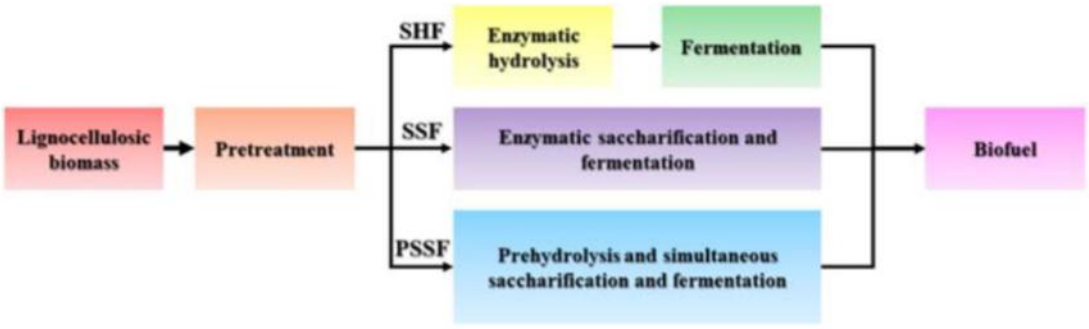

Figure 10.2 Process flow of separate hydrolysis and fermentation; simultaneous saccharification and fermentation, and prehydrolysis and simultaneous saccharification and fermentation.

inoculation of the microbial culture during fermentation (Morales-Martínez et al., 2017, Guerrero et al., 2018). The selection of these process types at an industrial scale remains contentious due to their advantages and limitations. For instance, the SHF system ensures that enzymatic saccharification and fermentation occur in different vessels at their optimal conditions. During these steps, the pretreated substrate is enzymatically hydrolyzed to fermentable sugars, followed by the fermentation of available sugars to the product of interest (Guerrero et al., 2018). The main limitations of the SHF process include the inhibitory effect on cellulase enzymes due to the accumulation of fermentable sugars (Portero Barahona et al., 2020). In addition, there is a risk of contamination when transferring the enzymatic hydrolysate to the fermentation reactor. The SHF process exhibits high operational costs due to long processing times in separate vessels (Giang et al., 2019).

Major drawbacks of the SHF process can be overcome by the implementation of the SSF approach since hydrolysis and fermentation occur simultaneously in a single vessel and eliminates long process times (Da Silva et al., 2018). Additionally, the SSF process does not cause cellulase inhibition due to the simultaneous conversion of simple sugars (glucose) to bioproducts while reducing the risk of contamination (Carrillo-Nieves et al., 2017). When compared to SHF, SSF necessitates a shorter process time, leading to higher productivity yields (Giang et al., 2019). Nevertheless, the SSF process type also presents limitations attributable to ineffective hydrolysis stages carried out under suboptimal conditions (temperature and pH) that may yield significantly lower sugar and have a negative impact on the enzyme. The PSSF system, therefore bridges the gap between the SHF and SSF process types since it includes a short prehydrolysis step followed by fermentation. The benefits of enzymatic saccharification at its optimum setpoint promote the release of fermentable sugars before fermentation (Carrillo-Nieves et al., 2017). However, the prehydrolysis stages incur higher costs than the SSF process due to energy consumption and longer processing times, reducing its feasibility on a large scale. Compared to the SHF and PSSF process types, the SSF system offers several advantages for large-scale applications since it reduces process time and energy required by completely omitting separate hydrolysis steps (David et al., 2020).

For this reason, the SSF process type has been widely investigated for bioethanol production from various substrates, including banana waste (Guerrero et al., 2018), mango stem bark (Carrillo-Nieves et al., 2017), corn cobs (David et al., 2020), sugarcane wastes (Jugwanth et al., 2020; Moodley and Gueguim Kana, 2019) and *Agave lechuguilla* (Morales-Martínez et al., 2017), among several others. To date, all three different process types (SSF, SHF, and PSSF) have been extensively explored for bioethanol production. However, the literature on other biofuels such as biobutanol, biogas, and biohydrogen remains scanty.

10.4 Kinetic modeling for bioprocess development

Kinetic models provide significant insight into understanding the rational design of microbial metabolic processes and explore the phenomena in complex biological

systems. This information leads to accelerated cell factory engineering and defines the parameters for bioprocess control when considering industrial-scale applications (Oliveira et al., 2016). Bioprocess modeling represents the biological, physical, and chemical aspects of a bioreactor and aims to select and optimize the process parameters affecting microbial growth and product formation (Fedailaine et al., 2015). The kinetics models provide insight into the information gathered from the empirical observations through the experimental output. Thus predicting the optimal conditions within a fermentation process assists in (1) increasing the product yield and productivity, (2) minimizing undesired byproduct formation, (3) managing resource utilization, (4) cost and time efficiency, and (5) transforming lab-scale reactions to industrial-scale reactions, while maintaining high product purity (Kucharska et al., 2018a).

The kinetic growth modeling approaches are required to account for the biological system's regulatory effects and dynamics at a metabolite level (Costa et al., 2016). These models are usually based on a set of mathematical expressions that quantitatively describe the systematic occurrences and predict the response to various input results. There are many types of kinetic models used to describe specific growth rates of microorganisms and product formation, namely, the Monod model, logistic model and Haldane model, modified Gompertz model, and modified logistic model, amongst others. These models interpret microorganisms as independent components that interact with the environment, and their growth rate is determined by the biomass and substrate concentrations (Dong et al., 2015; Panikov, 1999). The Monod model describes the relationship between the specific growth rate and substrate utilization within the bioreactor (Monod, 1949). This model type can interpret data based on the substrate and biomass concentration as well as the substrate concentration only (Muloiwa et al., 2020). Although the model is most widely used, it presents many limitations, such as assuming that the bioreactor only consists of one growth-limiting substrate and its inability to be used when substrate inhibition occurs. It does not consider the lag and death phase of the growth cycle of the microbes resulting in overestimating the specific growth rate (Kong, 2017).

On the other hand, the logistic model, also known as the Verhulst model, is a substrate-independent kinetic model that observes changes in biomass growth and relates it to the growth rate and initial and maximum concentrations over time (Muloiwa et al., 2020). The model also accommodates the toxicity of the substrate, which results in substrate inhibition (Ali et al., 2017). The Haldane model is an extension of the Monod model that addresses the discrepancies of the latter model. The model includes an inhibition constant that allows it to handle toxic and nontoxic substrates and specific growth rate inhibition at low and high substrate concentrations (Muloiwa et al., 2020). Furthermore, the model can account for all growth phases, including the lag, exponential, stationary, and death phases.

Regarding product formation, the modified Gompertz model has been widely used for nonlinear modeling of cumulative biofuel production. The resultant data that fits the modified Gompertz equation assumes that the biofuel production in a batch mode is a function of the specific growth rate of the microorganisms within the bioreactor. The model can determine the production lag time, maximum

production rate, and maximum production concentration on a given substrate (Dodić et al., 2012). The modified logistic model is similar to that of the modified Gompertz model, which contains many constants of biological meaning that facilitates a better understanding of the reaction.

The abovementioned kinetic models, amongst others, consider the reliability of the results, sampling methods, analysis, and degree of accuracy when conducting experiments. The resultant outcome of these procedures enables the model to predict the behavioral trends of biochemical reactions. This knowledge is a critical aspect that contributes significantly to the technoeconomic analysis and interprets the phenomena within a process more precisely, thus enhancing productivity (González-Figueredo et al., 2018).

10.5 Lignocellulosic biofuel production

The global energy demand has escalated in light of population spikes, modern living, and urbanization, with its consumption, predicted to reach over 106 quadrillions British thermal units (Btu) by the year 2050 (Tabatabaei et al., 2019; Energy Information Administration, 2019). However, the finite supply of fossil fuels has jeopardized energy security since it accounts for more than 80% of the total energy markets (Zabed et al., 2016). Additionally, the excessive release of anthropogenic greenhouse gases has triggered unexpected environmental fluctuations (Soltanian et al., 2020). These drawbacks have strengthened the need to switch from fossil-based fuels to clean, renewable, and cheaper alternative sources such as biofuels produced from lignocellulosic waste (Kumari and Singh, 2018). Kinetic modeling plays a key role during the development, optimization, and scale-up of bioprocesses and has been applied in the lignocellulosic production of different biofuels. The different biofuels and processes are briefly discussed below.

10.5.1 Bioethanol

Bioethanol is known for its excellent fuel properties, such as higher flame speeds, broader flammability limits, and a high-octane number (108) that increases the heat of vaporization and compression ratio. These characteristics enable bioethanol to be blended with gasoline, which results in the reduced emission of CO_2, unburnt hydrocarbons, and carcinogens. Bioethanol is a product of microbial metabolism that has shown promise as an effective and eco-friendly alternative to fossil fuels. During bioethanol production, hexoses (glucose, fructose, mannose, and galactose) undergo oxidation in the absence of oxygen to generate ethanol, CO_2, and adenosine triphosphate (ATP). When considering commercial bioethanol production, several factors must be considered. For instance, microorganisms must have a high fermentative activity for monosaccharides, provide high bioethanol yields, maintain genetic stability and tolerate environmental stressors (inhibitors, osmotic changes, and high alcohol concentrations, amongst others) (Robak and Balcerek, 2018).

Traditionally, *Saccharomyces cerevisiae* and *Zymomonas mobilis* have been most commonly involved in bioethanol production. *S. cerevisiae* is a robust and well-established microorganism used frequently in industrial processes (alcohol and baking). This microbe can metabolize hexoses using the Embden–Meyerhof–Parnas (EMP) pathway in which pyruvate is generated and converted into ethanol and CO_2 by specific enzymes (Marin et al., 2017). On the other hand, *Z. mobilis*, a Gram-negative bacterium, produces bioethanol at an efficient rate from glucose, fructose, or sucrose as a carbon source. This microorganism follows the Entner–Doudoroff (ED) pathway that converts the fermentable sugars into ethanol, making the ethanologenic process 50% more efficient than the EMP pathway (Majidian et al., 2018). Even though both these microorganisms possess favorable characteristics for bioethanol production, their ineffective pentose phosphate pathway does not accommodate the utilization of pentose sugars for bioethanol (Robak and Balcerek, 2018). This presents a major drawback since pentose sugars are abundantly available in LCB and can drastically reduce expenses within the biorefinery system. As a result, metabolic and genetic engineering aims to address this issue by manipulating these pathways within microbial species for enhanced biofuel production. In addition to bioengineering, kinetic modeling has provided insight into the parameters that will enhance microbial growth and product formation toward industrialization.

Numerous studies have evaluated the kinetics of bioethanol production from different lignocellulosic wastes, as noted in Table 10.1. For instance, Srimachai et al. (2015) varied the age of oil palm and evaluated the kinetics of the oil palm frond juice (OPFJ) utilization for bioethanol production. The OPFJ at 3–4 years gave a maximum specific growth rate (μ_{max}) and Monod constant (K_s) of 0.29/h and 47.05 g/L, respectively, for the Monod model while the maximum ethanol concentration (P_m) of 11.50 g/L and maximum ethanol production rate ($r_{p.m}$) of 0.24 g/L/h was observed for the modified Gompertz model (Srimachai et al., 2015). These results showed that as the oil palm age increased, the μ_{max}, K_s, P_m, and $r_{p.m}$ decreased (Srimachai et al., 2015). Phukoetphim et al. (2017) reported on the production of bioethanol from sweet sorghum juice under high gravity conditions with and without the supplementation of yeast extract. Under high gravity conditions with the supplementation of yeast extract, the u_{max} (0.185/h) and X_{max} (10.6 g/L) were higher than those with no supplementation, which indicates that yeast extract promoted cell growth (Phukoetphim et al., 2017). Furthermore, an increase in the P_m (112.06 g/L) and $r_{p.m}$ (5.25 g/L/h) show that the presence of yeast extract in media enhances cell growth and, in turn, leads to higher sugar consumption and ethanol production (Phukoetphim et al., 2017). Additionally, a comparison of the SSF and PSSF bioprocesses using pretreated corn cob waste was channeled toward bioethanol production under microaerophilic conditions (Sewsynker-Sukai and Gueguim Kana, 2018). It was observed that the SSF process produced a higher μ_{max} of 0.274/h while the PSSF bioprocess gave a higher P_m of 42.24 g/L (Sewsynker-Sukai and Gueguim Kana, 2018). In the same vein, Moodley and Gueguim Kana (2019) assessed the kinetics of two SHF process modes using either filtered enzymatic hydrolysate (SHF-filtered) and unfiltered enzymatic hydrolysate (SHF-unfiltered) for the production of bioethanol by *S. cerevisiae* BY4743. The

Table 10.1 Bioethanol production from various lignocellulosic waste.

Microorganism	Substrate	Process type	Product formation kinetics	Microbial growth kinetics	Reference
Saccharomyces cerevisiae	Oil palm frond juice (3–4 y)	ND	11.5 g/L[a], 0.24 g/L/h[b]	0.29/h[c], 47.05 g/L[d]	Srimachai et al. (2015)
S. cerevisiae	Oil palm frond juice (20–25 y)	ND	2.34 g/L[a], 0.05 g/L/h[b]	0.11/h[c], 1.82 g/L[d]	Srimachai et al. (2015)
S. cerevisiae NP 01	Sweet sorghum juice	SSF	88.48 g/L[a], 2.17 g/L/h[b]	0.164 h^{-1}[c], 6.31 g/L[e]	Phukoetphim et al. (2017)
S. cerevisiae NP 01	Sweet sorghum juice + yeast extract	SSF	112.06 g/L[a], 5.25 g/L/h[b]	0.185/h[c], 10.6 g/L[e]	Phukoetphim et al. (2017)
S. cerevisiae ATCC 24858	Sweet sorghum bagasse	SSF	16.64 g/L[a], 0.51 g/L/h[b]	ND	Wang et al. (2013)
S. cerevisiae BY4743	Sugarcane bagasse	SSF	3.12 g/L[a], 0.29 g/L/h[b]	0.15/h[c], 2.58 g/L[e]	Jugwanth et al. (2020)
S. cerevisiae BY4743	Corn cobs	PSSF	42.24 g/L[a], 2.39 g/L/h[b]	0.216/h[c], 3.65 g/L[e]	Sewsynker-Sukai and Gueguim Kana (2018)
S. cerevisiae BY4743	Corn cobs	SSF	37.87 g/L[a], 2.14 g/L/h[b]	0.274/h[c], 3.52 g/L[e]	Sewsynker-Sukai and Gueguim Kana (2018)
S. cerevisiae BY4743	Sugarcane leaf waste	SHF-unfiltered	31.06 g/L[a], 2.44 g/L/h[b]	0.153/h[c], 4.19 g/L[d]	Moodley and Gueguim Kana (2019)
S. cerevisiae BY4743	Sugarcane leaf waste	SHF-filtered	30.49 g/L[a], 2.81 g/L/h[b]	0.153/h[c], 4.19 g/L[d]	Moodley and Gueguim Kana (2019)
S. cerevisiae BY4743	Corn cobs	SHF	26.82 g/L[a], 1.48 g/L/h[b]	0.28/h[c], 0.70 g/L[e]	David et al. (2020)
S. cerevisiae BY4743	Corn cobs	SSF	16.49 g/L[a], 2.06 g/L/h[b]	0.23/h[c], 1.63 g/L[e]	David et al. (2020)
S. cerevisiae BY4743	Corn cobs	PSSF	18.28 g/L[a], 3.08 g/L/h[b]	0.61/h[c], 0.87 g/L[e]	David et al. (2020)

ND: not determined; *PSSF*: simultaneous saccharification and fermentation with prehydrolysis; *SHF*: separate hydrolysis and fermentation; *SSF*: simultaneous saccharification and fermentation.
[a] P_m—potential maximum bioethanol concentration (g/L),
[b] r_{pm}—maximum bioethanol production rate (g/L/h),
[c] μ_{max}—maximum specific growth rate (per h),
[d] K_s—Monod constant (g/L),
[e] X_{max}—maximum cell biomass concentration (g/L).

SHF-unfiltered process observed a higher P_m of 31.06 g/L compared to the SHF-filtered process (30.49 g/L) (Moodley and Gueguim Kana, 2019). However, the SHF-filtered and SHF-unfiltered processes showed a μ_{max} of 0.153/h. These results indicate that these systems had an insignificant effect on the μ_{max}. Therefore carrying out the SHF-unfiltered process for fermentation can reduce the costs attached to the biomass separation stage usually required in the conventional SHF system (Moodley and Gueguim Kana, 2019). In a recent study, David et al. (2020) reported on the kinetics of microbial growth and ethanol production of all three bioprocess types (SHF, PSSF, and SFF) using the logistic and modified Gompertz models, respectively. The PSSF system obtained the highest μ_{max} (0.61/h), while the SSF process showed the highest X_{max} (1.63 g/L) (David et al., 2020). The high μ_{max} was attributed to the initial glucose produced in the prehydrolysis stage and was readily available to the yeast cells for its growth (David et al., 2020). Furthermore, the high X_{max} for the SSF type results from low initial glucose concentrations that drive the microbial metabolism toward cell growth instead of ethanol production (David et al., 2020). On the other hand, the SHF system produced the highest P_m (26.82 g/L) due to the fermentable sugars readily available to drive metabolism to product formation (David et al., 2020). Even though the SHF process is known to be the most effective for producing bioethanol, its prolonged enzymatic hydrolysis, biomass separation, and risk of contamination due to reactor transfer may incur more expenses, and increase time and energy (David et al., 2020).

10.5.2 Biobutanol

Biobutanol has gained significant attention as a biofuel due to its similarities with gasoline, a well-established petroleum-derived liquid used in spark-ignited internal combustion engines (Majidian et al., 2018). The four-carbon primary straight-chain fuel is produced using the acetone–butanol–ethanol (ABE) fermentation process. The ABE process involves two distinct fermentation phases: acidogenesis and solventogenesis (Ibrahim et al., 2018). During the acidogenic phase, cell growth occurs and converts the substrate to acetic and butyric acids. The acids generated causes the pH of the fermentation media to decrease below 5.0, inhibiting cell growth (Majidian et al., 2018). The low extracellular pH signals and intracellular acyl phosphates signals shift the ABE fermentation from the acidogenesis phase to the solventogenesis phase (Jiang et al., 2019). This phase causes 70%–80% of the viable bacterial cells to sporulate (Al-Hinai et al., 2014). The organic acids are transformed into acetone, butanol, and ethanol in a 3:6:1 ratio (Nithyanandan et al., 2016). Biobutanol is typically produced by *Clostridium* species, including *C. acetobutylicum*, *C. beijerinckii*, *C. saccharoperbutylacetonicum*, and *C. saccahrobutylicum*, amongst others. However, the low production profile of *Clostridium* species poses many challenges in the commercialization of the ABE fermentation process. To improve biobutanol production during ABE fermentation, the focus has shifted towards genetically engineering the Clostridia fermentative pathway in microorganisms such as *Escherichia coli* and *Bacillus subtilis*. Despite these attempts, the lack of information regarding the metabolic shift from acidogenesis to solventogenesis

and its regulatory interactions hinders the functional butanol biosynthesis pathway. Biobutanol has several benefits compared to traditional biofuels, such as higher energy content, lower volatility, reduced ignition issues, and a higher heating value (Ibrahim et al., 2018). Furthermore, butanol presents similar chemical and physical characteristics to gasoline, allowing it to be easily blended in a pure or mixed form. It may be used in automobile engines without modifications (Ibrahim et al., 2018). When mixed with diesel, it could also decrease soot emission and improve thermal efficiency (Rakopoulos et al., 2010). However, butanol is not void of drawbacks such as higher fuel consumption and lower engine performance. Additionally, the industrial-scale application has been hampered due to the high expenses and low production of the ABE fermentation system. For this reason, researchers are searching for a system that will reduce the cost of production and increase the biobutanol yields for large-scale implementation. Recently, Rochón et al. (2020) carried out an isopropanol−butanol−ethanol (IBE) batch fermentation using in situ gas stripping-pervaporation to retrieve the biobutanol (Table 10.2). The modified Monod kinetic model was used to estimate the consumption of a sugarcane and sorghum juice mixture by *C. beijerinckii* DSM 6423 for the IBE solvent production. The IBE solvent fermentation gave a μ_{max}, K_s, product yield ($Y_{p/s}$), and $r_{p.m}$ of 0.23/h, 2 g/L, 0.22 g/g, 0.21 g/L/h, respectively, for the modified Monod model (Rochón et al., 2020). A previous study by Rochón et al. (2018) carried out an ABE batch fermentation using in situ gas stripping-pervaporation to recover biobutanol. The metabolism of a sugarcane and sorghum juice mixture by *C. beijerinckii* DSM 6423 was evaluated for ABE solvent production. This type of solvent fermentation gave a lower μ_{max} (0.22/h), K_s (0.2 g/L), and $Y_{p/s}$ (0.20 g/g) (Rochón et al., 2018). The results showed significant improvements in the microbial growth and productivity of the IBE fermentation process compared to the ABE process. In an earlier study, Chen et al. (2013) compared the product formation kinetics of the SHF process under sterile and nonsterile conditions. The SHF-sterile process gave a higher P_m (6.23 g/L) and $r_{p.m}$ (4.82 g/L/d) in comparison to the SHF-nonsterile process, which obtained a P_m and $r_{p.m}$ of 6.51 g/L and 3.21 g/L/d, respectively, for the modified Gompertz model (Chen et al., 2013). Regardless of the higher butanol concentration and the production rate for the SHF under sterile conditions, the study points out that fermentation under nonsterile conditions did not present a significant difference (Chen et al., 2013). Therefore the presterilization step can be omitted within the biobutanol production system, resulting in lower operation costs (Chen et al., 2013). Several studies have been reported on the production of biobutanol. However, there is a shortage of knowledge on the kinetic modeling of butanol bioprocesses. For this reason, bioprocess commercialization may be severely hindered since substantial information regarding microbial growth and product formation has not been established.

10.5.3 Biohydrogen

The depletion of fossil fuel reserves, combined with rising environmental concerns resulting from fossil fuel consumption, has prompted research into biohydrogen

Table 10.2 Biobutanol production from various lignocellulosic waste.

Microorganism	Substrate	Process type	Product formation kinetics	Microbial growth kinetics	Reference
Clostridium saccharoper butylacetonicum N1−4	Rice straw	SHF-sterile	6.23 g/L[a], 4.82 g/L/d[b]	ND	Chen et al. (2013)
C. saccharoper butylacetonicum N1−4	Rice straw	SHF-nonsterile	6.51 g/L[a], 3.21 g/L/d[b]	ND	Chen et al. (2013)
Clostridium beijerinckii DSM 6423	Sugarcane and sorghum juice	SHF	0.20 g/g[c]	0.22/h[d], 0.2 g/L[e]	Rochón et al. (2018)
C. beijerinckii DSM 6423	Sugarcane and sorghum juice	SHF	0.21 g/L/h[b], 0.22 g/g[c]	0.23/h[d], 2 g/L[e]	Rochón et al. (2020)

ND: not determined; *PSSF*: simultaneous saccharification and fermentation with prehydrolysis; *SHF*: separate hydrolysis and fermentation; *SSF*: simultaneous saccharification and fermentation.
[a] P_m—potential maximum biobutanol concentration (g/L),
[b] $r_{p,m}$—maximum bioethanol production rate (g/L/h or g/L/d),
[c] $Y_{p/s}$—biobutanol yield coefficient (g/g),
[d] μ_{max}—maximum specific growth rate (per h),
[e] K_s—Monod constant (g/L).

synthesis using a variety of biological processes (Giang et al., 2019). These include (1) dark fermentation with anaerobic bacteria, (2) photofermentation by photosynthetic bacteria, (3) direct/indirect biophotolysis using cyanobacteria and green algae, and (4) microbial electrolysis cell (MEC) (Hay et al., 2013). Dark fermentation has emerged as a promising technology for biological hydrogen production and offers several advantages over other processes. The transformation of LCB to biohydrogen is a multistep process that includes: (1) physical, chemical, and physicochemical pretreatment, (2) enzymatic hydrolysis of LCB to simple or complex sugars, (3) conversion of these sugars into organic acids, carbon dioxide (CO_2) and biohydrogen (H_2) through dark fermentation (Patil et al., 2021). During dark fermentation, LCB is converted to volatile fatty acids (acetic acid and butyric acid), H_2, and CO_2 by fermentative bacteria in the absence of light (Soares et al., 2020). Through the glycolytic pathway, bacteria convert the simple and complex saccharides obtained from the hydrolysis of cellulose to pyruvic acid by simultaneously producing ATP from ADP and NADH. Pyruvic acid is further converted to CO_2 and H_2 through the assistance of pyruvate ferredoxin oxidoreductase and hydrogenase enzymes. There are three types of hydrogenase enzymes primarily expressed by microorganisms (e−Fe, Fe−Fe, and Ni−Fe hydrogenase). The Fe−Fe hydrogenase can accelerate both the reduction of H^+ and the oxidation of H_2. Whereas Ni−Fe hydrogenase only increases the oxidation of hydrogen. Hence, Fe−Fe hydrogenase plays a primary role in fermentative H_2 production (Patil et al., 2021). Pyruvate can be further converted to acetyl-CoA, acetate, butyrate, and ethanol. In theory, Eqs. (10.1) and (10.2) exemplify that 4 mol of H_2/mol glucose can be produced when acetic acid is the byproduct, whereas 2 mol H_2/mol glucose is produced when butyric acid is the byproduct. However, not all glucose is converted to biohydrogen since some glucose is required to support and maintain microbial growth (Hay et al., 2013). In addition to LCB, pure sugars (glucose, xylose, lactose, galactose, sucrose), food waste, municipal solid waste, industrial wastewater, or glycerol can be utilized as feedstocks during dark fermentation (Soares et al., 2020). Furthermore, mixed cultures can be used, such as anaerobic sludge, bovine manure, and organic compost (Soares et al., 2020). Most biohydrogen-generating microorganisms are facultative anaerobes and obligate anaerobes (Sarangi et al., 2020). Examples of these anaerobic microorganisms include mesophilic (*Escherichia coli*, *Bacillus*, *Enterobacteria*) and strict anaerobic (*Clostridia*) and thermophilic bacteria (*Thermoanaerobacterium*).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CH_3COOH + 2CO_2 \quad (10.1)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2H_2 + CH_3CH_2CH_2COOH + 2CO_2 \quad (10.2)$$

Several benefits and limitations exist for biohydrogen as gas and its microbial production. For example, biohydrogen is a renewable and green energy resource that does not emit greenhouse gases (GHG) when combusted. It is also environmentally friendly since the combustion of biohydrogen produces only water vapor and heat as a byproduct (Giang et al., 2019). In addition, biohydrogen has a high

calorific value of (141 kJ/g) (Sarangi and Nanda, 2020); therefore it can be used to generate a vast amount of energy per unit mass (Hay et al., 2013). Furthermore, biohydrogen can be converted to hydrocarbon fuels or electricity by incorporating fuel cell technologies (Sarangi and Nanda, 2020). The microbial dark fermentation process can be carried out at ambient temperatures and pressures; therefore, it is less energy-intensive (Hay et al., 2013). The dark fermentation process also does not require a light source, making it economically viable and sustainable (Giang et al., 2019). However, major drawbacks arise from several aspects pertaining to the fermentation process. The fermentative bacteria are sensitive to pH and temperature as both fluctuations may negatively impact the metabolic pathway and growth rate of microorganisms (Hay et al., 2013). For biohydrogen production, an optimal pH of 5.5 during dark fermentation is required (Sarangi and Nanda, 2020). Dark fermentation can be operated at a temperature for mesophilic (25°C−40°C), thermophilic (40°C−65°C), and hyperthermophilic (> 80°C) microorganisms (Sarangi and Nanda, 2020). Compared to mixed cultures, pure cultures require a sterile environment to prevent contamination. However, this is problematic and expensive to achieve at an industrial scale (Soares et al., 2020). For this reason, mixed cultures have been given first preference for scale-up operations. In addition, mixed cultures are desirable because they do not require a sterile process and create synergies between microorganisms (Tapia-Venegas et al., 2015). Another underlying issue is the heterogeneity of waste materials, usually composed of various substrates. Furthermore, microorganisms present in the waste may consume these monosaccharides, thus leading to a lower biohydrogen yield than expected (Hay et al., 2013).

Several studies have focused on producing biohydrogen from various lignocellulosic substrates (Table 10.3). Cheng et al. (2015) pretreated water hyacinth with microwave-assisted dilute acid (1% H_2SO_4) and AC (activated charcoal detoxification) under dark fermentation and obtained a maximum hydrogen yield and production rate of 134.9 mL/g and 89.2 mL/L/h, respectively when the hydrogen-producing bacteria (HPB) were utilized for hydrogen fermentation. The findings indicated that AC could effectively remove the fermentative inhibitors produced in the acid pretreatment process. A slightly higher maximum biohydrogen yield of 150 mL H_2/g was obtained by Morales-Martínez et al. (2020) from Agave biomass using a synthetic *Clostridium* medium. Similarly, Wei et al. (2021) achieved a maximum biohydrogen yield and rate of 153.96 mL/g and 3.55 mL/h from sugarcane bagasse by employing ammonium-tolerant *Rhodobacter capsulatus*. In a study by Nasr et al. (2014), corn cobs were anaerobically digested, resulting in a maximum biohydrogen potential of 141 mL/g and biohydrogen yield of 265 mL/g. The highest biohydrogen production rate was 8.8 mL/h. A positive correlation between H_2 production rates and yields was observed. Moodley and Gueguim Kana (2018) assessed the pretreatment effectiveness of three acids (HCl, H_2SO_4, and HNO_3) on sugarcane leaf waste for the successive generation of biohydrogen. A potential maximum biohydrogen yield of 191.53 mL/g was obtained after optimized HCl (4.9%) pretreated corn cobs. A biohydrogen yield of 18.6 mL/g was attained. Previous studies have reported on the production of biohydrogen from various lignocellulosic

Table 10.3 Biohydrogen production from various lignocellulosic substrates.

Inoculum	Substrate	Process type	Parameter conditions	Biohydrogen production	Reference
Anaerobic sludge (HPB)	Water hyacinth	SHF	35°C, pH 6, 40 h	89.2 mL/L/h[b], 134.9 mL/g[c]	Cheng et al. (2015)
Synthetic medium (*Clostridium*)	Agave biomass	SHF	35°C, pH 5.5, 264 h	150 mL/g[c]	Morales-Martínez et al. (2020)
Anaerobic sludge (HPB)	Corn cobs	SHF	70°C, pH 5.5, 30 min	141 mL/g[a], 8.8 mL/h[b], 265 mL/g[c]	Nasr et al. (2014)
MedA medium (*Rhodobacter capsulatus* SB1003)	Sugarcane bagasse	SHF	30°C, pH 7, 108 h	3.55 mL/h[b], 153.96 mL/g[c]	Wei et al. (2021)
Anaerobic sludge	Sugarcane leaf waste	ND	37°C, pH 6.5, 180 rpm, 72 h	191.53 mL/g[a], 18.6 mL/g[c]	Moodley and Gueguim Kana (2018)

HPB: hydrogen producing bacteria; *ND*: not determined; *SHF*: separate hydrolysis and fermentation.
[a] P_m—potential maximum biohydrogen concentration (mL/g),
[b] $r_{p,m}$—maximum biohydrogen production rate (mL/L/h or mL/h),
[c] $Y_{p/s}$—biohydrogen yield (mL/g).

substrates; however, there has been a lack of microbial growth kinetics data due to the use of mixed microbial consortia.

10.5.4 Biogas

Biogas production has been encouraged globally because of its versatility and applicability for common purposes such as household uses (Meyer et al., 2021). In a recent study, Meyer et al. (2021) indicated that biodigesters can supply the energy required for cooking within households in remote areas and could result in monetary savings of approximately $2/d or $55/mon. Biogas consists of methane (55%−75%), carbon dioxide (CO_2) (25%−45%) and hydrogen (5%−10%) (de Mes et al., 2003; Kamali et al., 2016). This fuel can be upgraded to a higher quality (biomethane) or used for electricity and heat generation (de Mes et al., 2003). Biogas is a renewable energy resource derived from organic wastes (such as LCB) or industrial wastewater, mainly using anaerobic digestion (AD) (Moghaddam et al., 2019, Li et al., 2017). Prior to AD, a pretreatment process is required to enhance biogas production from LCB. Biogas production through AD (nonoxidative metabolism) is a naturally occurring biological process that can be divided into four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Xu et al., 2019). During hydrolysis, various microbes (*Bacteriodes, Clostridium*, and *Acetivibrio*) secrete hydrolytic enzymes that cleave the complex organic macromolecules (proteins, lipids, and carbohydrates) to their equivalent component units (amino acids, fatty acids, and sugars). Following hydrolysis, the monomers are converted by

Enterobacterium, *Acetobacterium*, and *Eubacterium* into various organic acids (acetic, propionic acid, butyric acid, succinic acid, and lactic acid), alcohols, and ammonia. The acetogenic bacteria (*Syntrophomonas, Syntrophus, Clostridium*, and *Syntrobacter*) further convert these compounds to acetate, carbon dioxide, and hydrogen, which are natural substrates for methanogenesis (Xu et al., 2019, Goswami et al., 2016). Methanogenesis involves the fermentation of various organic compounds (carbon dioxide and hydrogen gas) to generate biogas as the end product (Goswami et al., 2016). Common inoculum sources for AD and biomass gasification include agricultural waste, municipal solid waste, and waste-activated sludge (Li et al., 2017). In addition, animal manure and food wastes contain a significant amount of biodegradable organic carbon, which can be used for biogas production.

Biogas production has several benefits and drawbacks. An advantage of the AD process is the low requirements for heat and electricity. Furthermore, it is a sustainable process that generates a residual fertilizer (digestate rich in nitrogen and phosphorus), which can be used for soil conditioning (Ersahin et al., 2011). Moreover, mixing several wastes (codigestion) increases biogas yields and cost efficiency. Ammonia reduction, carbon to nitrogen ratio, and essential trace elements contribute to a controlled reaction environment for microorganisms, subsequently influencing the yield of biogas produced (Xu et al., 2019). Nevertheless, several disadvantages persist, whereby biogas produced from AD comprises a large amount of carbon dioxide (25%−45%). For biogas to be used as fuel for vehicles, it must be upgraded by removing CO_2 to achieve a high purity ($>95\%$) of biomethane. The cost of upgrading is relatively high and represents a significant bottleneck for increasing biogas yields (Li et al., 2017). Factors that affect the efficiency of the generation of biogas involve pH, temperature, alkalinity, hydraulic retention time and organic loading rates, presence of toxic and inhibitory compounds, type of substrate and total solids (TS) and volatile solids (VS) content (Ersahin et al., 2011).

Numerous studies have obtained biogas from various lignocellulosic feedstocks (Table 10.4). For example, Ugwu and Enweremadu (2019) mechanically pretreated Orka pod waste and fermented it with anaerobic digest for 25 days generating a biogas yield of 270.98 mL/g. Similarly, Mustafa et al. (2016) subjected rice straw to fungal pretreatment with *Pleurotus ostreatus* prior to AD to enhance the biodegradability of rice straw and biogas production yield. A biogas yield of 263 mL/g was obtained at a moisture content of 75% after 20 days. A 120% higher biogas yield was observed from pretreated rice straw than from untreated rice straw.

Similarly, Fu et al. (2015) pretreated corn straw using a thermophilic microaerobic pretreatment. Structural analysis showed disruption of cellulosic structures and pores of corn straw during pretreatment. A maximum biogas yield (325.7 mL/g) was obtained at an oxygen load of 5 mL/g substrates. The highest biogas value received was 16.24% higher than untreated corn straw. In another study, Oleszek et al. (2014) compared biogas yields from two types of reed canary grass (cultivated and wild). A higher biogas yield of 406 mL/g (cultivated reed grass) was obtained compared to 120 mL/g (wild reed grass). It was determined that the chemical composition and indigestible crude fiber and ash within the reed grass contributed to the

Table 10.4 Biogas production from various lignocellulosic substrates.

Inoculum	Substrate	Process type	Parameter conditions	Biomethane production	References
Anaerobic digest	Okra pod waste	SHF	37°C, pH 7.68, 25 d	268.4 mL/g[a], 270.98 mL/g[c]	Ugwu and Enweremadu (2019)
Anaerobic sludge (*P. ostreatus*)	Rice straw	SSF	37°C, 20 d, 75% moisture content	263 mL/g[c]	Mustafa et al. (2016)
Biogas slurry	Corn straw	SHF	37°C, 130 rpm, oxygen 5 mL/g substrate	325.7 mL/g[c]	Fu et al. (2015)
Sewage sludge	Reed canary grass (cultivated)	ND	35°C, pH 7.0, 20 to 40 d	406 mL/g[c]	Oleszek et al. (2014)
Sewage sludge	Reed canary grass (wild-type)	ND	35°C, pH 7.0, 20 to 40 d	120 mL/g[c]	Oleszek et al. (2014)
MPB (methane producing bacteria)	Water hyacinth hydrolysate	SHF	35°C, pH 7.0, 5 d	123 mL/g[c], 189.4 mL/L/d[b]	Cheng et al. (2015)

ND: not determined; *SHF*: separate hydrolysis and fermentation; *SSF*: simultaneous saccharification and fermentation.
[a] P_m—potential maximum biomethane concentration (mL/g),
[b] $r_{p,m}$—maximum biomethane production rate (mL/L/d),
[c] $Y_{p/s}$—biomethane yield (mL/g).

variations observed. Furthermore, these components could reduce biogas quantity and quality. The study by Cheng et al. (2015) subjected residual hydrolysates of water hyacinth to biogas production and produced a yield and production rate of 123 mL/g and 189.4 mL/L/d, respectively.

10.6 Current challenges of lignocellulosic biofuel production

Even though microbially produced biofuels from LCB sources have proven advantageous, their production presents many technological issues. These drawbacks occur at different biofuel processing stages and significantly hinder its large-scale implementation. Pretreatment is necessary to make the cellulose moieties within LCB accessible to enzymatic attack for enhanced conversion of polysaccharides to monosaccharides. Numerous pretreatments have been established with chemical methods displaying high efficacy since they can recover up to 80% of fermentable sugars compared to pretreatments without chemicals. However, these pretreatment strategies incur high costs due to the chemicals required, equipment, and reactor compatibility. Additionally, water is a finite resource required in large quantities during pretreatment, further placing strain on resources (Kumar and Sharma, 2017). Heating processes in pretreatment are also associated with high energy and cost (Moodley et al., 2020). Furthermore, the generation of inhibitors

due to chemical methods reduces the product yields during enzymatic saccharification and fermentation and reduces the product's purity during recovery. More specifically, regarding the biofuel production process, one major challenge is the significant disparity in the number of studies, with some fuels receiving higher priority than others. For instance, numerous studies have assessed the SHF, SSF, and PSSF systems for bioethanol production, while a significant knowledge gap exists for other biofuels (biobutanol, biohydrogen, and biogas). Likewise, kinetic modeling plays a fundamental role in understanding the metabolic fluxes occurring within the cell and product formation (David et al., 2020), but most studies have focused on bioethanol production with a lack of data for fuels such as biohydrogen and biogas. Moreover, the production of biofuels requires a significant amount of energy input for heating the bioreactors and thermal activation of biochemical reactions (Baruah et al., 2018). On the research front, most studies on biofuel production have been carried out at a laboratory scale; therefore there is a dearth of translatable data for scale-up. Apart from the drawbacks mentioned above, safety limitations of biofuels such as biohydrogen include its explosiveness when reacted with oxygen. Therefore it has to be stored in specialized vessels under pressure (Sarangi and Nanda, 2020).

10.7 Advancements in lignocellulosic biofuel production

The commercialization of biofuel production is still at an early and dynamic stage in the current markets due to the high costs attached. Many technologies have been developed to mitigate the limitations of lignocellulosic waste-based biofuel production with minimal capital investments and increased product outputs. To date, studies have aimed to improve the systematic approaches to the four stages of biofuel production: pretreatment, enzymatic saccharification, fermentation, and downstream processes. Pretreatment is imperative when considering LCB and accounts for 40% of the total production expenses. The pretreatment step is a key process that gets the ball rolling toward high product yields, improved downstream applications, and reduced capital costs (Kucharska et al., 2018b; Limayem and Ricke, 2012). Researchers have been investigating the suitability of new lignocellulosic waste sources, assessing new pretreatment strategies, and optimizing the existing methods to enhance this process (David et al., 2020; Rorke et al., 2021). For example, the development of alkali and inorganic salt pretreatments has emerged as a cheaper and more environmentally friendly alternative to more expensive chemicals such as NaOH (Moodley and Gueguim Kana, 2017a; Qing et al., 2016; Sewsynker-Sukai and Guegium Kana, 2018; Bhardwaj et al., 2020). Particularly, inorganic salts such as sodium chloride (NaCl) are emerging as efficient pretreatments (Moodley and Gueguim Kana, 2017b) that offer impurified equivalents in the form of table salt, that is widely accessible, noncorrosive, and do not negatively impact on the environment. However, recent studies have focused on using chemicals generated from industrial chemical lines to pretreat LCB. For instance, green and black liquor from

the Kraft paper and pulp industry has previously been investigated for their pretreatment abilities (Zhou et al., 2017; Goshadrou, 2019). However, these residues are usually recycled into the Kraft paper and pulp industry to be used again. On the other hand, green liquor dregs have been brought to the forefront as a potential pretreatment catalyst for lignocellulosic pretreatments since these residues possess alkaline characteristics that have been shown to be beneficial during pretreatment (David et al., 2020; Rorke et al., 2021). Moreover, these residues are discarded after the Kraft paper and pulping process since they cannot remain in the filter lines due to blockages and hydraulic conductivity issues. Therefore diversion of these waste residues to the pretreatment aspect of biofuel production will drastically reduce the expenses attached to the cost of pretreatment chemicals. Besides the high chemical cost, water usage also significantly impacts operation costs. To solve this problem, the use of industrial wastewater has been proposed to be coupled with pretreatment chemicals to omit the use of freshwater streams (Sewsynker-Sukai et al., 2020). Implementing these strategies may become a suitable method that can be assessed for scale-up of biofuel production. In conjunction with pretreatment strategies, important aspects such as process development, optimization, and mathematical kinetic modeling during fermentation are imperative for industrial-scale biofuel production. For this reason, researchers need to understand the kinetics of each process type (SHF, SSF, and PSSF) to enhance biofuel production's feasibility and productivity. Recent biofuel production studies have proposed the application of nanotechnology and surfactants as catalysts that could improve the biological dynamics such as enzymatic saccharification and microbial cellular processes of the system (Sanusi et al., 2021; Rorke et al., 2021). In addition to catalyst inclusion and kinetic studies, genetically modified fermentative microorganisms capable of resisting adverse conditions such as tolerance to inhibitors, high temperatures, and the ability to uptake co-ferment hexoses and pentoses should be explored (Kumar and Sharma, 2017). Furthermore, employing a single microorganism that can consume all available sugars (broaden substrate utilization) simultaneously at high rates in a medium could shorten fermentation time, consequently improving volumetric productivity and increasing productivity yields (Hay et al., 2013). With the abovementioned advancements in mind, it is interesting to note that the production of high-value industrial commodities such as itaconic acid, lactic acid, and citric acid, amongst others, may present another avenue to alleviate high costs incurred during biofuel production processes. Following the fermentation of value-added products, the effluent generated still contains residual components of vitamins, peptides, and carbon sources. This fermentation effluent can be channeled toward the production of biofuels such as bioethanol, biobutanol, biohydrogen, biogas, or other desired biofuels. This proposed concept of using the effluent from the production of other commodities intends to manipulate every aspect of waste to achieve a circular bioeconomy with complete waste valorization (Pinales-Márquez et al., 2021; Patel and Shah, 2021).

10.8 Conclusion and future perspectives

Recently, second generation (2G) biofuels from lignocellulosic feedstocks have gained widespread attention regarding their sustainability and nonpolluting nature. An abundance of literature on lignocellulosic biofuel production systems pertaining to pretreatment, enzymatic hydrolysis, and microbial fermentation toward biofuels and other bioproducts. Biorefinery systems incorporate various thermal, chemical, and biological processes to produce multiple products (biofuels and bioproducts) and utilize the feedstocks involved completely. Nevertheless, the interconnection between research and applied biotechnology requires swift action to identify the knowledge gaps to overcome these systems' technological, economical and environmental barriers. Multidisciplinary approaches that bring experts from various backgrounds seem to be the solution to achieving successful biorefineries, with a long path ahead toward industrialization.

Abbreviations

2G	second generation
ABE	acetone-butanol-ethanol
AC	activated charcoal detoxification
AD	anaerobic digestion
ADP	adenosine diphosphate
ATP	adenosine triphosphate
Btu	British thermal units
ED	Entner–Doudoroff
EMP	Embden–Meyerhof–Parnas
GHG	greenhouse gases
HPB	hydrogen-producing bacteria
IBE	isopropanol-butanol-ethanol fermentation
$\mathbf{K_s}$	monod constant
LCB	lignocellulosic biomass
MEC	microbial electrolysis cell
NADH	nicotinamide adenine dinucleotide hydride
ND	not determined
OPFJ	oil palm frond juice
P_m	maximum product concentration
PSSF	SSF with a prehydrolysis step
$r_{p.m}$	maximum product production rate
SHF	separate hydrolysis and fermentation
SSF	simultaneous saccharification and fermentation
TS	total solids
VS	volatile solids
X_{max}	maximum cell biomass concentration
$Y_{p/s}$	product yield
μ_{max}	maximum specific growth rate

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Integrated biorefineries: The path forward

13

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13.1 Introduction

The depletion of petroleum-based sources due to the increasing global population and the detrimental effect on the environment has intensified the search for suitable alternatives. Biofuels produced from microorganisms are being explored globally as the race towards alternative energy sources continues (David et al., 2020). While biofuels are renewable, sustainable, and reduce harmful effects on the environment, their production processes are currently expensive and generate low yields. Therefore integrated biorefineries have emerged as innovative multistaged systems for developing high-value bioproducts and biofuels (Pinales-Márquez et al., 2021; Patel and Shah, 2021). Remarkably, the biorefining of renewable lignocellulosic biomass (LCB) feedstocks has garnered significant attention towards achieving a circular bioeconomy (Banu et al., 2021). Unlike agricultural crops, LCB is a waste that is produced in large quantities and does not hinder food security, thus presenting an ideal feedstock for biorefinery systems (David et al., 2020; Ray, 2021). Wastes generated from corn, sugarcane, sorghum, rice, and wheat processing industries are examples of LCB that have previously been used for the microbial production of industrially relevant value-added compounds and biofuels (Moodley et al., 2020; David et al., 2020).

Three main steps, biomass pretreatment, enzymatic hydrolysis, and fermentation, are involved in lignocellulosic bioprocesses (such as bioethanol production). The pretreatment step is crucial since it unwinds the resistant lignocellulosic structures such as lignin and hemicellulose for cellulose release. The enzymatic hydrolysis step is essential for converting the complex polysaccharides to fermentable monosaccharides, which are metabolized into biofuels (David et al., 2020; Behera et al., 2022). Microbial biofuels and bioproducts can be produced using three process types: (1) separate hydrolysis and fermentation (SHF), (2) simultaneous saccharification and fermentation (SSF), and (3) simultaneous saccharification and fermentation with prehydrolysis (PSSF) (Sewsynker-Sukai and Gueguim Kana, 2018). Nevertheless, the high process cost and low product yields continue to plague the commercialization of lignocellulosic biofuel production.

Advances in Lignocellulosic Biofuel Production Systems. DOI: https://doi.org/10.1016/B978-0-323-91192-4.00019-5

Therefore integrated lignocellulosic biorefineries are being investigated globally to overcome these challenges and promote the commercialization of biofuels.

More recently, microbial-derived bioproducts such as lactic acid, itaconic acid, and succinic acid from lignocellulosic substrates have been assessed as high-value compounds of industrial significance (Chen et al., 2019; Krull et al., 2017; Anwar et al., 2021). These value-added compounds possess versatile applications and serve as precursors for producing biopolymers, chemicals, and fuels (Chen et al., 2019; Yang et al., 2020; Akhtar and Idris, 2017). Integrated biorefineries demonstrate the potential to alleviate the bottlenecks typically associated with biofuel production processes, with a different goal to achieve complete valorization of waste-based substrates and transform the landscape of lignocellulosic processes. Although biorefineries represent what could soon be ideal systems, several aspects must first be addressed to promote their feasibility at an industrial scale level.

This chapter provides an in-depth evaluation of integrated biorefineries by detailing the chemical and biological processes involved in forming these systems. First, feedstocks, pretreatment methods, and pretreatment selection criteria for biorefineries are outlined. Subsequently, the different fermentation process types are compared, followed by the microbial fermentative production of bioethanol, biohydrogen, and biogas. Then, microbial-derived high-value products (lactic acid, itaconic acid, and succinic acid) and value-added products (benzaldehyde, biochar, and bio-oil) are briefly outlined. Next, the innovations in lignocellulosic biorefineries are briefly highlighted. Additionally, the life cycle assessment (LCA) and techno-economic analysis of biorefineries are discussed. Lastly, the current challenges, trends, and future perspectives toward enhancing integrated biorefineries are presented.

13.2 Feedstocks for biorefineries

The evolution of integrated biorefineries has unraveled the conceptualization of biomass-based conversion processes analogous to petrochemical refineries. In this context, these substrates can be employed in a biorefinery system where several bioproducts are produced in a process. Interestingly, biomass contains an elementary composition of carbon, hydrogen, and oxygen similar to fossil resources, with the advantage of being renewable and abundant. Therefore several substrates have been extensively investigated to produce bioproducts ranging from biofuels, bioenergy, and commodity chemicals.

13.2.1 Lignocellulosic substrates

LCBs, such as postharvest forestry and agricultural residues, are an abundant resource available for the generation of biofuels and value-added products, with their global production reaching approximately 120 billion tons per annum (Abraham et al., 2020). Lignocellulose continues to attract global interest due to the

broad areas of application and variety of conversion methods available (Chen, 2015). The primary composition of lignocellulose involves an intricate complex of the polysaccharides hemicellulose and cellulose and the phenolic polymer lignin. This polymer contributes significantly to the strength and rigidity of plant cell walls and protects hemicellulose and cellulose from enzymatic and microbial degradation (Yoo et al., 2020). Cellulose and hemicellulose contribute between 40%–60% and 20%–35%, respectively, to lignocellulose weight and are composed of 500–1400 D-glucose units (cellulose) and 100–200 glucose units (hemicellulose) (Zoghlami and Paës, 2019), while lignin makes up 5%–40% of lignocellulose and contributes significantly to the recalcitrance (Yoo et al., 2020). There is a wide variety of lignocellulose resources, so variation in lignocellulosic composition is high (Table 13.1). Efficient conversion remains a research hotspot as a "one size fits all" approach results in low hydrolysis rates and, subsequently, low product yields. Efforts to valorize different lignocellulosic substrates and their components (lignin, hemicellulose, and cellulose) to reduce this waste and increase its value may be achieved by adopting a biorefinery concept.

13.2.2 Lignocellulose-starch substrates

Presently, significant efforts are being driven toward biofuel generation from renewable sources. These renewable sources include sugar crops, starch crops, and lignocellulosic and lignocellulose-starch biomass (LCSB) (Mojović et al., 2009; Aruwajoye et al., 2017). For all the substrates previously mentioned, biofuel

Table 13.1 Lignocellulosic composition of commonly used substrates.

	Cellulose (% w/w)	Hemicellulose (% w/w)	Lignin (% w/w)	Reference
Pinus spp. sawdust	52.7 ± 2.8	12.9 ± 2.6	26.7 ± 0.9	Morales-Máximo et al. (2021)
Miscanthus sinensis	37.7 ± 3.8	22.9 ± 3.7	17.4 ± 1.3	Xu et al. (2020)
Miscanthus floridulus	36.3 ± 2.6	22.0 ± 3.6	16.9 ± 1.2	Xu et al. (2020)
Miscanthus sacchariflorus	39.3 ± 3.1	26.4 ± 3.7	18.1 ± 1.4	Xu et al. (2020)
Sugarcane bagasse	35.2 ± 0.9	24.5 ± 0.6	22.2 ± 0.1	Rezende et al. (2011)
Sweet sorghum bagasse	36.1 ± 1.1	31.2 ± 0.8	24.8 ± 2.0	Camargo et al. (2019)
Corn stover	36.5 ± 2.1	22.1 ± 0.9	18.8 ± 1.7	Yang et al. (2016)
Wheat straw	38.2 ± 0.1	29.0 ± 0.1	15.7 ± 0.0	Tufail et al. (2018)
Eucalyptus grandis sawdust	43.6 ± 2.1	11.1 ± 0.5	30.5 ± 0.5	Rochón et al. (2022)

production is only achievable through fermentative processes that utilize simple sugars released from the disintegration of polymeric carbohydrates embedded in the biomass. Currently, LCBs are generally preferred as substrates for biofuel production, despite the progress of this technology still being in the developing stages. Up to 70% of LCB are usually agricultural wastes generated as by-products of food preparation, farming activities, and the remains of crops postharvesting (Agamuthu, 2009; Balan, 2014; Nagendran, 2011). The preference for LCB over sugar and starch crops is founded on their lack of interference with food availability and the preservation of food security (Ray and Ramachandran, 2018). Although LCBs are considered wastes, they consist of highly organized structural carbohydrates—hemicellulose and cellulose often intertwined with lignin (Mosier et al., 2005).

On the other hand, LCSB have starch as an additional and major polysaccharide component in their composition, which confers the "starch-based" characteristic. Various LCSB substrates have been reported, including cassava peels, leaves, stems and bagasse, sweet potato peels and bagasse (Aruwajoye et al., 2017; Ray and Naskar, 2008; Chohan et al., 2020; Ojewumi et al., 2018; Pooja and Padmaja, 2015). The compositional analysis of LCSB reveals a starch composition in the range of 2%−66% depending on the type of source crop (Table 13.2), nature of the postharvesting activity, and other environmental factors involved during their generation (Aruwajoye et al., 2020). Starch, the reference component of LCSB, is a giant polymer of monomeric sugars, usually in the form of amylose and amylopectin chains. While α-1−4 glycosidic bonds link the amylose polymer, the amylopectin contains α-1−6 bonds in addition to the presence of α-1−4. Often, the extent of cleavage of the starch bonds during the pretreatment of LCSB significantly determines the level of the simple sugar yield for fermentative processes.

13.3 Overview of pretreatment

In lignocellulosic biorefineries, the general purpose of pretreatment is to unlock the lignin framework and expose the carbohydrate polymers to enzymatic hydrolysis. The highly ordered structure of lignin acts as a physical barrier that protects cellulose and hemicellulose from being biologically or chemically degraded into their metabolically favored form (Kumar et al., 2020a,b). To reduce these limitations, lignocellulosic pretreatment aims to modify the complex structures and improve the biodegradability of the biomass by removing lignin, reducing cellulose crystallinity, and increasing the reactive cellulose surface area and porosity (Kim, 2013). These key LCB pretreatment approaches are necessary prior to its exposure to downstream biological processes such as enzymatic saccharification and fermentation. Various pretreatment methods such as physical, biological, chemical, and a combination of these different strategies have been implemented.

Physical pretreatment usually involves mechanical, hot water, and radiation-based strategies that reduce the particle size of the biomass, expand the contact surface area, and decrease the degree of polymerization and cellulose crystallinity

Table 13.2 Different lignocellulos-starch biomass types with their composition.

LCSB substrate	Starch (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	References
Cassava stem	15.0	22.8	28.8	22.1	Pooja and Padmaja (2015)
Cassava peels	2–45	9–18	7–28	1–10	Pooja and Padmaja (2015); Aruwajoye et al. (2017)
Potato peels	20–42	4–34	5–10	4–33	Ben Taher et al. (2017); Hijosa-Valsero et al. (2018); Chohan et al. (2020)
Sweet potato peels	32.05	13.31	13.32	8.15	Ray and Naskar (2008); Mithra and Padmaja (2016)
Pumpkin waste	65.3	NR	NR	NR	Chouaibi et al. (2020)
Pumpkin peels	24.61	21.05	17.74	10.66	Mithra and Padmaja (2017)
Elephant foot yam peels	28.96	15.63	14.00	7.01	Mithra and Padmaja (2016)
Tannia peels	30.46	17.32	14.48	8.26	Mithra and Padmaja (2016)
Greater yam peels	28.84	18.02	20.02	6.72	Mithra and Padmaja (2017)
Beetroot peels	27.13	18.94	19.17	3.87	Mithra and Padmaja (2017)
Banana peels	36.56	22.40	15.19	10.55	Mithra and Padmaja (2017)

Note: The starch, cellulose, hemicellulose and lignin are represented in wt.% and are determined from dry biomass.
NR: not reported.

(Kumar et al., 2020a). Several approaches have been discovered, including grinding, extrusion, sonication, steam explosion, microwave, and gamma rays. Even though physical methods are environmentally friendly and produce little to no toxic by-products, these regimes are limited to low lignin-containing substrates since they cannot remove lignin (Kumar et al., 2020a). Therefore these methods are often applied as a preparation step prior to or in conjunction with other pretreatments (Kumari and Singh, 2018).

On the other hand, biological pretreatment involves the action of microbial metabolism (fungi and bacteria) or bio-based products (enzymes) for the degradation of cellulose, hemicellulose, and lignin fractions within lignocellulosic feedstocks (Kumar et al., 2020a). The microbial degradation process involves two extracellular systems: the hydrolytic and ligninolytic (Wagner et al., 2018). The hydrolytic system is required to break down cellulose and hemicellulose, while the ligninolytic system is involved in depolymerizing lignin using enzymes such as peroxidases and laccases (Baruah et al., 2018). Biological pretreatments are known for low energy requirements, cost efficiency, easy downstream processing, little to no waste generated, and reduced enzymatic and fermentation inhibitors (Abraham et al., 2020). However, despite these advantages, this type of pretreatment requires long process times and continuous records of microbial growth, making it a tedious and inefficient process (Kumari and Singh, 2018).

Chemical pretreatments are most often adopted and mediated by either acid, alkaline, inorganic salts, ionic liquids, or organosolvents. These strategies function by engaging in chemical reactions within the aqueous solution, resulting in delignification and hemicellulose solubilization. The mechanism of action and the intended outcome depend significantly on the type of chemical used and the operating conditions during the pretreatment process (Abraham et al., 2020). Singhvi et al. (2014) reported that chemical pretreatments tend to improve the enzyme digestibility of the biomass and enhance the overall yield of fermentable sugars by approximately 80% in comparison to pretreatments that are void of chemical supplementation. Additionally, these methods are energy efficient, stable, and flexible with the choice of substrate. Although chemical pretreatment regimes offer numerous advantages, these methods are usually costly, may cause corrosion of reaction vessels, and release inhibitory compounds that affect downstream processes. Consequently, the chemicals used for pretreatment must be carefully selected and evaluated for commercial production of the desired products.

Several pretreatment studies have focused on a combination of physical, biological, and chemical strategies, with alkaline-based methods shown to be the most promising (Woiciechowski et al., 2020). Nevertheless, alkaline methods are costly and energy-intensive and may produce undesirable inhibitor compounds due to their harsh operating conditions (Balat et al., 2008; Hendriks and Zeeman, 2009). Recently, waste-based pretreatment strategies such as paper wastewater and green liquor dregs have been proposed to alleviate limitations associated with high energy, chemical costs, and water consumption (David et al., 2020; Sewsynker-Sukai et al., 2020).

13.4 Pretreatment selection criteria for microbial-derived products in biorefineries

The selection of a specific lignocellulosic pretreatment method, although frequently determined based on costs, energy, and sugar yield, as stated previously, has also

shown to be dependent on the target fermentation product to be produced. For instance, C5 sugars are derived from heteropolymer hemicellulose. Therefore a pretreatment that solubilizes this lignocellulosic structure must be applied. However, the application of C5 sugars in microbial bioprocesses remains limited since only a few microorganisms can metabolize sugars, such as xylose. Nevertheless, xylose has been widely used as an artificial sweetener in the food industry but may also be utilized for microbial biohydrogen production using the dark fermentation process (Mafuleka and Gueguim Kana, 2015).

On the other hand, the cellulose polymer gives rise to C6 sugars such as glucose, which is most often used in fermentation processes since microbial metabolic pathways typically follow glycolysis. Thus pretreatment methods that reduce cellulose crystallinity to enhance its enzymatic digestibility are selected. An array of microbially produced value-added compounds may be generated from glucose, such as biofuels (bioethanol, biohydrogen, biogas) and value-added products (lactic acid and itaconic acid), among several others.

Even with the selection of specific pretreatments that target cellulose and hemicellulose structures for the production of a particular compound, the aromatic lignin polymer causes a significant hindrance by acting as a resistant barrier that prevents and reduces hydrolysis of cellulose and hemicellulose structures. Because of this, the selected pretreatment must ideally be capable of degrading lignin and subsequently target cellulose, hemicellulose, or both. Apart from the desired value-added compound to be produced, the basis for selecting a pretreatment method should also consider the fermentation and/or enzymatic inhibitor compounds. These inhibitory compounds are formed as by-products of pretreatment and are primarily determined by the lignocellulosic feedstock composition and the severity and type of pretreatment method utilized (Rorke and Gueguim Kana, 2016). Some inhibitory compounds include aliphatic acids, phenolic compounds, and furan derivatives. More specifically, aliphatic acids (acetic acid) and furan aldehydes (furfural and 5-hydroxymethylfurfural) are inhibitors that are commonly found in pretreatment lignocellulosic hydrolysates and, to a lesser extent, certain phenolic compounds (2-methoxy-4-vinyl phenol and 4-hydroxy-benzaldehyde). The formation of acetic acid during chemical (acid, alkaline, oxidation, kraft pulping) and/or hydrothermal pretreatment occurs as a result of the degradation of acetyl and ester bonds in hemicellulose. Furan derivatives such as furfural and 5-hydroxymethylfurfural (HMF) form when carbohydrates (pentose and hexose) are degraded during acid- and/or hydrothermal-based pretreatments (Harmsen et al., 2010).

Moreover, under prolonged severe pretreatment conditions, including long reaction time and high temperature and/or acid concentration, HMF is further degraded to other inhibitory compounds such as levulinic and formic acids (Fengel and Wegener, 1989). On the other hand, phenolic compounds are formed when ether bonds in lignin are fragmented due to alkaline pretreatment. Inhibitor compounds negatively impact the cellulase enzyme and microbial fermentation process by interfering with enzyme activity and cell metabolism; therefore, their removal is imperative (Gupta et al., 2017). Generally, the application of harsh pretreatment conditions, such as high temperatures and high chemical concentration, in an

attempt to improve fermentable sugar yields leads to inhibitor compound formation. Various strategies have previously been evaluated to alleviate the detrimental effects of lignocellulosic fermentation inhibitors, which include preventing their formation, converting them to a nontoxic form, or detoxifying the pretreatment hydrolysate (Taherzadeh et al., 2000). Furthermore, the selected pretreatment must be cost-effective from a chemical and energy perspective, eco-friendly, and lead to a high sugar and fermentation yield.

13.5 Microbial fermentation

Biowastes have become a well-recognized carbon source for their conversion into biofuels and platform chemicals with a wide range of applications in various industries. The monomeric sugars released following pretreatment and enzymatic hydrolysis of these biowastes become the intermediates in the overall process channeled towards microbial metabolism to produce value-added products. Microbial conversion processes depend on fermentation or anaerobic digestion (AD) by specific microorganisms, including bacteria, yeasts, fungi, and microalgae (Gomez et al., 2021). The preferred microorganisms required for microbial product formation must be able to metabolize broader substrate spectrums, possess high specific growth rates, increased substrate to substrate, higher substrate to biomass yield, higher overall tolerances, and improved metabolic fluxes (Höffner and Barton, 2014). Several microorganisms may utilize a single hydrolyzed biomass stream to produce various bioproducts, significantly boosting the economics of a biorefinery. However, the hydrolyzed biomass may contain a mixture of both hexose (glucose, fructose, mannose) and pentose sugars (xylose, arabinose) depending on the type and composition of the feedstock (Raud et al., 2019). Most microorganisms require hexoses, mainly glucose, to carry out their metabolic processes towards product formation. At the same time, pentose metabolism is restricted to a few native strains with relatively low yields and high sensitivity to process conditions (temperature, pressure, pH) (Mosier et al., 2005). Therefore choosing a suitable inoculum source is imperative to the productivity and functionality of a biorefinery process. For this reason, various microbial strains may be introduced into the system to produce different bioproducts, while in other cases, a single microorganism source with the dual capability of utilizing both pentose and hexose sugars is employed (Moodley, 2021).

13.6 Lignocellulosic fermentation process type

Cellulosic product formation occurs following three key steps:

1. LCB pretreatment,
2. enzymatic saccharification, and
3. microbial fermentation.

The enzymatic hydrolysis stage has been deemed a fundamental step in the bioproduct formation process because the fermentable sugars that will undergo fermentation are released during this step. Microbial bioproduct formation using lignocellulosic wastes can be produced using three different process types:

1. SHF,
2. SSF and,
3. simultaneous saccharification and fermentation with a prehydrolysis step (PSSF) (Carrillo-Nieves et al., 2017).

The above-mentioned process has its merits and bottlenecks that are discussed below.

13.6.1 Separate hydrolysis and fermentation

SHF is a method by which enzymatic hydrolysis and fermentation are performed sequentially. The process principle of SHF includes the separation of hydrolysis and fermentation of the reactions in separate units. In SHF, both hydrolysis and fermentation are carried out at their optimal temperatures for hydrolysis and fermentation. Within SHF systems, enzymatic saccharification of pretreated LCB is carried out first at the optimal temperature of the saccharifying enzyme (40°C−50°C); fermenting microbes (30°C−40°C) are then added to ferment the saccharified sugar at its optimal process conditions (David et al., 2020). Since enzymatic hydrolysis is performed at optimal temperature, SHF may require a lower quantity of saccharifying enzymes than the SSF process but achieves a higher saccharification efficiency (Binod et al., 2010). The SHF system, however, requires two independent reactors for the different reactions (enzymatic hydrolysis and fermentation), thus placing an additional cost on equipment. Moreover, the sugar accumulation during the hydrolysis stage may result in carbohydrate feedback inhibition effects on cellulolytic enzymes, reducing their hydrolyzing capability and efficiency (Koppram et al., 2013; Binod et al., 2010).

13.6.2 Simultaneous saccharification and fermentation

SSF is a process that combines enzymatic hydrolysis with instantaneous fermentation to obtain desired bioproducts (Ballesteros et al., 2004). This method is based on the use of the enzyme complex for hydrolyzing cellulose and sugar production (Guilherme et al., 2019). Microorganisms simultaneously ferment the released sugars to produce a particular product. The SSF process has several advantages over other process types. Compared to SHF, some advantages are using a single vessel for fermentation and saccharification, reduction in residence times, and the capital costs of the process (Pratto et al., 2020). Another prominent advantage is the reduction of inhibitory compounds from enzymatic hydrolysis, which improves the process's overall performance (Shi et al., 2019). Due to these advantages, SSF has been widely employed for the production of biofuels such as ethanol and butanol from lignocellulosic and starchy feedstocks (Izmirlioglu and Demirci, 2012;

Sewsynker-Sukai and Gueguim Kana, 2018; Moodley and Gueguim Kana, 2019; Shi et al., 2019; Sanusi et al., 2021).

Furthermore, the SSF process eliminates the inhibition of saccharifying enzymes that could occur from high initial fermentable sugar concentration because the resulting sugars are converted to products by fermenting microorganisms. However, the SSF process has a significant disadvantage compared to the SHF process since the optimum temperature and pH for microbial fermentation differ from that for the enzymatic hydrolysis step (Olofsson et al., 2008). It is, therefore, necessary to find an equilibrium point where the process works optimally for both the enzymatic and fermentation processes to avoid adverse effects on either reaction (Binod et al., 2010).

13.6.3 Simultaneous saccharification and fermentation with a prehydrolysis step

The PSSF processes have been introduced to enhance the efficiency of SSF processes by including a short prehydrolysis stage as a prior technique. Prehydrolysis before simultaneous saccharification and fermentation is usually performed by subjecting the saccharifying enzymes to their optimal temperatures for a short period. The optimal temperatures during prehydrolysis are often at elevated levels ($>40°C$), which are higher than the selected conditions for the SSF when the microbe is introduced. This elevated temperature reduces the initial viscosity of the medium and allows for optimal performance of the saccharifying enzyme for a short time, leading to a higher bioethanol yield (He et al., 2016; Zhu et al., 2015). However, despite the short time usually involved in prehydrolysis stages, higher process costs are generally experienced compared to direct SSF because of the demands of the additional stage, energy, and operational units needed (Wingren et al., 2003). Besides the disadvantage of additional process costs, comparative studies and kinetic evaluation of PSSF and SSF processes on some substrates have revealed a lack of significant difference in their bioethanol concentrations (Aruwajoye et al., 2020; Sewsynker-Sukai and Gueguim Kana, 2018). Consequently, to achieve process profitability from PSSF, it may be necessary to consider critical factors such as substrate type, yeast strain, and other fermentation conditions.

13.7 Lignocellulosic biofuel production

The microbial production of biofuels from LCB has become a global research hotspot in the search for sustainable processes. Bioethanol, biohydrogen, and biogas are being exhaustively studied for supplementing current energy sources, and these biofuels are discussed below.

13.7.1 Bioethanol

Bioethanol is one example of liquid biofuels that can be obtained from LCB. Currently, bioethanol is one of the preferable liquid biofuels for the replacement of fossil fuels. The significant advantages of bioethanol over fossil fuels include its renewability, high octane rating, eco-friendly nature, and lower emissions (Sutjahjo, 2018). Lignocellulosic bioethanol can be produced in four stages: pretreatment, enzymatic hydrolysis, fermentation, and distillation/product recovery. Bioethanol product formation during the fermentation of LCB usually occurs in the cell of the fermentative microbe. The most common fermentative microbes for lignocellulosic bioethanol fermentation are *Zymomonas mobilis* and *Saccharomyces cerevisiae*. *S. cerevisiae*, with the greater preference, has highly specialized systems for converting glucose to ethanol (Behera et al., 2022). The specialized pathway for converting glucose to ethanol in *S. cerevisiae* is glycolysis, which occurs in the microorganism's cytoplasm. During glycolysis, the glucose is catabolized by enzymes in multiple steps, resulting in the formation of pyruvate and bioethanol (Bai et al., 2008; Zabed et al., 2017). Various lignocellulosic substrates have previously been used for bioethanol production with different pretreatments and process types (Table 13.3).

13.7.2 Biohydrogen

The biological production of hydrogen from lignocellulose offers an environmentally friendly alternative to the current thermomechanical and chemical processes. It is a clean-burning fuel that exhibits a high energy density of between 120 and 142 MJ/kg and is converted to water when utilized for electricity as a fuel cell (McCay and Shafiee, 2020). Hydrogen production can be carried out using several approaches, including dark fermentation, microbial electrolysis, photosynthetically photofermentation, and biophotolysis (Fig. 13.1). The primary mechanism of microbial hydrogen formation is proton reduction catalyzed by hydrogenase or nitrogenase (Hallenbeck and Liu, 2016). Dark fermentation has been the most extensively studied technology for hydrogen production as it exhibits a higher treatment capacity for various waste streams than photo-fermentation, including LCB. Additionally, dark fermentation does not require light and has a low energy requirement, is a simpler process, and exhibits increased stability (Mohan et al., 2011). Hydrogen is often produced by a mixed culture of facultative and obligate anaerobic microorganisms by carbohydrate conversion to acetyl-CoA through the glycolytic pathway. It produces organic acids such as butyrate and acetate, CO_2 and H_2 (Ghimire et al., 2015). Theoretically, a maximum of 4 mol H_2/mol glucose can be produced when acetate is the sole organic acid produced from glucose. However, a much lower yield of 2 mol H_2/mol glucose is achievable when butyrate is the favored organic acid. In lignocellulosic systems where both organic acids are produced, a theoretical yield of 2.5 mol H_2/mol glucose can be obtained (Dalena et al., 2017).

To achieve maximal yields, factors that affect productivity, such as pH, operational temperature, substrate type, inoculum source, and retention time, should be

Table 13.3 Bioethanol produced from different lignocellulosic substrates.

Microorganism	Substrate	Pretreatment conditions	Process type	Process conditions	Ethanol concentration	Reference
Saccharomyces cerevisiae PTCC 5052	Wheat straw	1:5 ratio biomass to [TEA][HSO_4] solvent, 0.5 h, 130°C	SHF	3 g/L microorganism concentration, 5% SL, 28 FPU (filter paper units)/g Cellulase CelluMax GFL enzyme, 30°C, 96 h	52.84%	Ziaei-Rad et al. (2021)
S. cerevisiae PTCC 5052	Wheat straw	1:5 ratio biomass to [TEA][HSO_4] solvent, 3 h, 130°C	SHF	3 g/L microorganism concentration, 5% SL, 28 FPU/g Cellulase CelluMax GFL enzyme, 30°C, 96 h	84.34%	Ziaei-Rad et al. (2021)
S. cerevisiae CCUG 53310	Rice straw	5% SL, 0.5 M Na_2CO_3, 3 h, 93°C	SHF	5 g/L microorganism concentration, 20% SL, 10 FPU/g Cellic CTec2 and Cellic HTec2, 37°C, 120 h	55.1 g/L	Molaverdi et al. (2019)
S. cerevisiae CCUG 53310	Rice straw	5% SL, 0.5 M Na_2CO_3, 10 h, 93°C	SHF	5 g/L microorganism concentration, 20% SL, 10 FPU/g Cellic CTec2 and Cellic HTec2, 37°C, 120 h	67.7 g/L	Molaverdi et al. (2019)
S. cerevisiae	Corn cobs	5% SL, 0.95 g/L $FeSO_4$ and 29.8 g/L H_2O_2, 30 min, 35°C, 0.75% NaOH, 1 h, 90°C	PSSF	5 g/L,[a] 10%,[b] 15 FPU/g[c] Spezyme CP cellulase, 48 h	19.3 g/L	He et al. (2016)

S. cerevisiae	Bamboo	10% SL, 0.5% NaOH, 2 h, 170°C	SHF	0.027 g/mL,[a] 5%,[b] 15 FPU/g[c] cellulase and 30 IU xylanase, 35°C, 24 h	4.8 g/L	Yang et al. (2019)
Escherichia coli FBR5	Wheat straw	86 g/L SL, 0.5% H_2SO_4, 10 min, 160°C	SSF	5% (v/v),[a] 8.6%,[b] 150 μl/g[c] Celluclast, Novozym188 and ViscoStar cocktail, 35°C, 24 h	17.4 g/L	Saha et al. (2011)
Scheffersomyces stipitis NRRL-Y7124	Sugarcane bagasse	5% SL, 0.3 mol/L NaOH, 20 min, 60°C	SSF	5 g/L,[a] 11%,[b] 20 FPU/g[c] Cellic CTec2, 30°C, 96 h	17.26 g/L	Hilares et al. (2017)
Saccharomyces cerevisiae BY4743	Sugarcane bagasse	9.69% SL, 1.73 M $ZnCl_2$, 1.36 M NaOH, 30 min, 121°C	SSF	0.06 g/L,[a] 10%,[b] 100 U/g[c] Cellic CTec2, 39°C, 60 h	4.88 g/L	Jugwanth et al. (2020)

Note: a = biomass concentration, b = substrate solid loading, c = enzyme loading. *SHF*: Separate hydrolysis and fermentation; *SSF*: simultaneous saccharification and fermentation; *PSSF*: simultaneous saccharification and fermentation with prehydrolysis; *SL*: solid loading; *[TEA][HSO$_4$]*: triethylamine hydrogen sulfate.

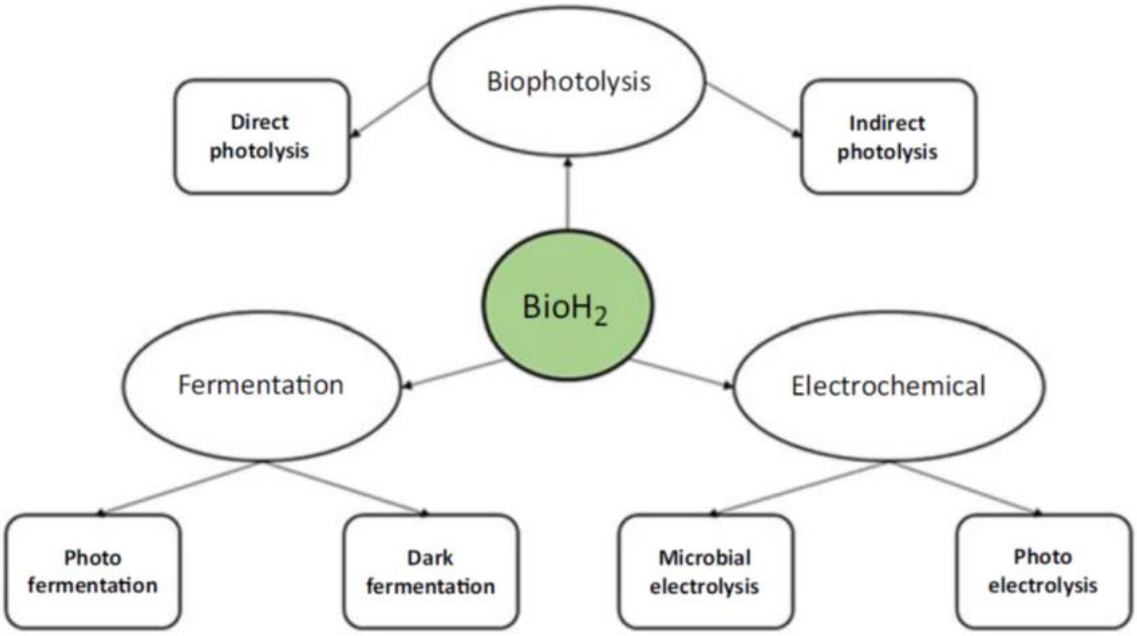

Figure 13.1 Biohydrogen production pathways from biomass.

considered and optimized (Ghimire et al., 2015). Some drawbacks of dark fermentation are low product yields, high by-product formation, and low substrate conversion efficiency (Osman et al., 2020).

LCB has been exhaustively used for biohydrogen production (Table 13.4). But the low yields remain a significant bottleneck. Recent studies have also focused on using industrial residues such as paper mill sludge to produce biohydrogen (Rorke et al., 2021; Moreno-Dávila et al., 2017).

13.7.3 Biogas

Biogas is an end product of the anaerobic microbiological conversion of organic material (Lin et al., 2016). It is a gaseous mixture of methane (55%−75%), carbon dioxide (CO_2) (25%−45%), and hydrogen (5%−10%) (de Mes et al., 2003; Kamali et al., 2016), which can be utilized for heating, upgraded to a higher quality (natural gas alternative) or used in the co-generation of electricity and heat (de Mes et al., 2003). AD is a process used for the generation of biogas from organic waste and wastewater and can be subdivided into four main characteristically unique phases (Angelidaki et al., 2011; Manyi-Loh et al., 2013; Zhang et al., 2016; Manchala et al., 2017) as seen in Fig. 13.2:

1. Hydrolysis converts complex biopolymers such as carbohydrates, lipids, and proteins to soluble organic compounds such as sugars, fatty acids, and amino acids. Hydrolysis is achieved by extracellular enzymes such as cellulases, proteases, lipases, and amylases, which are secreted by hydrolytic bacteria into the bulk liquid.
2. Acidogenesis—the anaerobic acid-producing process in which soluble organic compounds are converted to organic acids such as volatile fatty acids (VFAs) (butyrate, acetate, etc.), hydrogen, and carbon dioxide. The substrate serves as both electron donor and acceptor

Table 13.4 Biohydrogen produced from different lignocellulosic biomass and industrial waste sources.

Lignocellulosic substrate	Hydrogen yield (mol H_2/mol glucose)	Hydrogen yield (mL/g biomass)	Reference
Corn stover	1.5–3.0	–	Cheng et al. (2011)
Rice straw	0.8	–	Cheng et al. (2011)
Sugarcane bagasse	1.7	–	Cheng et al. (2011)
Potato steam peels	2.4–3.8	–	Cheng et al. (2011)
Sweet sorghum	–	15.1–127.3	Cheng et al. (2011)
Corn cobs	–	49.4	Zhang et al. (2021)
Sugarcane bagasse	2.3	–	Łukajtis et al. (2018)
Wheat straw	–	133.6	Zhu et al. (2022)
Corn stover	1.5–2.2	–	Yadav et al. (2020)
Sweet sorghum bagasse	1.3–2.6	–	Panagiotopoulos et al. (2010)

for obligate and facultative anaerobic bacteria within the species of *Clostridium*, *Propionibacterium*, *Lactobacillus*, etc.

3. Acetogenesis—organic acids [VFAs and long-chain fatty acids (LCFAs)] are converted to methane formation precursors such as acetate, hydrogen, and carbon dioxide. It can be achieved by hydrogen-utilizing acetogens, strictly anaerobic bacteria that use the acetyl-CoA pathway as the primary mechanism for reductive synthesis of acetyl-CoA from carbon dioxide. Organic acids are also oxidized to acetate by hydrogen-producing acetogens. These acetogens are obligate users of hydrogen ions and carbon dioxide as electron acceptors. Therefore the unfavorable energetics of the process limits them as energy derivation for growth is only possible when product concentration is low—creating an obligatory dependence on hydrogen scavengers such as sulfate reducers to remove the product. This conversion is, therefore, efficiently inhibited by high concentrations of products such as hydrogen ($>10-4$ atm) in the system.
4. Methanogenesis—acetate, carbon dioxide, and hydrogen are converted to methane by methanogenic bacteria such as acetoclastic/acetotrophic methanogens, which split acetate into methane and carbon dioxide, and hydrogenotrophic methanogens, which use hydrogen as an electron donor and carbon dioxide as an electron acceptor, thus producing methane.

In light of dwindling energy reserves, AD has gained significant interest, particularly in its potential for biogas production and solids reduction (Kamali et al.,

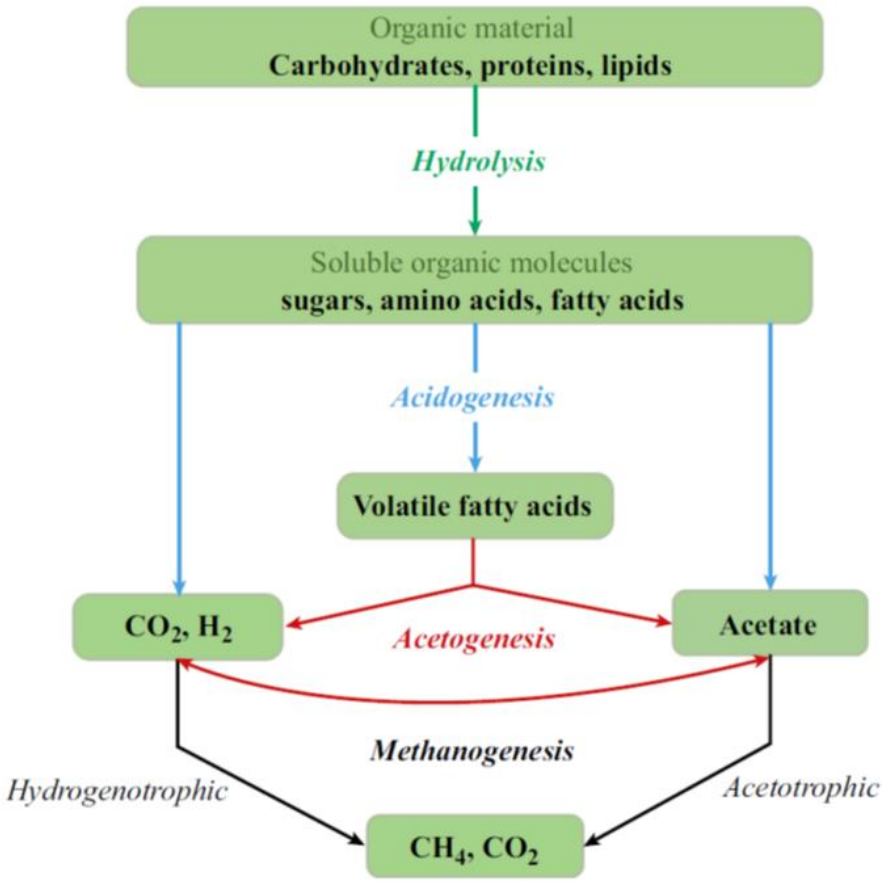

Figure 13.2 Schematic diagram of the anaerobic digestion process.

2016). Numerous advantages associated with the AD of organic wastes and LCB include (de Mes et al., 2003; Olatunji et al., 2021):

1. Low energy requirements of between 0.05 and 0.1 kWh/m^3 at mesophilic temperatures.
2. Significant retention of fertilizer nutrients (nitrogen, phosphate, and potassium).
3. Relatively low construction costs.
4. Stabilization of raw wastes.
5. Lower space requirements in comparison to conventional systems.

The efficiency of the AD process is dependent on start-up conditions and environmental factors such as a neutral pH, anaerobic conditions, and a suitable operating temperature, among others (Schirmer et al., 2014). Additionally, inhibitory compounds such as ammonia, hydrogen, sulfide, and VFAs can inhibit methanogens during AD (Zhang et al., 2016). Therefore, various methods have been applied to reduce inhibitory effects, such as gas stripping to reduce ammonia levels and ferric chloride to remove hydrogen sulfide (H_2S) and sulfate to reduce the inhibitory effect of propionate (Zhang et al., 2016).

Biogas is often produced to further upgrade the gas for use in applications requiring high methane concentrations. The content of the various gases present in biogas is determined by the nature of the substrate used as well as the pH of the process. The energy content of 50.4 MJ/kg—CH_4 (36 MJ/m^3—CH_4 at STP

conditions) has been reported for methane by Angelidaki et al. (2018). Therefore any additional gases present are considered pollutants and will reduce the energy content of biogas. Additionally, the presence of compounds like siloxanes can result in the generation of sticky residues in combustion engines, which may cause machinery malfunctions (Angelidaki et al., 2018). Various methods have been implemented to remove impurities, including the application of bottom ash from municipal solid waste incineration to adsorb CO_2 and H_2S from the resultant biogas stream, the removal of H_2S by biological oxidation by aerobic sulfate oxidizers and the conversion of CO_2 to methane by reacting it with H_2 (Angelidaki et al., 2018). Numerous LCB sources have previously been used for biogas production (Table 13.5). The AD process for biogas production is highly versatile compared to bioethanol and biohydrogen since the microbes can break down complex substrates without pretreatment (Abraham et al., 2020).

13.8 Microbial high-value products from lignocellulosic biomass

LCB accounts for many nonfood-based portions, including leaves, stalks, and husks that are either landfilled or burnt in fields (Kee et al., 2020). It poses a significant hindrance to environmental and health considerations; therefore utilizing the residual waste components to produce biofuel and other bioproducts will add value and aid in an effective waste management solution (Manna et al., 2018). This "waste to wealth" concept enables the manipulation of microorganisms to utilize these lignocellulosic residues as a carbon source to produce end products that can be tailored to suit global demands. To date, various studies have been carried out on the conversion of hydrolyzed LCB into biofuels (bioethanol, biohydrogen, biogas, etc.) and

Table 13.5 Biogas produced from different lignocellulosic feedstocks.

Lignocellulosic biomass	CH_4 potential (L CH_4/kg VS)	Reference
Corn	291–338	Sawatdeenarunat et al. (2015)
Wheat	351–378	Sawatdeenarunat et al. (2015)
Grass	286–324	Sawatdeenarunat et al. (2015)
Fodder beet	398–424	Sawatdeenarunat et al. (2015)
Rice straw	152–263	Mustafa et al. (2016)
Wheat straw	251	Schroyen et al. (2015)
Miscanthus giganteus	285–333	Wahid et al. (2015)
Corn straw	325.7	Fu et al. (2015)
Pulp and paper sludge	225	Granström and Montelius (2014)
Reed biomass	188	Lizasoain et al. (2016)

VS: Volatile solids.

value-added chemicals (lactic acid, itaconic acid, succinic acid, etc.) (De Bhowmick et al., 2018). Nevertheless, the production of biofuels has shown to be expensive with low yields, thus rendering it unfeasible on a large scale. Therefore in recent times, the lignocellulosic bioprocess trajectory focuses on producing a high-value product of commercial relevance (Fig. 13.3), followed by biofuel production. Some examples of valuable bioproducts that are imperative for the commercial sector and can be produced from LCB in biorefinery systems include lactic acid, itaconic acid, and succinic acid.

13.8.1 Lactic acid

Lactic acid, a fermentation product of *Lactobacillus* species and other genetically engineered microbial strains, is an important product for the food, chemical, cosmetic and pharmaceutical industries. This bioproduct is naturally produced using *Levilactobacillus brevis*, *Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus* (Zhang and Vadlani, 2015; Cui et al., 2011). Lignocellulosic-derived sugar to lactic acid has been driven by the abundance and cost-effectiveness of feedstock and the tolerance and selectivity of the microorganism to enhance both the economic and environmental aspects of the bioprocess (Banu et al., 2021). Lactic acid production from LCB is increasingly gaining popularity. For example, Cizeikiene et al. (2018) reported a lactic acid concentration of 101.8 g/kg from pretreated wheat straw using *Fructilactobacillus sanfranciscensis* MW15. Similarly, previous studies have observed lactic acid concentrations of 62 and 92.5 g/L when using pretreated beechwood (Karnaouri et al., 2020) and rice straw (Chen et al., 2019), respectively, as a carbon source. In an earlier study, Ray et al. (2009)

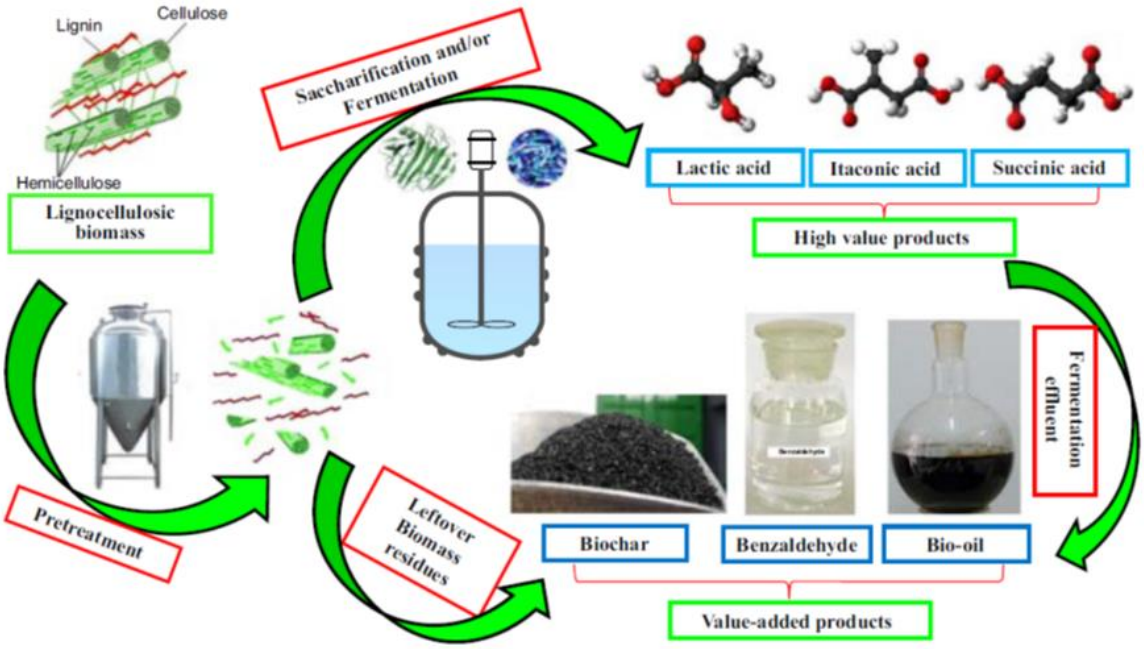

Figure 13.3 Integrated biorefinery process.

reported the production of 29.86 g of (L +) LA from 100 g of cassava bagasse (containing 60% starch) using *Lp. plantarum* MTCC 1407 as the fermenting organism.

13.8.2 Itaconic acid

Itaconic acid is an important precursor for polymers, chemicals, and fuels (Krull et al., 2017; Zhao et al., 2018). *Aspergillus terreus*, *A. itaconicus*, and *Pseudozyma antarctica* are microbes that naturally produce itaconic acid (Krull et al., 2017; Zhao et al., 2018; Kocabas et al., 2014). Other itaconic acid-producing microbes include *Ustilago maydis* and *Escherichia coli* (Maassen et al., 2014; Okamoto et al., 2014). Itaconic acid production using LCB as a substrate has gained prominence, with several aspects still to be investigated to maximize yields. Krull et al. (2017) and Yang et al. (2020) assessed itaconic acid production from alkaline pretreated wheat chaff and alkaline pretreated bamboo residues using *A. terreus* and observed maximum concentrations of 27.7 and 22.43 g/L, respectively.

13.8.3 Succinic acid

Succinic acid has versatile applications and can be used in the food, biodegradable polymer, pharmaceutical, and cosmetic industries (Yan et al., 2014). Major succinic acid-producing microbes include *Actinobacillus succinogenes*, *Mannheimia succiniciproducens*, and *Anaerobiospirillum succiniciproducens* (Guettler et al.,1999; Hong et al., 2004). Genetically engineered *E. coli* and *S. cerevisiae* have also been documented for succinic acid production (Yan et al., 2014; Thakker et al., 2013). Succinic acid from agricultural wastes such as LCB has been reported in the last few years (Zheng et al., 2010; Akhtar and Idris, 2017; Akhtar et al., 2020; Anwar et al., 2021). For example, Zheng et al. (2010) evaluated succinic acid production using corn stover and *A. succinogenes* as the substrate and inoculum source, respectively and obtained a yield of 47.4 g/L. In recent studies, Akhtar et al. (2020) and Anwar et al. (2021) reported succinic acid concentrations of 42.9 and 65.2 g/L from oil palm empty fruit bunch, respectively.

13.9 Value-added products from lignocelluloses: benzaldehyde, biochar, and bio-oil

Value-added products such as benzaldehyde, biochar, and bio-oil have gained popularity. They can be produced as a first, second, or third product for the complete valorization of the feedstock within integrated biorefineries. These bioproducts are briefly outlined below.

13.9.1 Benzaldehyde

Benzaldehyde is a bioproduct that can be obtained from the fermentation of different substrates by fermenting microbes (*Lp. plantarum*, *Pseudomonas putida*, and *Phanerochaete chrysosporium*) (Jensen et al., 1994; Nierop Groot and DeBont, 1998; Norliza and Ibrahim, 2005). For instance, benzaldehyde was obtained from the cultivation of *Rhizopus oligosporus* using mixed substrate (soya bean meal and rice husks). The maximum benzaldehyde concentration of 5.47 mg/g was obtained after 96 h (Norliza and Ibrahim, 2005). The biological-based benzaldehyde is a desirable flavoring agent next to vanillin (Welsh et al.,1989). Other flavoring compounds aside from benzaldehyde include vanillin, lactones, methyl ketones, alcohols, esters, terpenes, and cinnamaldehyde. These have been found to have industrial potential with commercial value. The biological-based benzaldehyde can also be obtained from plants as a glycoside (Norliza and Ibrahim, 2005). Chemical extraction of benzaldehyde results in the formation of toxic byproducts, limiting its usefulness. Therefore the production of biological-based benzaldehyde through fermentation processes is preferred. Furthermore, the low operational cost of fermentation, high productivity, low wastewater output, and improved product recovery has made fermentative benzaldehyde production attractive for commercialization (Bhattacharyya et al., 1998).

13.9.2 Biochar

Biochar is another bioproduct produced by heating choice biomass in the presence or partial absence of oxygen. Thermochemical conversion, such as pyrolysis, hydrothermal carbonization, gasification, and torrefaction, are standard techniques for biochar production (Lin et al., 2016; Pang, 2019; Yaashikaa et al., 2020). To obtain a high yield of biochar, the appropriate technique should be chosen as well as taking into consideration the biomass type (solid wastes, animal wastes, wood biomass, and agricultural residues), heating rate, carbonization temperature, biomass pretreatment, particle size and residence time (Yaashikaa et al., 2020). These conditions are vital because they affect the physical and chemical properties of the biochar produced. Biomasses are composed of cellulose, hemicellulose, and lignin. These components can be transformed into biochar under different reaction conditions and mechanisms (aromatization, condensation, repolymerization, crosslinking of intermediates, polymerization, and depolymerization) (Huang et al., 2012; Mu et al., 2013).

Biochar is usually characterized to determine its structural, elemental composition, surface area, porosity, and surface functional groups (Brewer et al., 2014). The techniques used to characterize biochar include scanning electron microscopy, Fourier transform infrared spectroscopy, X-ray diffraction, thermogravimetric analysis, nuclear magnetic resonance spectroscopy, Brunauer–Emmett–Teller and Raman spectroscopy (Yaashikaa et al., 2020). The stability of biochar during usage relates to its carbon structure since biochar's main constituent is the carbon structure (biochar's instability might be partly due to damage to the structural carbon)

(Huang et al., 2019). In addition, the stability of biochar should be considered due to environmental concerns; hence, it is important to determine in detail the toxicity effect(s) of produced biochar on the environment.

Biochar can play an important role in pollutant removal from the environment (soil and aqueous). It depends on porosity, functional group, surface area, pH, and hydrophobic nature. The eco-friendliness, inexpensive, and ease of biochar preparation from various biomass types make biochar an intensive area of interest. Biochar can also be used for other applications such as catalysts, wastewater treatment, composting, energy storage, carbon sequestration, and soil amendment (Yaashikaa et al., 2020).

13.9.3 Bio-oil

Bio-oil is a liquid fuel obtained through thermochemical processes (pyrolysis and hydrothermal liquefaction) from biomass materials, mainly from LCB. Biomass for bio-oil production includes crop residues, algal biomass, municipal wastes, and agricultural and forestry byproducts (Fu et al., 2009). Bio-oil is a promising feedstock to replace fossil fuel for engine generation. It is because it has a high energy density, is easy to store compared to gaseous products, is carbon neutral, biodegradable, and is environmentally friendly (Hu et al., 2008; Fu et al., 2009).

13.10 Innovations in lignocellulosic biorefineries

The industrial development of the global economy has presented unprecedented concerns regarding the over-utilization of nonrenewable energy sources and pressurized regulatory environmental guidelines (Usmani et al., 2021). Biomass-based energy can offset these challenges due to its sustainability, reduced greenhouse gas emissions, and abundance. LCB is a major renewable carbon source that may support carbon sequestration and the production of bioenergy and biomaterials (Banu et al., 2021). With significant interest attached to lignocellulosic bioproducts, the economic value of these waste residues may be maximized by potential conversion into multiple products through the valorization process of an integrated biorefinery approach (Thomsen, 2005). These links waste resources and the recovery of valuable products in closed-loop cycles, thus contributing to a sustainable environment (Nizami et al., 2017; Harrison et al., 2016). Feedstock contributes about 60% of the total costs of a typical biorefinery system (Botero et al., 2017). A constant and cost-effective supply of renewable and reliable feedstocks (such as lignocellulosic, microalgae, municipal solid waste, and wastewaters) is very important to implementing the biorefinery system (Cherubini et al., 2009). On the other hand, biorefinery platforms are intermediaries that link feedstocks and products (Cherubini et al., 2009). Well-known biorefinery platforms include sugar, biogas, carbon-rich chains, syngas, and plant product platforms (Harrison et al., 2016). The processing of these platforms involves a wide range of technological processes, including

biochemical, thermochemical, mechanical, and chemical (Cherubini et al., 2009). Furthermore, biorefinery has been classified using various standpoints such as feedstock, platform, and processes (Harrison et al., 2016). The primary classifications are first, second, and third-generation biorefineries (Harrison et al., 2016).

1. First-generation biorefineries are based on "a single raw material, a single major product" systems (Cherubini et al., 2009; Harrison et al., 2016).
2. In the second-generation biorefinery, a single raw material or feedstock, often lignocellulosic materials, is converted to a wide range of products (Cherubini et al., 2009).
3. Third-generation biorefineries are a complex use of varieties of feedstocks and their transformation to generate multiple bioproducts (Harrison et al., 2016). A typical third-generation category is wastewater biorefinery (Harrison et al., 2016). Using wastewater as substrate minimizes waste emissions and promotes environmental sustainability (Harrison et al., 2016).

Particularly, the production of biofuels and value-added compounds from LCB faces significant economic challenges that need to be addressed by searching for low-cost feedstocks and pretreatments, suitable microbial inocula, robust enzyme cocktails, and bioprocess optimization. Recently, lignocellulosic biorefineries have emerged as potentially profitable systems due to the production of multiple products (low- and high-value) from a single waste stream. The biorefinery approach helps alleviate some economic barriers but still requires enhancement for its successful commercialization at an industrial scale. Biorefinery research is underway and has previously been documented (Banu et al., 2021). For example, in an earlier report, Kaparaju et al. (2009) investigated a biorefinery system in which biohydrogen was produced as the mainstream product, while lactic acid, acetic acid, and bioethanol were generated as by-products using hydrothermally pretreated coffee waste as a substrate.

Similarly, Kocabas et al. (2014) investigated the biorefinery concept by the production of xylanase enzymes and itaconic acid from corn cobs in a two-step fermentation and demonstrated yields of 70 IU/mL (xylanase) and 18 g/L (IA). Kuglarz et al. (2016) used hemp as a substrate for bioethanol and succinic acid production in a biorefinery system, while the latter authors also generated biogas. In a recent study, Moodley and Gueguim Kana (2019) assessed the kinetics of bioethanol production from sugarcane leaf wastes and proposed using effluent as animal feed. Other wastewater biorefinery applications consist of microalgal wastewater biorefinery. Microalgal biomass could be a source of multivaluable bioactive compounds in bioenergy and bioproducts recovery (Odjadjare et al., 2017). Microalgal wastewater biorefinery could transform microalgal biomass into a wide range of products such as biohydrogen bioethanol, biogas, biodiesel, polyhydroxyalkanoate (bioplastic), polyhydroxybutyrate, and animal feeds (Liu et al., 2020).

13.11 Life cycle assessment

LCA is essential in bioprocessing to increase economic sustainability and environmental friendliness. To achieve this, it is vital to determine the interconnectivity of

every component of the process from the very beginning (Khang et al., 2017). The LCA has been used in different endeavors; these include energy, chemistry, food, and agriculture (Nemecek et al., 2011; Restianti and Gheewala, 2012; Garrett and Ronde, 2013). LCA as an assessment tool could be obtained by following four assessment steps:

1. scope and goal,
2. inventory analysis,
3. impact assessment, and
4. interpretation (ISO, 2006; Khang et al., 2017).

Equally, the LCA process can be considered as the environmental management accounting for all aspects of ecological releases, an array of environmental interactions, and resource use associated with the complete production process (Norris, 2006; Khang et al., 2017). These include various standpoints, from the extraction of feedstocks, the release of production energy, and recycling and disposing of waste. However, LCA is a vital tool to compare the different aspects, but not a complete exact, precise assessment. Highly reliable standard methodologies (International Organization for Standardization) are used in LCA (ISO, 2006). LCA as a tool can be implemented mainly in four analytical approaches: LCA scope and goal, LCA inventory analysis, Life cycle impact assessment, LCA interpretation. Consequently, final decisions and recommendations can be made.

13.12 Technoeconomic analyses

The process efficiency of many bioprocesses has shown that their industrial implementation is desirable and potentially feasible. Nevertheless, the economic implication of their industrial potential should be assessed through technoeconomic analyses (TEA) to determine the overall cost and identify the steps contributing to the highest expenses (Juneja et al., 2019). The initial costing assessment of a biological process usually contains an investment estimation detailing the capital, operating costs, and investment profitability. This economic evaluation is expected to include research and development (R and D) expenditures for both successful and unsuccessful products. The R and D spending is crucial to the overall cost assessment. Hence, engineers and scientists need to develop effective process designs and methodologies that can cost-effectively eliminate projects or ideas with the potential to be unsuccessful (Koutinas et al., 2016). From an economic perspective, achieving this at the early stages of product and process development is desirable. It is because the probability of new projects never reaching commercialization is over 90%. Such assessment would assist investors in decision-making as well as the implementation of the chosen project. Capital investment for an innovative bioprocess plant will include fixed capital, start-up capital, operational capital, and certification cost (Peters and Timmerhaus, 1991; Seider et al., 1999). The capital investment cost is best estimated by taking note of the wide range of multiplier

values (such as equipment, maintenance, heating, ventilation, and air conditioning systems of processing space, laboratory, and office area). While the operational cost also covers a wide range of recurrent expenses such as raw materials, labor, energy cost, utility bills, waste disposal, wages, and salary. Process costing or TEA should result in a cost-competitive building block required for the project commercialization or industrial scale-up (Panda et al., 2018).

Furthermore, a TEA of bioprocesses is estimated using literature-cited (primary or secondary) data, conventional process design brochures, technical pamphlets, market prices, process flowsheets, and sensitivity analysis. Previous studies that are generally selected are based on data with high: process efficiencies, productivity, yield, and high substrate conversion rate (Koutinas et al., 2016). The reliable assessment might require the evaluation of different feedstocks or techniques to obtain the least expensive approach. Some of these feedstocks are available in substantial quantities and regarded as waste; hence, they are economically attractive. Moreover, there is a growing interest in their utilization for the production of various bioproducts such as bioethanol, biohydrogen, biogas, bioplastics, lactic acid, 2,3-butanediol, 1,3-butadiene, butanol, benzaldehyde, and bio-oil (Norliza and Ibrahim, 2005; Sriroth et al., 2000; Koutinas et al., 2016; Sivamani et al., 2018). The sensitivity assessment is equally crucial in technoeconomic assessment, which examines the impact of various key factors on the process. It reveals the variation in production cost associated with the influence of these fundamental factors and how each potentially alters the cost of production individually or interactively (Ong et al., 2012). Operational sensitivity analysis is performed on different process scenarios to show the influence of the diverse operation parameters (substrate type, temperature, pH, process time, enzyme, solvent concentrations, and product yields) to determine the most sensitive parameter in the process (Ong et al., 2012). Afterward, benefit/cost analysis would be considered to determine the most profitable approach for the best economic return.

13.13 Present challenges of integrated biorefineries

Despite the significant advances in developing lignocellulosic-based biorefineries, several aspects still need to be addressed related to the cost, resources, and energy.

1. For instance, the choice of feedstock plays a key role. It can prove detrimental to the entire biorefinery system when it is seasonal-dependent or affected by fluctuating weather conditions. This disrupts the steady year-round supply necessary for biorefinery operations (Oke et al., 2016).
2. In addition, other limitations of these feedstocks are also linked to the requirement for pretreatment processes, which is an integral step in degrading the tightly interwoven, recalcitrant matrice of LCB for enhanced accessibility to cellulose (Patel and Shah, 2021). The pretreatment process accounts for approximately 40% of the expenses within the biorefinery, thus posing a significant shortcoming (Kucharska et al., 2018). These costs include pretreatment chemicals, compatible reactors, energy resources, detoxification

technologies, and biomass separation processing, amongst others (Sewsynker-Sukai et al., 2020).

3. Another major concern is the high water consumption, a finite resource that remains a constraint in developing biorefineries. Lignocellulosic feedstocks are water-insoluble; therefore, substantial quantities of water are needed to enhance mass transfer during pretreatment, enzymatic hydrolysis, and fermentation processes (Sewsynker-Sukai et al., 2020).
4. Like the pretreatment process, enzymatic hydrolysis reactions play a key role in the biorefinery system by releasing sugars essential for microbes during fermentation but also face shortcomings. These may include: (1) costly enzymes that are negatively affected by various factors (inhibitor compounds, pH, temperature), (2) long process times, (3) low saccharification rates, and (4) reduced enzymatic activity as time progresses (Aguilar-Reynosa et al., 2017; Sewsynker-Sukai and Gueguim Kana, 2018).
5. Apart from the bottlenecks associated with pretreatment and enzymatic hydrolysis reactions, bioprocess development and optimization present their limitations. Of main concern is the lack of understanding of the network of metabolic fluxes for microbial cell growth and product formation within bioprocesses, which remains a limiting factor for optimization and scale-up (David et al., 2020; Sewsynker-Sukai and Gueguim Kana, 2018). The scarcity of information related to the microbial fermentation process has led to (a) poor sugar consumption by the fermenting microbes, (b) low product recovery, (c) high cost, and (d) energy-intensive bioprocesses (David et al., 2020).
6. Additionally, adequate research on LCA and TEA in lignocellulosic biorefinery processes has been scanty, which deters scale-up procedures and potential investors (Moodley, 2021).

13.14 Current status and future trends in integrated biorefineries

Integrated biorefineries aim to produce continuous streams of bioenergy and other valuable bioproducts while recycling all the waste outputs with little to no discharge, thus achieving a circular bioeconomy. The recent efforts to enhance integrated lignocellulosic biorefineries are leaning towards developing multistream processes that yield multiple products (Banu et al., 2021). For example, concerning substrate selection, either a single LCB substrate or a mixed feedstock approach can be adopted (Oke et al., 2016). While the single substrate method utilizes one feedstock type, the mixed substrate approach combines two selected wastes. The mixed feedstock approach has demonstrated the potential to reduce challenges associated with logistics issues (harvesting, collection, preprocessing, storage and transportation), intrinsic characteristics (low bulk density and nutrient-deficient biomasses), and extraneous factors (competing uses for the biomass and constant supply regardless of season and weather conditions) (Oke et al., 2016). Interestingly, the mixed biomass method has previously been used for the production of bioethanol (Ferreira et al., 2015), biohydrogen (Prakasham et al., 2009; Kim and Lee, 2010), biogas/methane (Lehtomäki et al., 2007; Kalra and Panwar. 1986; Li et al., 2014) polyhydroxyalkanoates (Sangkharak and Prasertsan, 2013; Shamala

et al., 2012) and microbial enzymes (Shamala et al., 2012; Azin et al., 2007; Shamala and Sreekantiah, 1986). Further research on the mixed substrate approach in lignocellulosic biorefineries for complete valorization is recommended to negate feedstock limitations. Additionally, the pretreatment front has made significant strides with the appearance of green liquor dregs (David et al., 2020; Sewsynker-Sukai et al., 2020; Rorke et al., 2021) and paper wastewater (Sewsynker-Sukai et al., 2020) that are both generated from the paper industry as potential methods to replace chemicals and water, respectively. Furthermore, commercial biotechnology conglomerates have developed high-performing cellulase cocktail enzymes for LCB. They have demonstrated extraordinary saccharification efficiencies (Aguilar-Reynosa et al., 2017) and exhibit improved endo/exo activity ratio, resulting in lower end-product inhibition and higher tolerance to nonspecific binding (Ramos et al., 2015). Even with these breakthroughs that promote the implementation of these systems, effective isolation and procurement of the valuable aromatic lignin polymer remain an untapped goldmine in lignocellulosic biorefineries (Chio et al., 2019). The lignin macromolecule represents up to 30% of the LCB. It is usually an unexploited resource from a chemical perspective since it could potentially be used for the commercial production of plastics, pharmaceuticals, and paints. On the other hand, current trends in bioprocess development and optimization deal with evaluating fermentation process type (SHF, SSF, and PSSF) and kinetics of cell growth and product formation (David et al., 2020; Sewsynker-Sukai and Gueguim Kana, 2018; Moodley and Gueguim Kana, 2019), that may provide substantial information for translation to pilot scale. The bioprocess type has commonly been defined and associated with bioethanol production (David et al., 2020) but has also been applied to other biofuel processes (Rorke et al., 2021). The ideal biorefinery system should produce high-value commodities such as lactic acid, itaconic acid, and succinic acid, followed by biofuel production (bioethanol, biogas, and biohydrogen) using the effluent generated. Another innovative advancement in fermentation entails the use of industrial wastewaters (such as dairy, food, or pharmaceutical) that provide a dual purpose of (1) trace nutrients and (2) water sources to curb the use of this scarce resource in biorefinery systems. Other promising approaches include the supplementation of nanoparticles (Sanusi et al., 2021) and surfactants (Rorke et al., 2021) that improve both the enzymatic and fermentation processes. Utilization of industrial wastes from the food, agricultural and chemical sectors, in addition to the generation of multiple products, make provisions for: (1) the potential development of suitable LCA that provides significant insight into the environmental sustainability of the processes and (2) additional revenue streams that reduces the technoeconomic barriers of lignocellulosic biorefineries, thus promoting the circular bioeconomy approach. Innovations to speed up the circular bioeconomy encompass a range of different methods. More recently, virtual experimentation using machine learning models have increasingly been documented for several processes such as lignocellulosic pretreatment (Moodley and Gueguim Kana, 2019; Rorke and Gueguim Kana, 2016) and biofuel production (Sewsynker et al., 2015; Sewsynker and Gueguim Kana, 2016; Sewsynker-Sukai and Gueguim Kana, 2017). The convergence of machine learning models and biorefinery processes such as

pretreatment, enzymatic hydrolysis, and microbial fermentation pave the way towards transitioning from resource-intensive, laborious, and time-consuming wet-lab experiments toward virtual environments. Artificial intelligence tools have gained popularity for application in biotechnological processes, which may be attributable to their effective encapsulation of biological systems known to be non-linear and dynamic. Artificial intelligence tools can extract functional relationships between the process inputs and corresponding outputs (product yields), thus allowing for the prediction of maximum productivity and reduced inhibitor yields. Experts can then assess the viability of the biorefinery process before scale-up procedures. However, this does not follow a straightforward approach but necessitates using robust artificial intelligence tools and large data sets to develop models that can equally predict defined chemicals and wastes.

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