UTILISATION OF BAGASSE FOR THE PRODUCTION OF C_5- AND C_6- SUGARS

by

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DECLARATION

I hereby declare that this is my own work and has not been submitted for a degree at any other University.

R. C. TRICKETT
January, 1982

A C K N O W L E D G E M E N T S

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ABSTRACT

Surplus sugarcane bagasse, estimated at a maximum of $0,9x10^6$ tons/year, represents an annual renewable resource which is readily available at the mill site and is a suitable potential source of alternative fuels and chemical feedstocks.

This work contains an extensive literature survey which covers the production of C_5- and C_6- sugars from lignocelluloses by chemical hydrolysis and the pretreatment of cellulosic materials for enzymatic hydrolysis of the cellulose fraction. This survey was then used to determine the final direction of this research into the utilisation of bagasse for the production of fermentable sugars.

It was decided that research should be directed at the dilute acid hydrolysis of the bagasse hemicellulose fraction to determine whether this fraction could be selectively hydrolysed from the complex lignocellulose structure and to obtain xylose yields under different hydrolysis conditions.

Acids, especially acetic acid, are liberated from bagasse by steaming at elevated temperatures. In this acid medium the hemicelluloses are hydrolysed and become soluble. Autohydrolysis tests on whole bagasse indicate that hemicellulose hydrolysis becomes significant at temperatures above 140°C. However, the autohydrolysis liquor would still require dilute mineral acid hydrolysis to convert the pentose oligomers to their monomeric forms.

Dilute sulphuric and batch hydrolysis of whole bagasse hemicellulose has thus been investigated at a solid to liquid ratio of 1:15 over the following temperature and acid concentrations ranges : 80° to 150°C and 3 to 40 g/ℓ acid. Xylose, glucose, furfural and acetic acid formation and sulphuric acid consumption were monitored during these hydrolyses.

Hemicellulose hydrolysis to produce mainly xylose is readily achieved over the entire range of acid hydrolysis conditions tested with little removal of the other bagasse components (lignin and cellulose). At the upper end of the temperature range acid concentrations below 20 g/ℓ are sufficient for hemicellulose hydrolysis due to the effect of temperature on reaction rate.

The bagasse hemicellulose consists of two fractions, an easily hydrolysable portion containing 165 mg of potential xylose/g bagasse and a resistant fraction containing 105 mg of potential xylose/g bagasse. A first order reaction model has been developed using the batch acid hydrolysis results. It is based on two hemicellulose fractions reacting simultaneously to give a common product (xylose) and predicts total xylose yield as a function of hydrolysis time for a given set of hydrolysis conditions.

The encouraging xylose yields obtained during the batch hydrolyses led to the design of a continuous hydrolysis reactor to process bagasse at low liquid to solid ratios to determine whether xylose yields similar to the batch hydrolysis yields could be obtained at the same hydrolysis conditions.

The continuous hydrolyses showed that for the conditions tested the xylose yields are unaffected by the decrease in liquid to solid ratio (down to 3,6:1) and it would appear that reactor performance is still controlled by reaction kinetics.

A number of reactor configurations for the industrial production of pentoses from bagasse hemicelluloses are also proposed.

CONTENTS

CONTENTS Page No.

CONTENTS

CONTENTS Page No.

CONTENTS

CONTENTS

CONTENTS

APPENDICES

FIGURES

FIGURES

FIGURES

FIGURES

TABLES

TABLES

TABLES

1. INTRODUCTION

An enormous amount of solar energy is incident on the earth's surface. However, global photosynthesis is able to convert only 605×10^{15} kcal of this energy which is less than 0,07% of the costless energy the earth receives[1]. The annual net yield of photosynthesis has been estimated at $1,8 \times 10^{12}$ tons of biodegradable substances[1], 40% of which is cellulose and this enormous quantity of cellulosic materials represents an annual renewable resource.

However, a maximum of 20% of these cellulosic materials are readily available for conversion to chemicals and energy[2] Much of the cellulosics are too widely distributed for easy collection and transport to large economical conversion centres.

At present, the most readily available sources of cellulose are residues or wastes i.e. municipal solid waste and agricultural residues, which contain between 45 - 56% cellulose, 10 - 25% hemicellulose and 16 - 30% lignin[3]. Municipal residues are readily available at a central point but require extensive classification to remove non-organics. Agricultural residues, on the other hand, are seasonally produced (storage necessary) and collection is a problem unless they are waste from a conversion process.

In 1977 Woodburn[4] suggested that sugarcane waste could be utilised to supply some of South Africa's motor fuel requirements. As a result there has been a vigorous research programme aimed at the utilisation of sugarcane bagasse for the production of C_5- and C_6- sugars at the Department of Chemical Engineering at Natal University in Durban.

This work contains an extensive literature survey which covers the production of C_5- and C_6- sugars from lignocelluloses by chemical hydrolysis and the pretreatment of cellulosic materials for enzymatic hydrolysis of the cellulose fraction. This survey was then used to determine the final direction of this research into the utilisation of bagasse for the production of fermentable sugars.

It was decided that research should be directed at the dilute mineral acid hydrolysis of the bagasse hemicellulose fraction to determine whether this fraction could be selectively hydrolysed from the complex lignocellulose structure and to obtain xylose yields under different hydrolysis conditions.

2. LITERATURE SURVEY

2.1 Bagasse Supply

South African sugar mills vary in capacity and each produces between 76 000 and 580 000 tons of moist bagasse per year (50% moisture); ten mills produce more than 300 000 tons per year[5].

According to the S.A. Sugar Yearbook [6] an average of 18,477 x 10^6 ton/year of cane was cut over the years 1975-1980. Dry bagasse production amounted to approximately 15% of this total; thus representing an annual production of about 2,75 x 10^6 tons. This bagasse is traditionally used as a boiler fuel at the mills. However, in the large modern mills which operate a more energy efficient process[7,8], there is a surplus of bagasse even after the combustion requirements.

It has been estimated that a maximum of one third of the total bagasse production would be available for conversion to chemical feedstocks[5], i.e. approximately 0,9 x 10^6 tons/year.

This surplus lignocellulosic material represents an annual renewable resource which is readily available at the mill site and is a suitable potential source of alternative fuels and chemical feedstocks.

2.2 Structure and Composition of Cellulosic Materials

2.2.1 Structure of Plant Cells

A knowledge of the structure of cellulosic materials is essential to the exploitation of the hemicellulose and cellulose components as renewable chemical and energy resources.

Cellulose makes up about 90% of cotton fibres but only 45% of typical wood cell walls. The cellulose in cotton

and wood is very similar in molecular structure. Cotton fibres are formed independently and thus contain no intercellular substance. Wood and bagasse fibres, on the other hand, form a cohesive three dimensional structure whose integrity is assured by large amounts of intercellular substance[9].

Most cellulose fibres have a general construction theme[10]. The outermost layer of the fibre is called the primary wall (P) which was formed on cell division. The secondary wall, formed during the growth and maturation of the cell, is subdivided into the transition lamella (S1), the main secondary wall (S2) and the inner secondary wall (S3). Surrounding the fibre and heavily lignified and stiff is the middle lamella (M) which is about 1 to 2μ thick and is shared by adjacent fibres. The primary wall is usually very thin (300 Å) corresponding to three layers of cellulose elementary fibrils. The secondary wall, (S1, S2 and S3) which thickens during cell growth contains the majority of cellulose. It is anywhere from 1 to 10μ thick consisting of cellulose microfibrils in a helical arrangement (Figure 2.1).

This compounding is perfect; the linear but hydrophilic chains contribute tensile strength while hydrophobic, amorphous lignin brings about resistance to chemical and biological degradation[11].

2.2.2 Composition of Cellulosic Materials

Most cellulosic solids contain three major components : cellulose, hemicellulose and lignin in ratios of roughly 4 : 3 : 3[12]. These are only approximate figures. For instance, soft wood contains typically 42%, 25%, and 28% cellulose, hemicellulose and lignin respectively while corncobs contain about 40%, 36% and 16%.

Venter[13] has summarised the approximate chemical composition of bagasse reported in the literature and these are

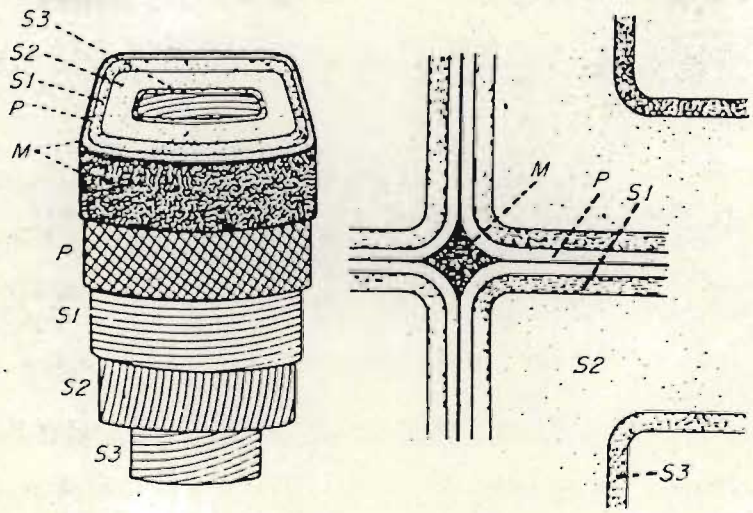


Figure 2.1. Sketch showing the various layers of wood cell walls.

P = primary wall
M = middle lamella
S1 = transition lamella
S2 = main secondary wall
S3 = inner secondary wall

reproduced in Table 2.1. Average values for hardwoods and softwoods and for South African grown pines, eucalyptus and bagasse are included for comparison.

2.2.3 Hemicellulose

The hemicelluloses provide the link between lignin and cellulose and consist of polysaccharides with complicated, exactly defined structure of fixed sequence. In wood, hemicelluloses consist of relatively short, mainly branched heteropolymers of glucose, xylose, galactose, mannose and arabinose as well as uronic acids of glucose and galactose, linked by 1,3 ; 1,6 and 1,4 glucosidic bonds with an appreciable number of acetyl groups[9, 11].

Bagasse hemicelluloses consist primarily of the polymers 4-O-methyl-glucuronoxylan acetate and 4-O-methylglucurono-arabinoxylan, commonly called xylans, which have a straight chain backbone of xylose monomers connected with 1 : 4-β bonds[13a,14]. Attached to these chains are substituents at the hydroxyl groups preventing crystallisation. These substituents are 4-O-methylglucuronic acid, arabinose and acetate groups[15]. (See Figure 2.2).

The chemical composition of hemicelluloses makes them strong hydrogen bonders with cellulose. Furthermore due to their 'loose' structure (nearly no crystallinity) and high content of OH-, CO- and COOH- groups they are highly hydrophilic and can be regarded as a kind of 'glue' for the linear cellulose chains[11].

Various researchers have investigated the formation of sugars during hemicellulose hydrolysis in an attempt to characterise the hemicellulose sugar components.

Slutzky *et al*[16] have detected xylose, arabinose, glucose, galactose and a homologous series of oligosaccarides in acid hydrolysates from bagasse. The presence of glucose was related to a random attack of the cellulose or that the arabinoxylans could have a bound structure between glucose and xylose. They also concluded that there are two different

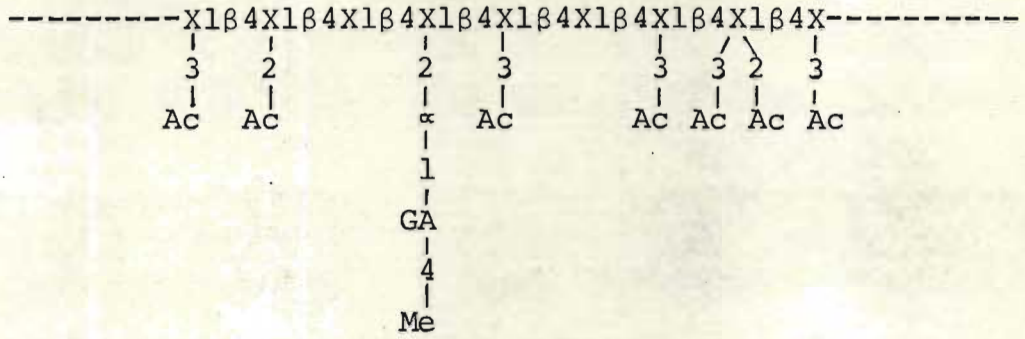
TABLE 2.1. : Average Chemical Composition of Bagasse and Pulpwood

	Whole Bagasse	Depithed Bagasse	Pith	Softwood	Hardwood	SA Pine[xx]	SA Eucalyptus	SA Whole Bagasse
llulose ross & evan) %	52 (41-63)	61 (56-63)	52,5 (46,0-55,4)	58,5 (54-61)	60 (51-64)	44,0[x] (39,3-47,2)	39,6[x]	43,6[x] (41,7-45,3)
Cellulose %	35,2 (26,6-38,9)	41,5 (38,3-43,3)	34,2 (26,6-36,4)	47,4 (45,0-48,9)	49,0 (44,5-56,5)			
lo- llulose %	76 (75,2-78,3)	77,8 (76,9-78,6)	77,7 (76,6-79,4)	71 (68-73)	81,0 (76-85)			
ntosans %	25,8	28,5	29,4	9,8	20,4	10,6	18,9	25,6
gnin %	19,5	20,8	20,8	27,0	22,4	27,6	23,3	21,4
n %	2,2 (1,0-5,8)	1,1 (0,6-2,2)	2,9 (1,8-4,9)	0,25 (0,2-0,5)	0,45 (0,3-0,8			1,8 (1,6-1,9)
cohol- nzene ractives %	4,8 (3,2-10,8)	2,2 (1,6-3,6)	2,5 (1,7-3,0)	4,0 (1,4-8,3)	3,0 (1,7-9,1)	3,3	4,9	
twater ubles %	3,4 (2,8-11,2)	1,3 (0,4-4,5)	2,1 (1,0-4,6)	4,0 (3,0-5,0)	2,9 (2,0-5,0)	3,9 (0,5-7,6)	4,0	

x Seifert cellulose (see section 3.1.)

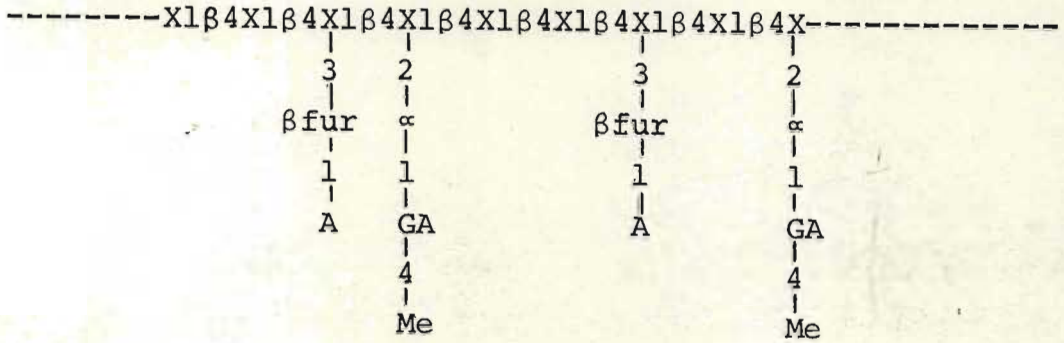
xx Average values for a number of species, covering ages of 6 to 30 years

4-O-methylglucuronoxylan acetate



X = xylose monomer
GA = glucuronic acid monomer
Me = methyl
Ac = acetyl

4-O-methylglucuronoarabinoxylan



X = xylose monomer
A = arabinose monomer
GA = glucoronic acid monomer
Me = methyl
fur = furanosidic bond (all others pyranosidic)

Figure 2.2. Structure of bagasse xylans

types of hemicellulose ; an arabinoxylan richer in arabinose and easy to hydrolyse and another arabinoxylan poor in arabinose and more difficult to hydrolyse.

The bagasse hemicelluloses on acid hydrolysis were found by Banerjee et al[17] to yield D-galacturonic acid together with D-glucose, L-arabinose, D-xylose and another sugar in minor amount. This sugar was identified as xylulose. That this sugar was not an artifact was proved by the fact that it was not produced when xylose was heated for an extended period with dilute sulphuric acid.

Partial acid hydrolysis of xylans containing 4-O-methyl glucuronic acid side chains results in the formation of a polymer homologous series of β(1 : 4) xylodextrins and a closely related acid series[18]. The linkage between the glucuronic acid and xylose in the polymer backbone is especially acid resistant. High yields of the aldobiuronic acid[19], 2-O-(4-O-methylαDglucopyranosyl uronic acid)-D-xylose, are thus always obtained even on drastic acid hydrolysis of 4-O-methylglucuronoxylans.

The hemicelluloses have also been studied by extraction with alkali from bagasse holocellulose followed by precipitation into various fractions. Blake et al[20] isolated hemicelluloses from sugarcane bagasse by alkali extraction using the conventional procedure. In this convention hemicellulose A is the water insoluble fraction which is precipitated on neutralisation of the alkaline extract to pH 4,5 to 5,0 with acetic acid; hemicellulose B is extracted by precipitation with three volumes of ethanol and the remaining portion is designated hemicellulose C. Hemicellulose A was found to have the following composition: arabinose 4,5%, xylose 70,0%, galactose 0,7%, glucose 8,2% and 4-O-methylglucoronic acid 6,2%.

El-Ashmawy et al[21] fractionated alkali extracted bagasse hemicellulose into hemicellulose A and B. These fractions were then acid hydrolysed to produce the following netural

sugars: hemicellulose A - xylose, arabinose and glucose; hemicellulose B - xylose, arabinose, glucose and galactose. Fraction A was richer in pentoses than B with xylose the predominant sugar in both fractions.

2.2.4 Lignin

Lignin is a three dimensional macromolecule of high molecular weight. Since its units are extensively cross-linked, an individual molecule cannot even be defined : the total lignin of one middle lamella must be regarded as one molecule[11]. There is no fixed formula for lignin but rather a random three dimensional arrangement of p-hydroxycinnamyl alcohols. These phenylpropane type groups include coniferyl units in the case of gymnosperms and both coniferyl and syringyl units in the case of angiosperms, which bring about considerable chemical and microbial resistance[9, 11].

Only hydrogen, carbon and oxygen are present in lignin in the approximate weight ratio 6 : 30 : 60. The high carbon content thus indicates the aromatic nature of lignin[15].

Figure 2.3 is a schematic model of the monomeric units and bonds in lignin[9]. These main structural features of lignin have been elucidated mainly by degradation of lignin and analysis of the degradation products. An excellent review of lignin functional groups and lignin degradation products has been presented by Rydholm[15].

2.2.5 Cellulose

Cellulose is a linear polymer of D-anhydroglucopyranose units linked by β(1-4) glucosidic bonds. The number of glucose units per molecule (degree of polymerisation) of native cellulose lies in the range 10 000 to 14 000[9], which means that the molecular weight of native cellulose is above 1,5 million. The glucose units have also been shown to adopt a chain conformation with the hydroxyl groups occupying the stable equatorial position. As the length of the anhydroglucose unit is 0,515 nm the total length of the native cellulose molecule is about 5 microns[22].

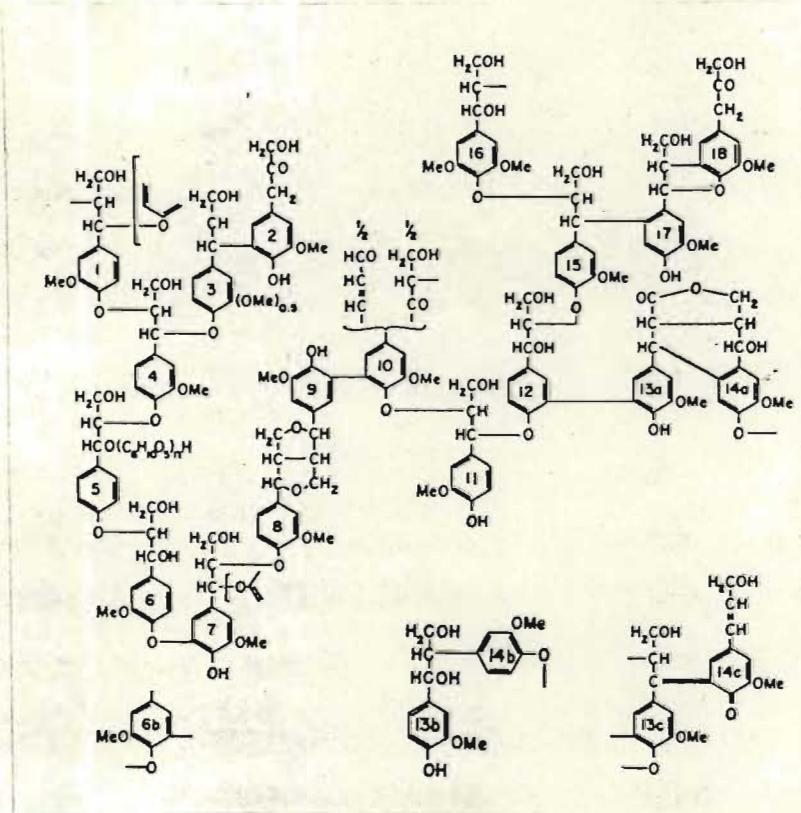


Figure 2.3. Schematic model showing the various types of monomer units and inter-monomer bonds known to exist in spruce lignin(9)

As is the case with all hydrophilic linear polymers, cellulose tends to form elementary fibrils, in which the polymer chains are orientated in parallel and firmly bound together by a large number of strong hydrogen bonds. The elementary fibrils are the smallest structural units of microfibrils and fibres[22].

In the elementary fibrils, areas of complete order, the crystallites alternate with less ordered amorphous regions. On average cellulose is 15% amorphous and 85% crystalline[12]. There is no sharp boundary between the crystallites and amorphous areas. As the average length of crystallites in native cellulose is 100 ±20 nm and that of the amorphous areas 30 - 40 nm, the cellulose chains in the elementary fibril run through large numbers of both crystalline and amorphous regions, binding them by covalent forces[22].

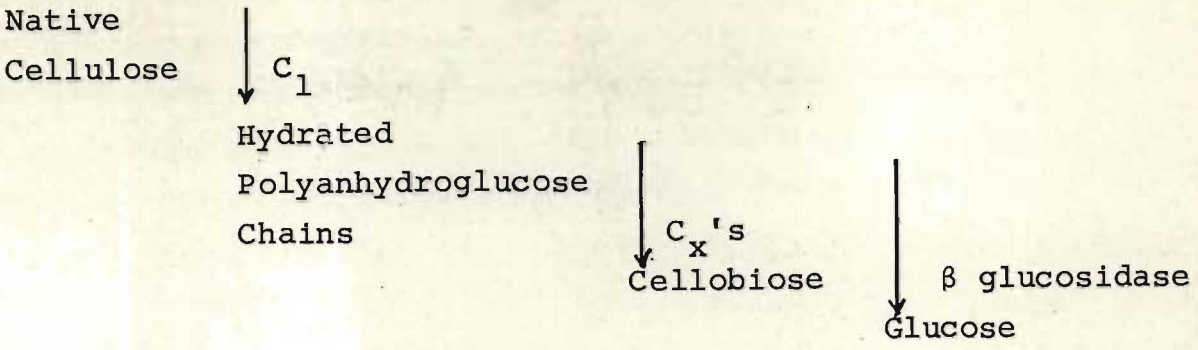
The crystalline structure of cellulose is still a subject of considerable controversy. Two major schools of thought exist[10]. Models of cellulose fibrils involving folded chains have been suggested. On the other hand, models based on extended cellulose molecules are also found in the literature. However, if cellulose molecules are fully extended, the length of elementary fibrils is expected to be much longer than 1000 Å measured for those of wood and cotton[12]. Consequently, cellulose molecules are believed to be more likely folded in some fashion.

Recent evidence suggests[10] that a cellulose molecule folds back and forth on itself within a plane with fold length corresponding to between 100 and 200 anhydroglucose units.

2.3 Properties of Cellulose Affected by Pretreatment

2.3.1 General

The action of the cellulose enzyme complex (C_1, C_x) has been approximated by the following reaction scheme[23]:



However, it has recently become apparent that the cellulase system has the following composition[24, 25]:

1) Endo-β1,4 glucanases, the old C_x enzymes, can hydrolyse soluble derivatives of cellulose or swollen and partially degraded celluloses. Attack usually occurs at random and cellobiose and cellotriose are the major products of endo-β1,4 glucanase action.

2) Exo-β1,4 glucanases are present in varieties : a) β1,4 glucan glucohydrolase, which removes single glucose units from the nonreducing end of the cellulose chain and b) β1,4 glucan cellobiohydrolase (CBH) which removes cellobiose units from the nonreducing end of the chain. This CBH is currently being equated with the old C_1 enzyme.

3) β-glucosidase hydrolyses cellobiose and short chain cello-oligosaccharides to glucose but has no effect on cellulose.

The susceptibility of cellulose and cellulosic wastes to enzymatic hydrolysis is greatly affected by a number of features of the cellulose-enzyme system. These have been discussed by Cowling[9, 26, 27] and are summarised below:

i) moisture content
ii) size and diffusibility of the enzyme molecules in relation to the capillary structure of cellulose
iii) degree of crystallinity of cellulose
iv) unit cell dimensions of the crystallites present
v) conformational and steric rigidity of the anhydro-glucose units

vi) degree of polymerisation of the cellulose

vii) nature of the substances with which the cellulose is associated

viii) nature, concentration and distribution of substituent groups.

These factors can be altered to a greater or lesser degree by physical and/or chemical means prior to hydrolysis in order to increase susceptibility to enzymatic attack.

Four aspects of cellulosic materials are considered to be particularly significant with respect to susceptibility to enzymatic hydrolysis and as potential bases of effective pretreatment. The capillary structure of a cellulose fibre determines the accessibility of the cellulose to cellulase enzymes. Secondly, highly crystalline (ordered) regions of a substrate are more resistant than the amorphous regions.

The presence of lignin and its manner of association with the cellulose can also interfere with the hydrolysis. Finally, reduction in the degree of polymerisation of cellulose leads to increased susceptibility to enzymatic attack. The following summary indicates previous work that has been directed to these subjects.

2.3.2 Capillary Structure and Surface Area

Cowling[9] has postulated that one of the most important structural features that affects the enzymatic hydrolysis is the capillary structure of a cellulose fibre, because the susceptibility of cellulose to hydrolysis is determined largely by the accessibility of the cellulose surface to cellulolytic enzymes. Direct physical contact between the enzyme and the cellulose substrate is a prerequisite for hydrolysis. From size and shape measurements of cellulytic enzymes it was also evident that they are able to diffuse into gross capillaries of plant fibres but had difficulty in penetrating the cell wall capillaries.

The pore volumes of cotton linters swollen by increasing concentrations of phosphoric acid were measured by Stone et al[28]. A linear relationship existed between initial hydrolysis rate using cellulase enzyme and the surface area within the cellulose gel which was accessible to a molecule of 40 Å.

On the other hand, Fan et al[29] investigated the effect of specific surface area on the enzymatic hydrolysis rate. They deduced from their data that the specific surface area didn't significantly affect the hydrolysis rate as reported by Cowling. Each type of cellulose showed a distinct range of hydrolysis rates. It was concluded that the structural nature or treatment history determines the extent of hydrolysis to a greater degree than specific surface area. However, the specific surface area was measured on a BET apparatus using nitrogen as the adsorbate gas. The surface area accessible to a nitrogen molecule was measured, which is not necessarily the area accessible to a larger enzyme molecule.

Water also has a profound effect upon the structure of cellulose. The specific surface area of natural cellulose is known to increase upon wetting due to water penetration into the amorphous regions of the cellulose (intercrystalline swelling)[11]. Considerable osmotic forces are generated which open up the capillary system, providing access to the enzymes. On air drying from the water swollen state, the capillary structure collapses thus decreasing the specific surface area[28, 29] and decreasing enzymatic susceptibility[30].

The treatment of native cellulose with sodium hydroxide solutions of increasing strength (above 20%) has a similar effect, producing extensive swelling and separation of structural elements[31].

2.3.3. Crystallinity

By X-ray diffraction analyses, cellulose has four known stable crystal lattices[9]. Cellulose I is the crystal form in native cellulosic materials, while cellulose II is

formed by treating cellulose I with alkali and is found in regenerated cellulose such as viscose filaments, cellophane and mercerised cotton.

Cellulose III is formed by treating cellulose I or II with liquid ammonia and cellulose IV is prepared by heating cellulose III in glycerine at 250°C[32].

Walseth[33] has shown that cellulolytic enzymes rapidly degrade the more accessible amorphous portion of regenerated cellulose but are less able to attack the less accessible crystalline portion. Consequently a significant increase in crystallinity is observed during enzymatic hydrolysis of cellulose. A similar trend is also evident during acid hydrolysis[29]. Thus as the cellulose becomes more crystalline (ordered), it becomes more resistant to further hydrolysis.

Ball milling has been demonstrated to induce crystalline modifications in cellulosic materials. Dry ball milling results in complete destruction of the crystalline lattice as determined by X-ray diffraction. However, upon wetting and redrying, reversion to the native cellulose I form occurs in cases where ball milling time was insufficient (less than $\pm$ 3 hrs). In the Caulfield and Steffes report[34] ball milling times of six hours gave a product whose crystalline structure was entirely cellulose II after being soaked in water at 40°C and dried. The extent of crystallinity as measured by the crystallinity index was roughly one-half of the original non-milled cellulose pulp. After three hours of ball milling the wetted and dried products crystalline phase was a mixture of cellulose I and cellulose II.

Fan et al[29] investigated the effect of crystallinity on the hydrolysis rates of various celluloses. The crystallinity index influenced the hydrolysis of the microcrystalline cellulose, the Solka Floc and the ball milled Solka Floc in

the same way. Thus it was concluded that the inverse relationship between crystallinity index and hydrolysis rate follows the same trend for all pure cellulose substrates, regardless of treatment history.

In a more recent publication Fan et al[35] examined the relationship between hydrolysis rate and two structural parameters; specific surface area (SSA) and crystallinity index (CrI). Solka Floc was pretreated with various methods to obtain a wide range of crystallinity indices and specific surface areas. It was observed that independent of the method of treatment, the rate of hydrolysis would tend to increase with an increase in SSA and a decrease in CrI. A linear regression analysis of the data produced the following relationship for the extent of hydrolysis after eight hours:

$$X_8 = 0,38\ (SSA)^{0,195}\ (100-CrI)^{1,04}$$

This expression indicates that the rate of hydrolysis is more sensitive to crystallinity index than to specific surface area.

Various chemical and waste paper pulps were subjected to pulping and beating by Thonart et al[3]. The effectiveness of these pretreatments were evaluated by enzymatic hydrolyses with cellulase enzymes. Cellulose crystallinity was not affected by this treatment, yet there was an increase in digestibility. It was concluded that the crystalline structure was not an insurmountable obstacle to hydrolysis though it affected the hydrolysis speed and that the increase in digestibility was due mainly to an increase in specific surface area.

Water is known to cause an increase in crystallinity of cellulose. This increase is due to a recrystallisation effect which becomes more pronounced for highly amorphous cellulose[29].

The mercerisation process and anhydrous liquid ammonia treatment of cellulosic materials also result in swelling and transitions in crystalline form (usually to cellulose II). An excellent review of the literature on this subject is provided by Spano et al [32].

2.3.4 Lignin Carbohydrate Complex

The nature of the association between lignin and carbohydrate in wood has been debated for many years. Three main theories are prevalent :

a) hydrogen bonding between constituents
b) covalent chemical bonds and
c) incrustations where the three dimensional lignin network encases the cellulose, thereby preventing easy access of enzyme molecules.

Olson et al [36] investigated the fermentability of ball milled groundwoods. Ball milling (100 hours) did not improve the susceptibility to fermentation. The presence of a toxic substance in the extractive-free ball milled wood was also ruled out. It was thus concluded that a chemical combination between lignin and cellulose was the probable explanation for the non-fermentability of wood since ball milling appeared to exclude the possibility of a physical interaction.

The stability of the lignin carbohydrate complex (LCC) of milled spruce wood has been investigated by Brownell [37] to resolve conflicting reports of its acid-and alkali-lability The lignin-carbohydrate linkage is alkali stable below 100°C, at which point hydrolysis of the lignin becomes measurable. Below 100°C the liberation of lignin from milled wood results from alkali 'peeling' of the hemicellulose and not from hydrolysis of ester or other alkali-labile lignin-carbohydrate bonds. Mild acid treatments show that the acid-lability of LCC is a lability of the hemicellulose rather than of the lignin carbohydrate linkage. It was thus concluded that the L-C linkage, if it is not simply physical entanglement, is probably not an acetal, hemi-acetal, ketal, ester or glycoside bond, but it may be an ether bond.

Pew and Weyna[38] have been advocates of the incrustation theory. They contend that the evidence presented for the existence of covalent bonds is incomplete and that chemical bonds alone could not explain the total resistance of wood to cellulolytic enzymes.

It is their contention that even ball milled wood particles as small as several microns constitute a 'cage' of lignin in which the carbohydrate molecules are enmeshed. A model experiment was performed in which holocellulose was impregnated with a water soluble phenolic resin, cured and then subjected to enzymatic hydrolysis after ball milling for 5 hours. Very little digestion occurred and this tended to support the theory of interpenetration of lignin and carbohydrate.

Bellamy[39] discusses the carbohydrate lignin bond. There appear to be very few covalent bonds between lignin and cellulose. There are, however, ester bonds between the uronic acids of hemicellulose and the phenol groups of lignin. Lignin appears to surround cellulose fibres as a three dimensional net whibh may inhibit enzyme degradation of the cellulose fraction of the lignin-cellulose complex.

Lignin contains a considerable portion of alcoholic-, phenolic- and ether-oxygen groups. Such groups frequently give hydrogen bonds and it is likely that hydrogen bonds are formed with the carbohydrate hydroxyl groups. Hydrogen bonds are, however, very weak unless systematically arrayed in a highly oriented cellulose. Pew and Weyna[38] concluded that the hydrogen bonding of lignin to carbohydrate would be weak due to the amorphous nature of lignin and is thus only a contributing factor in the lignin-carbohydrate bond.

It would appear that the lignin carbohydrate complex can be attributed to the encrustation of the carbohydrate molecules by the amorphous lignin. Removal of this lignin should thus result in improved enzymatic susceptibility.

2.3.5 Degree of Polymerisation

Cowling[9] has noted that the length of cellulose molecules in a fibre vary over a wide range. This variation would be expected to affect the rate of hydrolysis very considerably, particularly by enzymes that cleave the cellulose molecules by an endwise mechanism. When the degree of polymerisation (DP) of cellulose is reduced to a point that the molecules become more soluble and they no longer maintain their structural relationships with one another, there is a great increase in the susceptibility to enzymatic hydrolysis. Whether such a change is due to chain shortening or increased solubilisation enhancing the hydrolysis is a matter not yet fully understood[1].

Cotton linters and alpha sulphate pulps have been depolymerised extensively by high voltage cathode rays at dosage levels above 5×10^6 equivalent roentgens[40]. They depolymerised at practically the same rate resulting in products with nearly average chain lengths. At a dose of 5×10^8 equivalent roentgens the cotton linters were converted to water soluble materials.

Caulfield and Steffes[34] noted that ball milling of pure celluloses resulted in substantial decreases in degree of polymerisation. This fact is well documented and a review of this topic is presented by Spano *et al*[32].

During acid hydrolysis there is also a rapid decrease in degree of polymerisation which levels off at a constant value between 100 and 200[12].

2.4 Pretreatment Processes

Early work in enzymatic hydrolysis reported very slow reaction rates obtained with native untreated lignocellulosic materials and the extent of the reaction was low, often under 20%[41]. All processes for enzymatic hydrolysis currently proposed incorporate some type of pretreatment of the lignocellulose in order to obtain reaction rates that can support economic operation.

2.4.1 Mechanical Pretreatments

2.4.1.1. High Temperature

It has been claimed[31] that modifying cellulose fine structure by simple thermal exposure offers an appealing approach to improve reactivity. A three hour treatment at 200°C in a non-polar liquid such as kerosene, or in dry air is claimed to give a product with a greatly enhanced rate of acid hydrolysis.

However, Millet and Goedken[40] reassessed the thermal pretreatment. They confirmed 200°C as being the optimum temperature but the maximum increase in rate of dilute acid hydrolysis was only about 35% with an increase of 27% in maximum sugar yield. Considering that a 32 hour treating period was required to obtain even these modest benefits on cotton linters and softwood pulp, it is unlikely that simple thermal pretreatment has much to offer as a commercial venture.

2.4.1.2. Hammer Milling

A hammer mill is an impact mill consisting of a rotor to which a set of hammers is attached. As the rotor turns rapidly, the hammers impact the material against a breaker plate exerting compressive, shear and tensile forces. This process is repeated until the material is small enough to pass through a screen at the bottom of the mill.

Mandels *et al*[42] noted that hammer milling of newspaper gave good size reduction and increased bulk density but virtually no improvement in susceptibility using a variety of screen sizes. In fact, for the finer screen sizes, susceptibility was less than for the newspaper feed material.

Andren *et al*[43] also investigated the effect of hammer milling on more than thirty representative cellulosic

wastes. Although there was a decrease in particle size with a resulting increase in surface area, results showed only marginal increases in reactivity over un-milled substrates.

2.4.1.3. Fluid Energy Milling

In a fluid energy mill the particles to be reduced in size are entrained in high velocity gas or vapour streams and impacted against a target plate or opposing entrained particles. The product is removed using an internal air classifier.

Mandels et al[42] fed newspaper at between 0,036 kg/hr and 2,8 kg/hr using both the target plate/nozzle and double opposed nozzle combinations into a fluid energy mill and only modest susceptibility improvements were noted over the untreated newspaper. For this reason, plus the high operating costs and low production rates, this type of milling was considered unsatisfactory.

2.4.1.4 Colloid Milling

A colloidal mill is an attriting machine consisting of two disks revolving in opposite directions at peripheral speeds between 10 and 50 m/sec and set close to each other. Feed in the form of a slurry is passed between the disks where particles impact each other and in the process are reduced in size and dispersed.

Mandels et al[42] operated at gap settings between 0,025 and 0,127 millimeters and with 2% water slurries of newspaper and only modest improvements in susceptibilities were observed. This fact, along with high operating costs, led the authors to conclude that colloidal milling would not be a satisfactory method of substrate preparation.

2.4.1.5 Two Roll Milling

A two roll mill consists of two cast-iron tempered surface rolls placed horizontally in bearings and held in place by mill housings. The rolls are set close together and are adjustable by mill screws. These rolls also operate at different speeds resulting in a shearing, crushing effect.

Differential speed two roll milling is an effective pretreatment for increasing the susceptibility of cellulose to enzymatic hydrolysis[32, 44]. Using mills with three, six and ten inch diameter rolls and processing times of 10 minutes or less resulted in the following percentage increase in susceptibility over untreated controls : cotton, 1100; maple chips, 1600 ; white pine chips, 600; newspaper, 125. In comparison, ball milling of newspaper for 24 hours gives only 62% increase.

Tassinari et al[45] maintain that the reduction in degree of crystallinity and degree of polymerisation of cellulose and partial destruction of the structural integrity of lignocellulosics brought about by compression milling significantly increase the susceptibility of cellulose to enzymatic hydrolysis. The enzymatic hydrolysis yield was found to be directly related to the specific energy input to the cellulosic substrate (controlled by milling time). The power requirements for compression milling which render equivalent hydrolysis yields depend on the source and kind of lignocellulosics to be pretreated. For newspaper, the specific energy input required for 55% sugar yield was estimated as 0,66 kWh/kg substrate including 15% power loss. The additional sugar gained from the enzymatic hydrolysis of compression-milled newspaper (above sugar yield of untreated substrate) was 453 g sugar/kWh energy input.

Spano et al[46] also found that a four minute compression milling pretreatment of whole sugarcane bagasse

resulted in a 7,5 times increase in susceptibility to T. reesei cellulase (19 IU/g substrate and 5% slurry) after 24 hours of hydrolysis.

Further advantages of the roll mill is the increased wet density of the product permitting higher slurry concentrations during hydrolysis and the power requirement per kilogram is 20% less than for ball-milled newspaper with comparable reactivity[13]. Important parameters of mill effectiveness are roll clearance and processing time. However, the heavy erosion of the rolls which must occur as a result of the grit in the bagasse would not make this a practical pretreatment method.

2.4.1.6. Vibratory Ball Milling

A vibratory ball mill consists of a cylindrical shell, approximately one-third of its volume filled with balls (steel or ceramic). The reciprocating movement of the mill causes the balls to vibrate and the abrasive conditions result in size reduction.

Pew and Weyna[38] investigated the effect of vibratory ball milling on the digestion (4 - 6 days) of spruce and aspen sawdust using Trichoderma viride cellulase in 5% slurries at pH 4,6 and 40°C. As grinding time increased, solubilisation of carbohydrates by enzyme increased to 96% for both woods after 8 hours (spruce) and 5 hours (aspen) of vibratory milling.

The effect of vibratory ball milling on the digestibility of four wood species using a commercial enzyme preparation has been measured by Matsumura et al[47]. Both softwoods and hardwoods were investigated and the extent of enzymatic hydrolysis increased with reduction of particle size.

Extensive research has been conducted to increase the digestibility of various wood residues to make them suitable as sources of dietary energy for ruminants[48, 49]. The first 30 minutes of vibratory milling appear to have the major influence on digestibility of hardwoods. A digestibility plateau is apparently attained beyond which additional milling is of little value. Softwoods are not very responsive to vibratory milling and a digestibility maximum of 18% was obtained for five different softwoods milled for 120 minutes. It was concluded that this species selective response is due to the quantity, chemical nature and distribution within the cell walls, of lignin.

Shimizu[30] also found that vibratory milling was an effective pretreatment for thermorechemical pulp rejects and ground wood papers (newsprint). A 30 minute milling increased the hydrolysis extent of newsprint from 36 to 70%, i.e. 90% polysaccharides susceptible to cellulase enzyme (23% lignin content of paper). A susceptibility plateau was also reached after 30 minutes of grinding.

However, vibratory ball milling requires high energy demands and not being brittle, bagasse is unsuitable for processing in vibratory mills.

2.4.1.7 Ball Milling

A ball mill consists of a cylindrical vessel approximately half filled with balls (steel or ceramic) which is rotated about its axis using a tumbling motion to the balls. The impact and rolling action of the balls results in size reduction of the mill feed.

Mandels et al[42] found that ball milling was the best mechanical pretreatment they investigated which gave good size reduction, maximum bulk density and maximum susceptibility. Hammer milling, fluid energy milling and colloidal milling were all less satisfactory. Newspaper after one day of ball milling was 46,8%

saccharified at 24 hours as compared to the hammer milled newspaper control which was only 23% saccharified.

Andren et al[43] reported on the susceptibility of more than thirty representative cellulosic wastes to enzymatic hydrolysis before and after ball milling. Ball milling increased the bulk density of the cellulosic materials as well as their susceptibility to saccharification by a Trichoderma viride cellulase. Oat hulls and cotton linters showed an increase in saccharification from 7,9% and 8,5% respectively to 59,9% and 68,0% after ball milling. The major drawback to ball milling as a pretreatment is that it is expensive and energy intensive.

2.4.1.8. Refining

The disk refining principle of one stationary disk and one rotating disk fitted with various plate configurations is in increasing use by the wood pulp industry to produce refined mechanical pulp from steamed wood chips. By the combination of shearing, rolling and cutting, the fibre bundles are separated into virtually individual fibres thus greatly increasing exposed surface area.

Han et al[50] investigated the effect of refiner defibrizing on the fermentability of rye grass straw. The in vitro rumen digestibility and susceptibility to cellulase of defibrized straw was significantly higher than for hammer milled straw. The defibrizing process exposed the inner fibre structure of the straw making it more reactive to chemical, enzymatic and microbial attack. Reducing the plate clearance produced a finer fibre exposing more surface area to enzymatic attack.

However, the distribution of lignin in these thermo-mechanical fibres will be of paramount importance when considering susceptibility to enzyme hydrolysis.

Atack[51] has shown that spruce fibres produced at 115°C (69 kPa) were separated at the S1 layer whereas those produced at 170°C (690 kPa) were separated at the interface between the primary wall and the middle lamella. Because of the high concentration of lignin at the middle lamella, surfaces produced at 170°C were quite heavily encrusted with lignin. This change in fibre separation was shown to be related to the fact that under refining conditions lignin in wood chips can undergo a glass transition over the temperature range 120 to 135°C. Thus fibres produced above the transition temperature are heavily case hardened with lignin after quenching to reduce the temperature and pressurised refining at a temperature below the transition temperature leads to the production of a pulp in which the fibre surface has a more cellulosic character.

Kerr and Goring[52] also investigated the distribution of lignin in spruce (softwood) and aspen (hardwood) thermomechanical fibres. The wood chips were refined at 210 kPa and 690 kPa. In both cases the 690 kPa fibres seemed to be separated at the primary wall middle lamella boundary and the middle lamella lignin remained intact on the fibre surfaces.

It is thus obvious that refining conditions must be carefully chosen to result in any improvement in enzymatic susceptibility. In fact, refining under adverse conditions could result in an actual decrease in susceptibility as the fibre surfaces could become heavily encrusted with lignin at temperatures above the glass transition temperature.

2.4.1.9 Simultaneous Wet Milling

Kelsey and Shafizadeh[53] have investigated the effectiveness of combined wet milling and enzymatic hydrolysis of cellulosic substrates. These substrates were wet milled with a variety of grinding elements such as sand, glass beads and stainless steel beads, agitated in a shaker bath and simultaneous hydrolysis

was achieved with a 2% substrate slurry using a commercial Trichoderma viride cellulase. Newsprint, lignocellulose (prehydrolysed to remove hemicellulose) and wood (all initially <20 mesh) exhibited enhanced hydrolysis rates above those observed for ball milling and subsequent hydrolysis and yielded a more extensive saccharification.

The effectiveness of the process was dependent upon the lignified matrix of the cellulose microfibrils, the grinding elements and the oscillation frequency of the shaker bath. For lignocellulose 93% saccharification could be attained by wet milling with cellulase for 24 hours. This was three times greater than that of ball milled and ten times greater than that of the unmilled substrate.

The advantage of simultaneous wet milling was attributed to a continuous generation of accessible sites and a sustained rapid hydrolysis rate, whereas in the ball milled substrates the hydrolysis rate slowed down as the number of active sites was reduced.

However extensive size reduction of the substrate is required before the simultaneous wet milling process and the economic and industrial feasibility require further research and development.

2.4.1.10. Attritor Milling (Agitation bead milling)

Attritor mills are essentially ball mills in which the balls are stirred with an agitator whilst the drum remains stationary. These mills are more energy efficient than ball mills and are extensively used in the paint industry for the grinding of pigments.

Horton et al[54] have found attritor milling to be an effective pretreatment for municipal solid waste for the S.S.F. Gulf Process. The increase in ethanol production was attributed to the production of a finely ground substrate with increased surface area and perhaps

increased hydration of the cellulose particles. They concluded that attritor milling was a borderline economic pretreatment but that it represented a processing treatment with promise.

Attritor milling has also been found to enhance the susceptibility of acid treated bagasse to cellulase enzymes. After acid treatment the bagasse was brittle and more amenable to grinding. Purchase[5] at the Sugar Milling Research Institute has indicated that a ten minute grind using 0,453 kWh/kg of material milled produced a significant increase in sugar yield. He concluded that this power consumption was comparable with figures obtained for twin roll milling, which was regarded as an efficient pretreatment.

2.4.2 Irradiation

In general radiation initiates an oxidative degradation of the cellulose molecule. Dehydrogenation, destruction of anhydroglucose units to yield carbon monoxide and carbon dioxide and cellulose chain cleavage to yield a homologous series of products are observed. Oxidative products include carboxyl and carbonyl groups[32].

Saeman et al[55] used high voltage cathode ray electrons to treat alpha-cellulose wood pulp and cotton linters. Extent of dilute acid hydrolysis (0,1 N H_2SO_4 at 180°C) was used to evaluate the treatment effectiveness. The optimum dosage of 10^8 equivalent roentgens resulted in sugar yields (carbohydrate decomposition included) of 70,2% and 62,1% for the wood pulp and cotton linters respectively. Untreated wood pulp and cotton linters gave respective overall sugar yields of only 33% and 28%.

A combination of thermal and linear accelerator high energy electron beam radiation treatment was investigated by Millet and Goedken[40] using cotton linters and a nitration grade softwood pulp. The effectiveness of the treatment was

determined by measuring sugar production by dilute acid hydrolysis (0,1 N H_2SO_4 at 180°C). A dosage of 10^6 equivalent roentgens, the level at which irradiation begins to significantly effect cellulose structure, was applied as it is also the maximum permissible economic dosage. Best results were obtained by irradiation first followed by heat treatment at 200°C for up to 16 hours. Maximum sugar yield increased from 22,5% to 28,5% and irradiation alone only improved sugar yield from 22,5% to 24,5%

Even though the hydrolytic activity of cellulose was enhanced by dual pretreatment, the authors concluded that the improvement was not sufficient to be of potential commercial significance.

Efforts have also been made to increase the in vitro digestibility of various wood residues by electron irradiation[48, 49]. Dosages of between 10^6 and 10^8 rads were applied to both hard and softwoods. Aspen (hardwood) digestibility was increased from 55% to 78% and spruce (softwood) from 3% to 14%. It was concluded that the cost of an electron irradiation pretreatment was prohibitive.

The effect of irradiation on the accessibility of cellulose in bagasse to enzymatic hydrolysis using Trichoderma reesei cellulase has been investigated in the Chemical Engineering Department at the University of Natal[56]. Dosages of up to 10^6 rads have no effect on the susceptibility of bagasse to hydrolysis.

This irradiation treatment is costly and in view of its failure to enhance susceptibility it is not considered as a viable pretreatment process.

2.4.3 Chemical Pretreatments

Chemical pretreatments of cellulosic materials are of potential benefit in the preparation of substrates for enzymatic hydrolysis. Factors that may be modified in

order to improve susceptibility to hydrolysis include swelling, degree of polymerisation, hydrogen bonding, relative extent of crystalline and amorphous regions, amount of lignin present and the nature of its association with cellulose. Disadvantages of chemical pretreatment are the possible occurrenceof undesirable side reactions such as derivative formation and oxidation. Also, the chemical media themselves may present disposal problems.

2.4.3.1 Alkali Treatment

Millet et al[57] reported that mercerisation of cotton and ramie yielded 40 - 50% increase in acid hydrolysis rates using constant boiling hydrochloric acid. However, mercerised hemlock sulphite pulps showed no improvement. The dilute alkali pretreatment (5 - 10% NaOH) of a variety of woods was also investigated by determining the substrate weight loss following incubation with rumen fluid. Softwoods (26 - 35% lignin), in general were more resistant to alkali treatment than hardwoods (17 - 28% lignin), indicative of the effect of lignin content.

Matsumura et al[47] investigated the effectiveness of alkali pretreatment on four wood meals with 1 - 24% aqueous sodium hydroxide at 5 - 100^{o}C for 1 hour. The alkali treated wood meals were subject to enzymatic hydrolysis with Trichoderma viride cellulase. In general, an increasing alkali charge resulted in increasing digestibility. Once again the softwoods were less responsive than the hardwoods to alkali pretreatment.

Various methods of alkali pretreatment of sugarcane bagasse have been assessed in terms of digestion rates using Trichoderma viride cellulase by Gray et al[58].

Two liquid to solid ratios were used (20 : 1 and 2 : 1) and the higher ratio was found to be more effective. In both cases increased alkali concentration led to increased weight loss and glucose release on saccharification.

Many efforts have been made to increase the nutritive value of cellulosic materials. Han et al[59] improved the feed value of ryegrass straw by treatments with various concentrations of sodium hydroxide or anhydrous ammonia followed by fermentation of the treated straw. The optimum condition for alkali treatment of straw was 4 - 6% NaOH (based on dry weight of straw) for one hour or 3% ammonia for four weeks at room temperature. However, the ammonia treatment was not as effective as the sodium hydroxide.

Pretreatment of wood residues to enhance rumen digestibility by both anhydrous ammonia and aqueous sodium hydroxide have been investigated[48, 49]. Softwoods were unresponsive to alkali treatment and hardwoods exhibited a maximum in vitro digestibility at 5 - 6 g of NaOH per 100g of wood. Anhydrous ammonia treatment was only found effective on aspen, increasing its digestibility from 33% to 48% in ½ hour at 30°C.

An excellent review of efforts to upgrade the nutritive value of forage and forest residues by either sodium hydroxide or ammonia treatment is given by Millet et al[31, 57].

Mandels, Hontz and Nystrom[42] treated hammer milled newspaper with 2% NaOH at 70°C for 90 minutes and noted moderate improvement in respect to susceptibility to enzymatic hydrolysis by Trichoderma viride cellulase. If the treated sample was dried, susceptibility was markedly reduced and in fact, less than the untreated sample, perhaps indicative of crystalline changes within the cellulose microstructure.

Alkali treatment can also significantly increase microbial growth. Han and Callihan[60] noted that alkali treatment of bagasse increased the growth rate of Cellulomonas bacteria. Treatment by 4% NaOH for 15 minutes at 100°C increased the carbohydrate utilisation from 29% to about 73% whilst treatment with 5,2% aqueous NH_3 only increased digestibility to 57%.

Bellamy[39] also investigated the 48 hour growth at 55°C of Thermoactinomyces on pretreated feed lot waste fibre. With no pretreatment only 15-20% of the cellulose was utilised. Sodium hydroxide treatment (0,05M, 23°C, 4 hours) resulted in 74 - 81% cellulose utilisation while treating with anhydrous liquid ammonia for 10 hours at 23°C at 10 atmosphere netted a 72 - 80% cellulose utilisation.

The work of Dunlap et al[41] on the alkali treatment of sugarcane bagasse demonstrates a typical response of digestibility to increasing amounts of alkali per unit of cellulosic solid. Maximum digestibility was reached at an alkali application level somewhere between 0,1 and 0,15 g NaOH/g solids.

The effectiveness of the anhydrous liquid ammonia pretreatment of cellulosic materials has been studied by Spano et al[32]. Samples of Avicel, ball milled newspaper, two hardwoods and a softwood were held in liquid NH_3 for three hours at 60°C (24 atm). In general, there is an increasing effect of ammonia pretreatment with increasing severity of experimental conditions. The liquid ammonia treatment also rendered the hardwood sawdusts more susceptible to enzymatic hydrolysis than the softwood samples.

The physicochemical mechanism for increased digestibility of lignocellulosic materials after dilute sodium hydroxide or liquid ammonia pretreatment (without

delignification) has been elucidated[28, 61]. When treated with dilute alkali the important chemical reaction is a saponification or ammonolysis of the esters of 4-O-methylglucuronic acid attached to the xylan chains. Since these esters bridge polymeric units, the effect of this reaction is the breaking of crosslinks. Consequently a marked increase in swelling capacity and pore size occurs. This increase not only provides increased diffusivity for the hydrolysing enzymes but also improves enzyme substrate interactions.

Using a Trichoderma viride derived cellulase, Toyama and Ogawa[62] examined the effects of the alkaline and peracetic acid treatment of rice straw, bagasse and two wood species on decomposition (weight loss). Partial delignification of bagasse and rice straw specimens by boiling in 1% NaOH for 3 hours or alternatively 1 hour at 120°C, showed 55% and 48% weight losses at 48 hours respectively as a result of enzymatic hydrolysis. Boiling in 20% peracetic acid for 1 hour followed by autoclaving at 120°C in 1% NaOH resulted in decomposition of 94,1% for the hardwood and 77,9% for the softwood at 48 hours. Residual lignin contents were 18% for the softwood (originally 32%) and 15% for the hardwood, down from 30%.

Delignification and swelling by aqueous sodium hydroxide is thus an effective pretreatment for lignocellulosic materials. It is more effective than either aqueous or anhydrous ammonia treatment and raises the possibility of using a mild soda pulping process prior to enzymatic hydrolysis.

2.4.3.2. Acid Pretreatment

Olson et al[36] treated white spruce sawdust with sulphuric, hydrochloric and nitric acids (4% concentration, 100°C, S/L = 1 : 10). Each acid pretreatment failed to yield a fermentable material but a combination of nitric acid and sodium hydroxide treatments was able to increase f[illegible]

The effect of 50% H_2SO_4 treatment on bagasse followed by dilute acid hydrolysis has been investigated by Han and Callihan[60]. The lignin content was higher than the starting bagasse due to acid hydrolysis of the hemicellulose and cellulose fractions. The low digestibility of the residue was attributed to the high lignin content and the fact that the acid treatment solubilised the easily digestible bagasse fraction.

Knappert et al[63] investigated the partial acid hydrolysis of cellulosic materials as a pretreatment for cellulosic material. The pretreatment was performed in a continuous flow reactor on oak, corn stover, newsprint, and Solka Floc at temperatures ranging from 160° to 220°C, sulphuric acid concentrations ranging from 0 to 1,2% and a residence time of 0,22 minutes. The effectiveness of the pretreatment was measured by hydrolysis using Trichoderma viride cellulase. For all substrates except Solka Floc, increased saccharification was achieved during enzyme hydrolysis. This increase after pretreatment was due to the removal of hemicellulose, reduced degree of polymerisation and possibly due to a change in the crystal structure of cellulose (more amorphous than initial material).

Wilke[64] also pretreated a number of agricultural residues with 0,09M H_2SO_4 at solid/liquid ratios of 6 to 8,5 : 100. The stirred mixture was boiled for 5,5 hours. A portion of each acid hydrolysis residue was then subjected to enzymatic hydrolysis and improved glucose yields were observed in all cases except cotton gin trash and rice hulls which had the highest initial lignin contents. Moderate amounts of lignin appeared in some of the acid straw liquors which is desirable, since it represents a moderate delignification of the substrate.

Disrupting the lignin-carbohydrate complex without significant removal of either component can be accomplished with sulphur dioxide. Operating with moist sawdust (water/wood = 3/1) and gaseous SO_2 under pressure for

2-3 hours at 120°C Millet et al[57] have obtained impressive results on both hardwoods and softwoods with Onazuka cellulase (derived from Trichoderma viride). Almost quantitative conversions of carbohydrates to sugars were attained after 48 hour saccharification of hardwoods and at this time the two softwoods were 70-85% converted.

Lee et al[65] investigated the effectiveness of a dilute acid hemicellulose hydrolysis on a hardwood residue (Southern red oak) as a pretreatment process in glucose production by Trichoderma viride cellulase. Significant increases in both reaction rate and glucose yield resulted from the pretreatment. The spent residues from sulphuric acid treatment had the highest rate enhancement - a 6 fold increase over the untreated control. A somewhat lower, but still quite significant enhancement ranging from 2,4 to 4,5 was observed for the residues treated with SO_2 or acetic acid.

A similar pattern was shown in glucose yield, the sulphuric acid treated residue again having the highest enhancement over a 50 hour hydrolysis. It was claimed that the susceptibility enhancement in sulphuric acid treatment was mainly a result of an increase in surface area whereas in SO_2 treatment the benefit came mainly from reduced crystallinity.

A dilute acid hydrolysis pretreatment of bagasse has one major advantage. In general, up to 75% of pentosans hydrolyse readily under moderate conditions to form xylose with very small losses due to decomposition reactions[66]. This xylose solution would then be suitable for fermentation.

2.4.3.3. Cellulose Solvents

Cellulose solvents are an effective pretreatment for lignocellulosic materials as the lignin appears in the residue and the cellulose is regenerated in an amorphous state.

Mandels et al[42] noted that cuprammonium regneration of newspaper was an effective pretreatment for enzymatic hydrolysis with Trichoderma viride cellulase. The cuprammonium treatment increased saccharification from 18% for the control to 57% provided that the product was not dried before hydrolysis.

Tsao at Purdue University has extensively investigated the potential of various cellulose solvents[10, 12, 67, 68]. The use of three solvents has been investigated.

Cadoxen is a solution of 5% cadmiun oxide in 28% aqueous ethylene diamine and can readily dissolve micro-crystalline cellulose up to concentrations of 3%. Regeneration is achieved by dilution with water or methanol. Enzymatic hydrolyses were performed on both solvent pretreated bagasse and corn stover[10, 68]. Almost quantitative glucose yields were obtained and it was noted that the rate, but not the extent of hydrolysis, was affected by enzyme or solvent concentration.

CMCS is an aqueous solution of 17% sodium tartrate, 6,6% ferric chloride, 7,8% caustic and 6,2% sodium sulphite which can dissolve up to 4% micro-crystalline Avicel cellulose at room temperature. Regneration is also achieved by dilution with methanol or water. Almost quantitative yields of glucose were obtained from CMCS pretreated corn stover after 100 hours of enzymatic hydrolysis using 16,7 I.U./g of cellulose as the enzyme : cellulose ratio[10, 68].

Concentrated sulphuric acid is a powerful swelling and solvation agent for cellulose. A 70% solution will dissolve cellulose which can be regenerated in a disorganised amorphous form by dilution with methanol. A glucose yield of above 90% has been achieved by enzymatic hydrolysis of corn stover in one hour[68].

Tsao has patented this process for the recovery and utilisation of the cellulose fraction using sulphuric acid[69]. The lignocellulosic material is hydrolysed by dilute sulphuric acid to remove the hemicellulose

fraction and is then contacted with 75% H_2SO_4 for one hour at room temperature to dissolve the cellulose (97% recovery of cellulose in the amorphous form). High glucose conversions (±90%) are claimed using the amorphous cellulose in dilute acid and enzymatic hydrolyses.

All these solvent pretreatments do, however, require an effective, efficient washing procedure to remove the solvent from the regenerated cellulose as these expensive solvents are used in large amounts and solvent losses must be avoided.

2.4.3.4. Steam Treatment and Auto-hydrolysis

Acids, especially acetic acid, are liberated from lignocelluloses by steaming at elevated temperatures [70]. In the acid medium xylans and other hemicelluloses are hydrolyzed and become soluble. The degree of solubilisation depends on the raw material as well as the temperature and duration of steaming.

Dietrichs et al [71] investigated the potential of steaming hardwoods and straw at 180-200°C for feed and food production. On average 20% carbohydrates, mainly xylan, were extracted and the accessibility of the washed material was increased, although the relative lignin content was about the same as in the raw materials. Enzymatic hydrolysis by a commercial cellulase preparation gave up to 68% hydrolysis of hardwoods and about 70% conversion of straw.

Puls et al [72, 73, 74] have also treated lignocelluloses of lower lignin content like hardwoods and agricultural residues with saturated steam at 170° to 210°C for some minutes followed by steam explosion or hot stage defibration. Under these conditions 10-25% hemicelluloses (based on raw material) were extracted with water as xylan and xylan fragments which had to be further hydrolysed to pentose sugars. The cellulose fraction became accessible for ruminant and enzymatic degradation

(70-80% and 50-60% digestion respectively) despite the presence of lignin and the lignin could be extracted with alkali or organic solvents. A pilot plant processing 100 to 400 kg of lignocellulose per hour has been established in Munich in 1978 using this steaming process.

Extensive studies towards upgrading the nutritive value of lignocellulose residues by steaming have been undertaken[31]. Steam treatment of aspen chips for about 2 hours at 160-170°C yielded a product readily acceptable by sheep up to 60% of the total ration. Steam treatment also showed a strong species response, hardwoods exhibited a much greater improvement in digestibility than softwoods.

Nesse *et al*[75] treated the fibre fraction of cattle manure in an autoclave for 5 - 30 minutes at temperatures ranging from 130-200°C. The reactivity of the cellulose measured by incubating samples with a commercial cellulase preparation was increased by a factor 4 - 6 compared to 1% NaOH treatment and 10-12 compared to untreated fibre which had a digestibility of 4,1%. The increased susceptibility was probably due to an increase in cellulose availability to enzymatic attack, as structural hemicelluloses are removed during the treatment.

Spano *et al*[46] found that steaming was an effective pretreatment for agricultural residues and hardwoods but not softwoods. Bagasse showed a six fold increase in susceptibility to *T. reesii* cellulase (19 IU/g substrate and 5% slurry) after steam treatment at 200°C for 15 minutes followed by water washing.

Samples of Douglas fir and redwood obtained from the masonite high pressure steam treatment process were evaluated as substrates for enzymatic hydrolysis by Wilke[64]. Comparison of results obtained on the treated and untreated materials indicated that little benefit to subsequent enzymatic hydrolysis resulted

from high pressure steam. The fibre produced from the masonite process is heavily case hardened with lignin and thus is unsuitable for enzymatic hydrolysis. This case hardening results from refining at temperatures above the lignin transition temperature (which lies in the range 120-135°C) and then quenching to reduce the temperature[51].

The delignification of hardwoods by autohydrolysis and extraction has been studied by Lora and Wayman[76]. For aspen and eucalyptus at temperatures of 175-220°C lignin solubility is induced in 9 : 1 dioxane : water or in 1% sodium hydroxide solution. There is an optimum time at each autohydrolysis temperature for maximum lignin solubility - longer reaction times render the lignin insoluble due to lignin recondensation. This phenomenon during autohydrolysis can, however, be prevented to some extent by the presence of 2-napthol or other aromatics which act as blocking agents and prevent lignin repolymerisation[77].

Wayman et al[78] claim that an autohydrolysis - extraction treatment of hardwoods produces an extremely reactive pulp for enzymatic hydrolysis. The wood was treated in a continuous reactor at 195°C for 30 minutes followed by explosive decompression to atmospheric pressure. The lignin in the residue was then extracted with caustic soda under mild conditions.

A continuous high pressure steaming process for the autohydrolysis of lignocellulosic materials has been developed by Stake Technology[79, 80, 81]. This system, which incorporates a plug flow reactor, converted crop residues into high energy ruminant animal feeds (in vitro digestibilities increased to greater than 60%). The process is also claimed to break down the lignin - cellulose complex rendering the cellulose fraction susceptible to enzymatic hydrolysis and saccharification yields greater than 90% have been reported using Trichoderma reesei cellulase. The hemicellulose and lignin fractions could be readily extracted with water

and alkali or organic solvents respectively (greater than 85% of hemicellulose fraction water soluble and greater than 90% of lignin alkali soluble).

Iotech Corporation has also developed an autohydrolysis process[82] which uses a high pressure gun from which the lignocelluloses, after brief steam treatment (245°C, 30-90 seconds), are exploded. This is a batch process but has a very rapid cycle time of approximately two minutes. The hemicelluloses can be extracted by a water wash and the lignin is largely soluble in ethanol. The cellulose is also susceptible to enzymatic hydrolysis[83,84].

Water at elevated temperatures has been shown to cause depolymerisation of lignin and to induce its water solubility[85, 86]. These aromatic lignin degradation products have been analysed and the following compounds detected : vanillin, coniferyl aldehyde, syringaldehyde, sinapaldehyde, p-hydroxybenzoic acid, vanillic acid and syringic acid from aspen hydrolysis liquors[87] and coniferyl aldehyde, vanillin, vanilloyl methyl ketone, p-coumaraldehyde and quaiacyl acetone from hemlock aqueous hydrolysis liquor [88].

Since depolymerisation of lignin occurs during aqueous hydrolysis of lignocellulosic materials, it could thus be expected that this would also occur to some extent during dilute acid hydrolysis. This should result in a mild delignification and depolymerisation of the lignin matrix thus making the bagasse more susceptible to further delignification.

2.4.3.5. Ozone Treatment

Pelloni[89] has described the use of ozone for the pre-treatment of lignocellulose. He subjected an aqueous suspension of 1 mm milled straw (50 g/ℓ) to an ozone stream (40 mg/ℓ) for twelve hours at a rate of 260 ℓ/h. It was claimed that with the solution of ozone into the aqueous phase, first lignin and then hemicellulose were solubilised. 60% of the lignin was removed and an

enzymatic hydrolysis of the residue yielded 240g reducing sugar per kg of straw. Similarly, a culture of Trichoderma viride grown on ozone treated straw at 28°C utilised 85% of the cellulose over four days (15% of cellulose utilised using untreated straw).

The effect of ozone treatment on acid treated sugarcane bagasse has been investigated by De Wilde and Lussi[90]. A prehydrolysed bagasse suspension (50 g/ℓ) was treated with an ozone stream (5 mg/ℓ) for six hours at a flow rate of 420 ℓ/h. The bagasse was also treated with ozone in the presence of ultraviolet radiation. In both experiments no attack of any of the bagasse constituents was observed and enzymatic hydrolysis of the ozone treated material did not show any improvement over untreated material.

From these poor results for bagasse it was not considered worthwhile to undertake further work on ozone treatment of bagasse.

2.5 Pulp and Paper Industry

In the pulp and paper industry the major objective in chemically treating wood is to produce a long fibered paper product of high strength and varying degrees of whiteness, depending on end use. To accomplish these aims chemical processes such as bleaching and delignification are used.

In the pretreatment (delignification) of bagasse for enzymatic hydrolysis, the major objectives are delignification and the prevention of cellulose degradation with little regard for fibre length or strength. Pulping processes achieve delignification and have reached an advanced state of technology. It is for this reason that they are reviewed. Excellent reviews of the numerous bleaching and delignification processes are provided by Rydholm[15] and an F.A.O. publication[91]. A brief summary follows :

2.5.1 Soda Pulping

Wood chips are cooked in pressurised vessels containing aqueous solutions of 17 to 25 precent sodium hydroxide on a dry wood basis for periods ranging from 2 to 6 hours and at temperatures of 165° to 175°C. Typical pulp yields are from 43 to 48% of original weight. A significant portion of the cellulose and hemicelluloses are solubilised and lignin is removed. The waste liquor contains cooking chemicals, fermentable sugars and lignin fragments.

Saad et al[92] have studied the manufacture of unbleached high yield pulp from depithed bagasse using the following conditions: 4-8% NaOH, temperature 70° and 90°C, solid : liquid ratio = 1 : 7 and a cooking time of 1 - 3 hours. The alkali consumption was due to the neutralisation of organic acids and hydrolysis of acetyl linkages between the hemicellulose and lignin during pulping. Only a small amount of alkali was required to dissolve the lignin but a minimum residual alkali concentration was required to keep the dissolved lignin in solution(pH >9). The delignification rates using 8% NaOH at 70° and 90°C were also determined and the delignification reaction using NaOH was found to be first order.

An excellent summary of the reactions of polysaccharides during alkaline pulping has been presented by Nunn[93]. Three principle reactions occur in the alkali medium :

(i) Hydrolysis of acetyl groups in the acetylated hemicelluloses.
(ii) Removal of methyl and glucuronosyl groups from arabino- and methylglucuronoxylans.
(iii) Degradation of the main polysaccharide chain.

The degradation of the polysaccharides depends on the presence of a reducing group at the end of the polysaccharide chain and is commonly referred to as the peeling reaction, because the terminal aldose units are removed in a stepwise manner until a stopping reaction occurs which stabilises the polysaccharides.

This degradation of the cellulose chain during alkaline pulping has been inhibited by various additives. Ghosh[94] worked with anthraquinone additives in the soda pulping of hardwood and showed that there was an improvement in delignification and carbohydrate stabilisation. Saad et al[95] also found that nitrogen containing additives were effective in inhibiting the peeling reaction during soda pulping of bagasse pith.

Optimum conditions for high temperature soda pulping of bagasse are claimed to be as follows[96]:

Temperature	170°C
Time at 170°C	10-30 min
Chemical	10-16% as Na_2O
Liquid/Solid ratio	4 to 5:1

2.5.2 Kraft Pulping

In the kraft or sulphate process the same conditions as the soda process are utilised. The only difference is that a mixture of sodium hydroxide and sodium sulphide is charged to the reaction vessel. As a result the polysaccharide fraction is stabilised and a greater yield with the same amount of delignification is obtained. The odour resulting from this process is a major disadvantage.

Optimum conditions for bagasse using the kraft process are claimed to be[96]:

Temperature	170° - 175°C
Time at temperature	5 - 20 minutes
Chemical	12 to 14% as Na_2O
Liquid/Solid Ratio	3 to 4:1

2.5.3 Neutral Sulphite Pulping

In this delignification process, wood chips are cooked at approximately 150°C in pressurised vessels containing water, sodium sulphite and sodium or ammonia as bases. The pulp yield is higher than the soda or kraft process since the

hemicellulose and lignin are not as easily attacked and removed in this process - bases act as buffers to maintain neutral pH.

Optimum conditions for neutral sulphite pulping of bagasse are[97]:

Temperature	170 °C
Time	75 minutes
Chemical	12% Na_2SO_3 + 3% Na_2CO_2
Liquid/solid ratio	4 to 7:1

2.5.4. Semi Chemical Pulping

Semi chemical pulping involves a two stage production process[98, 99]. The first is a mild chemical action which loosens and partly removes the lignin; the second step completes the separation of fibres by mechanical means. The first stage commonly involves the use of the neutral sulphite process. Modified kraft or soda processes are also used. The second stage involves the use of mechanical equipment such as refiners and hydropulpers.

With all the semi-chemical processes, high pulp yields (60-70%) can be expected as less hemicellulose and cellulose is attacked than in chemical pulping processes. On the other hand, less lignin is removed.

2.5.5. Bleaching

Bleaching can be achieved in two ways - lignin bleaching or lignin removal. In lignin bleaching, chromophores of lignin and other impurities are destroyed without sub-substance removal. Multistage processing, including an alkali extraction stage, is used to oxidise and remove lignin with minor cellulose loss. Hence this type of bleaching represents a continuation of the cooking process using much more selective and at the same time expensive chemicals.

Among the most commonly used bleaching agents, are elemental chlorine, oxygen, chlorine dioxide, hypochlorites and chlorites.

2.5.6 Miscellaneous Pulping Processes

2.5.6.1 Nitric Acid Pulping

Aqueous solutions of nitric acid (2-8%) have been found effective in delignifying agricultural residues such as rice straw and bagasse, but have produced low yields (37-44%), due to partial removal of cellulose and hemicellulose resulting from hydrolysis[32]. The process involves oxidation and nitration of the lignin fragments which are removed by an alkaline extraction step.

Optimum conditions for nitric acid pulping appear to be[13]:

(i) three hours steeping in 5% HNO_3 solution at room temperature with a liquid/solid ratio of 5 : 1
(ii) the moist bagasse is heated to 80°C for approximately one hour after drainage
(iii) boiling of the heated stock for 30 to 45 minutes in a 2% NaOH solution.

Through the use of ethyl alcohol in various proportions with aqueous nitric acid, it is possible to increase pulp yield to 40-50% due mainly to the inhibitory action of the alcohol on carbohydrate hydrolysis by nitric acid[100].

Favourable aspects of alcoholic nitric acid pulping of bagasse include :

(a) low pulping temperatures (80°C)
(b) atmospheric pressure
(c) short pulping time (2-4 hours)
and (d) low acid concentration.

2.5.6.2 Hydrotropic Pulping

Aqueous solutions of organic solvents such as sodium xylene sulphonate or other aryl sulphonates are used to dissolve lignin in hydrotropic pulping[101, 102]. The rate of dissolution is governed by pH since the hydrolysis rate of the lignin to cellulose bonds increases with decreasing pH. However, the pH must not fall below 5 since at lower pH levels acid splitting of cellulose and pentosan chains occurs. The dissolved lignin precipitates out on dilution of the reaction mixture with water. Thus hydrotropic pulps must be washed with a dilute sodium hydroxide solution to prevent lignin precipitation.

Procter[103] has reviewed hydrotropic pulping and concluded that the process can only be used effectively to pulp hardwoods and non-woody plant material. However, the technical problems of pulp washing, to prevent lignin reprecipitation, and liquor reconstitution and recovery have not been solved on a continuous operating scale. No commercial ventures had been operated to date and he concluded that the process offered no significant advantages over established pulping processes.

2.5.6.3 Soda- Nitrobenzene Process

Nitrobenzene is an excellent lignin oxidant. The addition of 10% nitrobenzene based on bagasse[104] or rice straw[105] to the soda liquor resulted in an accelerated rate of delignification at a given temperature and increased pulp yield. The increased carbohydrate yield is due to the stabilisation of the carbohydrate fraction against the stepwise removal or "peeling off" reaction by oxidation of aldehyde end groups to a more stable aldonic structure[105].

2.5.6.4 Soda- Oxygen Pulping

The soda-oxygen pulping of bagasse has been investigated[106, 107]. The loose structure of bagasse makes it possible to accomplish oxygen pulping in one step without fiberisation in contrast to wood which requires initial defiberising due to the difficulty of oxygen penetration. Soda-oxygen pulping gives higher yields due to stabilisation of the carbohydrate fraction and lower lignin contents than soda pulping.

2.5.6.5 Bisulphite Process

In bisulphite pulping the cooking liquor is prepared by adding sulphur dioxide to a sodium sulphite solution of known concentration until a pH of between 3,5 and 7 is achieved[97].

Bisulphite bagasse pulps are inferior to neutral sulphite pulps. They have higher lignin contents and require a longer cooking cycle of approximately four hours.

2.5.6.6 Celdecor Process

The Celdecor process is a continuous pulping process using caustic soda to produce a semipulp followed by wet gaseous chlorine treatment to produce a fine pulp[98,108]. This process has the advantage that the only non-fibrous raw material is salt which is electrolysed to produce NaOH and chlorine gas in roughly the required proportions.

The bagasse is treated in a mild soda cook (8% NaOH at 132^{o}C) and then chlorinated at either low or high consistency. The chlorinated lignin with low water solubility is then extracted in a sodium hydroxide extraction stage (3,5% NaOH at 60^{o}C) to produce a low lignin content pulp.

2.5.6.7 Organosolv Pulping

Aronovsky and Gortner[109] investigated the pulping action of aqueous solutions of aliphatic monohydroxy and polyhydroxy alcohols and found that n-butyl alcohol, n-amyl alcohol, isoamyl alcohol and ethylene glycol will delignify wood chips. Using aspen, a cooking time of 1,5 to 2 hours at 188°C and a 1 : 1 solution of butyl alcohol in water, yields of 48-55% with lignin contents of 6-10% were obtained.

The Organosolv pulping process for pulping subdivided plant material using water and water miscible, volatile organic solvents such as lower aliphatic alcohols and ketones has been patented by Kleinert[110]. Ethanol in the range 25-75% weight is the most suitable delignifying agent using temperatures from 150° - 200°C and times from one or more hours at the lowest temperature to a few minutes at the highest temperature. There is no consumption of alcohol during pulping and it can be recovered by distillation.

The steps involved in delignification using alcohols are believed to be alcoholysis causing partial depolymerisation followed by dissolution. If the solvent power is poor, only the lower molecular weight lignin fragments will dissolve, the remainder undergo condensation reactions and repolymerise[32].

April et al[111] have investigated the delignification of pine with aqueous-organic solvents. The effects of butanol- and phenol-water mixtures (1 : 1) were investigated at 175° and 205°C and treatment times of 30 minutes to 12 hours. The wood could be separated into three components - hemicellulose soluble in the aqueous phase, lignin soluble in the organic phase and cellulose remaining in the residue. The phenol-water mixture was more effective than the butanol-water and reduced the

lignin content from 30% to 3%, giving a 36% yield based on dry pine wood.

An excellent review of the various organosolv pulping processes which operate with or without acidic or basic catalysts has been presented by Sarkanen[112]. He noted that these organosolv pulps were exceptionally reactive in enzymatic conversion to glucose. Five pulping systems were investigated for bagasse to determine the lignin selectivity of these processes. For a given lignin content in the pulp the processes had the following comparative yields (in descending order) - ammonium sulphide in $EtOH/H_2O$, ammonium sulphide in water, soda-anthroquinone process, soda process and aluminium sulphate (acid catalyst) in $EtOH/H_2O$. He concluded that the bagasse was pulped with unsatisfactory selectivity in an acid catalysed organosolv system. The ammonium sulphide processes are also unsuitable due to air pollution problems (smell).

However, the organosolv pulping process is likely to prove too costly for the recovery of bagasse components although it might become competitive for pulp and paper. Katzen et al[113] have estimated an air dry bleached pulp price of $242/ton from wood at $46,4/ton for a 47 600 ton/year plant. This cost of pulp is reasonable but such a cost is clearly too high to be considered in the enzymatic conversion of cellulose to glucose.

2.6 Effect of Lignin Content on Digestibility

With lignin being the major roadblock to widespread enzymatic utilisation of the carbohydrate content of the abundant lignocellulose residues, delignification would appear to provide a straightforward solution to the problem. That it can be was indicated by the more than 1,5 million tons of sulphate and sulphite wood pulps from pine, spruce and fir consumed by ruminants during World War II in the Scandinavian countries[31].

Andren et al[43] have tested commercial sulphate and kraft process pulps for susceptibility to Trichoderma viride cellulase hydrolysis. Both were considered excellent substrates with the sulphite pulp completely saccharified in 48 hours. Beremberg rayon (wet) was also evaluated and found to be excellent with a 72% saccharification at 48 hours.

Shimizu[30] has also tested various grades of commercial paper and fibre residues from pulp mills for enzymatic susceptibility to T. viride cellulase. Their hydrolysis extents ranged from 6 to 88%. The chemical pulps (low lignin content) showed excellent susceptibility. The groundwood and thermomechanical pulps, however, gave low sugar yields (high lignin contents). Susceptibility thus strongly correlated with lignin content.

However, the use of expensive, high quality wood pulps as substrates for enzymatic hydrolysis is not economically feasible. Thus inexpensive delignification procedures must be developed that do not necessarily preserve fibre length and strength and the degree of delignification required for a reasonable level of carbohydrate utilisation must be determined.

Olson et al[36] found that the nonfermentability of wood by thermophilic organisms was associated with the lignin content. In order to obtain a good fermentation (85% destruction) the lignin content had to be less than one percent.

Sudo et al[114] have investigated the degree of delignification required to increase the susceptibility of various woods to T. viride cellulase. Hardwoods and softwoods, delignified by acid

chlorite treatment, showed different trends. The hardwoods only showed increases in susceptibility after 60% delignification and cellulose conversion reached 70% on complete delignification. Softwoods showed an increase in susceptibility after 50% delignification and cellulose conversion reached 90% on complete delignification. This difference was attributed to pore size differences between the woods. Kraft pulping of woods, however, gave a rapid increase in digestibility with lignin removal due to hemicellulose removal which increased the median pore size compared to acid chlorite treatment.

The lignin contents of various pretreated lignocellulosic materials appear to relate to the digestibility of these materials - the higher the lignin content, the lower the digestibility. Millet et al[31] have reviewed the literature concerning the relationship between lignin content and digestibility and concluded that it is primarily the degree of delignification that governs pulp digestibility, not the method of pulping.

Cowling and Kirk[26] also maintain that effective and economically viable procedures to remove or modify lignin must be developed to decrease the protective association between lignin and cellulose. However, not all the lignin must be removed or altered to significantly increase susceptibility to enzymes. Depending on the source of cellulose involved, only 20 to 65% of the lignin need be changed or removed.

Research performed in the Chemical Engineering Department at the University of Natal[115] confirms a linear relationship between lignin content and digestibility of cellulose by cellulosic enzymes. The various degrees of delignification were obtained by the alkali treatment of bagasse.

The use of a relatively simple delignification process, such as the soda process, should thus provide a solution to improve cellulose utilisation provided that processing costs and cellulose losses can be kept low.

2.7 Acid Hydrolysis Processes

2.7.1 Concentrated Acid

The Bergius process[116, 117] was developed in Germany between World Wars I and II. It required kiln dried wood to prevent acid dilution and used 40% HCl at atmospheric pressure in expensive acid-resistant equipment. The HCl was recovered by vacuum stripping. In spite of the high glucose yield (±90%) the operating costs were excessive and the plant is no longer operational.

The Russians[118] have developed a process in which dry cellulosics (5% moisture) are treated with HCl gas at 10 to 20 atmospheres to obtain high yields. The HCl diffuses into the substances forming concentrated HCl with the small moisture fraction. A powder containing oligosaccharides is obtained which is converted to monosaccharides by heating the powder in dilute acid solution.

Hamor[119] has patented a process for converting the hemicellulose and cellulose fractions of lignocelluloses into their respective sugars using concentrated formic acid as a catalyst (70-90%). The hemicellulose hydrolysis is achieved at 20°-30°C and the cellulose hydrolysis at 60°C. The formic acid is recovered by distillation and high glucose yields are claimed for this process.

Concentrated sulphuric acid processes such as the Hokkaido process which treats the cellulose fraction of wood with concentrated H_2SO_4[118, 120] are also in use. In this process the wood chips were prehydrolysed to remove the hemicellulose fraction before treating the remaining 'wood' with 80% sulphuric acid at room temperature at a liquid to solid ratio of approximately 1 : 1. Glucose yields of above 90% were obtained and the sulphuric acid was recovered by dialysis.

2.7.2 Dilute Acid

The American process[121] hydrolysed wood chips in tile-lined digesters using 0,5% H_2SO_4, a steam pressure of 9 atmospheres and a digestion time of 15 minutes. The sugars produced mainly from the hemicellulose were then extracted in a diffusion battery. Alcohol yields were only 83,3 litres/ton of dry wood and as a result of the decrease in price of blackstrap molasses the plant was forced to close down.

The Germans developed the Scholler process[117, 122, 123] between the two World Wars. Wood chips were placed in large cylindrical percolators and compressed by successive steam shocks. Hot dilute sulphuric acid solutions ranging from 0,4 to 1,2% at 170° to 180°C were passed through the wood chips. Dilute sugar solutions only suitable for fermentation were produced.

Extensive research into the dilute acid hydrolysis of wood was conducted by the Americans during the Second World War in an attempt to alleviate their ethanol shortage[124, 125]. The Madison Wood Sugar Process, based on the Scholler process, was then developed[122, 126]. Wood wastes were hydrolysed by allowing dilute acid to flow continuously through the wood charge. This was an improvement over the batch addition Scholler process yielding 244,15 litres/ton in 2,8 hours compared to 208,19 litres/ton in 13 to 20 hours.

Dunning and Lathrop[127] have developed a two stage continuous process for the acid hydrolysis of agricultrual residues. The first stage involves a dilute acid hydrolysis to remove the hemicellulose fraction in a countercurrent diffusion battery. Between 90 and 95% of the pentosans could be extracted from the corncobs in 80 to 120 minutes at 100°C depending on the sulphuric acid concentration. The cellulose fraction was then saccharified by concentrated sulphuric acid in a discontinuous screw digester.

Tsao[67, 68] at Purdue University has also developed a

process for the utilisation of hemicellulose and cellulose fractions of cellulosic wastes. The first stage is a dilute acid hydrolysis of the hemicellulose fraction (121°C for 15 minutes). This is followed by a solvent treatment in which the cellulose is dissolved in concentrated H_2SO_4 and then regenerated by dilution with methanol. This regenerated cellulose contains sufficient acid for a dilute acid hydrolysis to glucose at 125°C for 15 minutes or can be subjected to enzymatic saccharification.

Twin screw extrusion technology has recently been applied to the high temperature dilute acid hydrolysis of cellulose in various lginocelluloses[128]. The Werner and Pfleiderer Corporation has been operating a continuous pilot plant at New York University[129, 130]. Agricultural wastes are treated in a 53 mm diameter twin screw extruder at 230°C with 0,5% H_2SO_4 for ±20 seconds and produce a dark brown paste consisting of approximately 30% glucose. It is also claimed that this process is economically viable[131].

Tampella Engineering have proposed a continuous hydrolysis process for plant materials[132, 133] using a Tampella-type digester which is presently used for pulping of various raw materials. The process involves dilute acid hydrolysis followed by steam explosion which reduces the particle size. (The finest particles have the highest lignin content due to the fact that lignin-rich regions of plant materials are amorphous and most brittle). A one or two stage process is proposed. In the single stage process the hemicelluloses are converted into furfural and the cellulose is hydrolysed to glucose. The two stage process incorporates a prehydrolysis stage to remove the hemicelluloses followed by the main cellulose hydrolysis. In this hydrolysis cellulose recycle is practiced after steam explosion - the hydrolysate together with the fine lignin-rich particles is separated from the coarse cellulose fraction by a cyclone. It is claimed that 20% of the dry weight of straw can be recovered in the prehydrolysis step as monomeric sugars and 26% can be recovered as glucose in the main hydrolysis using this process.

A dilute acid hydrolysis process has been developed at the Georgia Institute of Technology for the conversion of cellulosic materials to glucose[134, 135]. In this process the lignocelluloses are pretreated by "steam explosion" followed by ethanol extraction to remove the lignin at low pressure and temperature. The cellulose is then converted in a Stake Technology[80] plug flow reactor, employing cellulose recycle with an 80% conversion as contrasted with limiting conversions of 55% in conventional dilute acid hydrolysis processes (e.g. Madison Process).

Church and Wooldridge[136] have developed a 1 ton/day isothermal plug flow pipe reactor fed by a high-solids twin ram pump for the continuous acid hydrolysis of unpretreated lignocellulosics and starchy biomass. Coarse oak sawdust, newspaper, straw and bagasse at 35% input solids were hydrolysed at 200 to 225°C. Glucose and furfural yields of 40-42% of theory were obtained from oak sawdust at 205°C, 4,5 minutes residence time and 1,5% H_2SO_4. Starchy material gave glucose yields up to 73% and xylose yields of 75-80% could be obtained by continuous prehydrolysis at 170°C, 1,5% H_2SO_4 and 2 minutes residence time.

A two stage process for the hydrolysis of cellulosic materials has been patented by Ant-Wuorinen[137]. A dilute SO_2 solution (less than 2% by weight) is used in both stages. Hemicellulose hydrolysis is performed at 155° to 165°C and cellulose hydrolysis at 185° to 205°C. Yields of 57% to 62% of reducing sugar are claimed for this process.

Springer and Libkie[138] have investigated the prehydrolysis of birch wood with sulphur dioxide to produce a relatively concentrated pentose solution. The key ideas are to conduct the prehydrolysis reaction at a very low liquor-to-wood ratio (vapour phase cooking) and to obtain a relatively concentrated solution by countercurrent extraction of the xylose, xylose oligomers and other solubilised material from the reacted chips. A two hour treatment at 120°C at a water/wood ratio of 0,57 to 1 using a 13% SO_2 solution extracted 88% of

the potential xylose and yielded a 21% sugar concentration in the wood chips which should produce a xylose solution with concentration above 10% by countercurrent washing.

Veeraraghavan et al[139] have investigated the acid hydrolysis of hemicellulose from a hardwood using several different acids including acetic, sulphurous, phosphoric and sulphuric acids. Sulphuric acid was found to be the most efficient catalyst. Batch hydrolysis tests were then carried out over the range 0-0,5% H_2SO_4 and 140° to 180°C at solid to liquid ratio of 1 : 10. There were three main products from the reaction ; xylose, xylose oligoners and glucose. (The glucose yield was attributed to the hydrolysis of amorphous cellulose). A maximum yield of 83% of the total xylose content of the feed was achieved in the batch hydrolyses. There are, however, two main problems associated with an industrial batch process ; separation of liquid from the solid residue and recovering the sugar in a concentrated form. The authors thus suggested that a 'percolation' reactor would be more suited to an industrial hemicellulose hydrolysis.

Xylose recovery from hemicellulose by selective acid hydrolysis has been investigated by Limbaugh et al[140]. A maximum xylose yield of 83% was achieved at 150°C and 0,2% H_2SO_4 at a solid to liquid ratio of 1 : 10 using oak chips. As the temperature was increased at constant acid concentration maximum xylose yields occurred at shorter reaction times and xylose decomposition was more rapid (decrease in maximum xylose yield with increasing temperature). They also found that there was significant xylose oligomer production from 140° to 180°C at a low acid concentration (0,05% H_2SO_4).

Pfeiffer[141] has recently patented a process for producing xylose from absorbent xylan containing raw materials such as agricultural residues. The residues are treated with acid by spraying or by acid vapour which results in xylan hydrolysis without complete saturation of the raw material by the hydrolysing liquid. The xylose is then removed by washing and a concentrated xylose solution can be produced.

A reactor system for the hydrolysis of pentosan containing materials has been patented by Savo and Nyman[142]. The hemicelluloses are continuously hydrolysed by dilute sulphuric acid flowing either co-current or counter-current to the lignocellulosic material. Hydrolysate recycle is used to increase the xylose concentration of the hydrolysate and the bagasse residue wash water is also recycled via the acid make-up. Pentose concentrations of 3,1 to 4,7% have been achieved with this reactor system.

Friese et al[143] have patented a method of producing xylose solutions low in acetic acid from the hemicellulose fraction of lignocelluloses. 1,1 to 1,2 moles NaOH/mole of acetic acid in the hemicellulose at 0,6 to 0,8% is added to the lignocellulose at 100°C to remove the acetic acid. The residue is then treated with dilute sulphuric acid to remove the hemicellulose. The xylose solution produced is low in acetic acid and thus requires less treatment to remove this acid. It is also claimed that xylose yields are increased by this process.

Other recent developments in the dilute acid hydrolysis field include the development of a plug flow reactor system for the hydrolysis of cellulosic wastes[144, 145] and the Silvichem process[146] for the recovery of hemicellulose from cellulosic materials without any damage to the cellulose fibre.

2.8 Reaction Kinetics in Dilute Acids

2.8.1 Kinetics of Pentosan Hydrolysis

Nee and Yee[147] have investigated the kinetics of pentosan hydrolysis in bagasse pith. Using dilute sulphuric acid at a concentration of less than 20 g/ℓ and at temperatures lower than 165°C, pith was hydrolysed to pentoses in a yield of 80-90% based on potential pentose in the pith. Hydrolysis of pentosans in pith appeared to be a first order reaction.

However, the semilogarithmic time plot for the hydrolysis of potential pentoses in the residue consisted of two straight lines of different slope. This may be explained by the assumption that bagasse pith contains two major pentosan fractions that are hydrolysed at different rates. Saeman's equation[148] for wood hydrolysis with H_2SO_4 was adapted to represent dependence of rate constant K on concentration C and temperature T of the two pentose fractions, i.e.

$$K_1 = 6,4 \times 10^5 \, C^{1,02} \exp(-6378/T)$$
$$K_2 = 10,7 \, C^{0,363} \exp(-2826/T)$$

The rapidly hydrolysable hemicellulose portion (K_1) thus has an activation energy of 12 675 cal/gmole and the more resistant hemicellulose portion (K_2) an activation energy of 5 615 cal/gmole.

This phenomenon of two hydrolysis rates has also been noted by Nagalyuk and Chalov[149] in the hydrolysis of hemicellulose of pine wood pulps using 12 g/ℓ HCl at temperatures of 120° and 130°C at a solid to liquid ratio of 1 : 0,84. However, at 140°C the hydrolysis reaction was found to proceed at a constant rate. All these hemicellulose hydrolyses proceeded in accordance with the mechanism of a first order reaction.

Grant et al[150] investigated the kinetics of straw hydrolysis at temperatures of 121° to 150°C, sulphuric acid concentrations of 5 to 30 g/ℓ and the periods of up to 40 minutes. They found that xylose, glucose and mannose were the predominant sugars released. Sugar yield data were fitted to a linear kinetic model which considered the hydrolysis of hemicellulose and the breakdown of the resultant monomeric sugars as first order reactions with reactions rates dependent on temperature and acid concentration. As the temperature and acid concentration were increased the rate of hydrolysis increased more than the rate of sugar breakdown for glucose and xylose so that their yields were highest at the more severe conditions

investigated. The reverse was true for mannose, its yield was highest at the milder hydrolysis conditions studied. However, the rate parameters were determined by nonlinear regression analysis and it is difficult to define confidence limits for the parameters themselves. How closely they approximate the true sugar release and decomposition rates could not be determined.

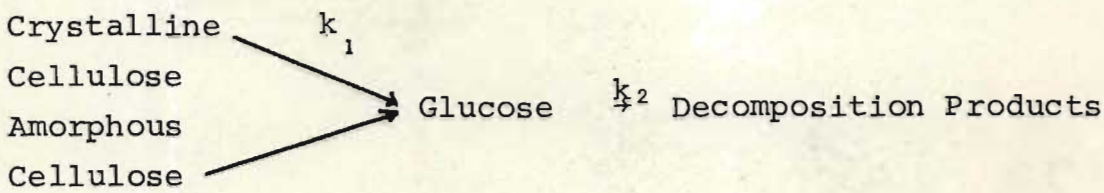
The hydrolysis of bagasse hemicelluloses under static conditions has been studied by Alemán et al[151]. Dilute sulphuric acid (5 g/ℓ) was used over a temperature interval of 100° to 160°C. The hemicellulose hydrolysis of bagasse was described by a kinetic model consisting of two simultaneous first order reactions with a common product, assuming that the hemicellulose consisted of two fractions of easily hydrolysable polysaccharides. The kinetic parameters for the description of the hydrolysis of easily hydrolysable polysaccharides were calculated as well as the relative content of each of the two fractions at 120° and 130°C. The dynamics of furfural and acetic acid formation during hydrolysis were also studied.

2.8.2 Kinetics of Cellulose Hydrolysis

Saeman[148] showed that the hydrolysis of crystalline wood cellulose is described by a $A \xrightarrow{k_1} B \xrightarrow{k_2} C$ reaction. Although this reaction is heterogeneous, it can be treated as a homogeneous reaction when the reactant cellulose is dispersed as fine particles of 20 mesh or less. The scope of his studies included temperature ranging from 170° to 193°C with a fourfold change in liquid to solid ratio and a tenfold change in acid concentration and he concluded that the hydrolysis of cellulose in dilute acid followed the laws of a first order reaction. The maximum yield of reducing sugar from cellulose also increased as the ratio k_1/k_2 increased (the ratio increases with increasing temperature and acid concentration). Thus increasing acid concentration and temperature resulted in an increased efficiency in conversion of cellulose to reducing sugar.

The kinetics of acid hydrolysis of cellulose in paper refuse have been investigated by Fagan et al[152]. A sequential peeudo-first-order model : cellulose → sugar → decomposed sugar developed by Saeman for the hydrolysis of wood chips was found to fit the data. The kinetic parameters were evaluated up to 240°C for acid concentrations of 2,5 and 10 g/ℓ. At 230°C with 10 g/ℓ sulphuric acid, 52% of the cellulose was converted to sugar in 20 seconds.

Thompson and Grethlein[144] modelled the hydrolysis of cellulose by the following :



The amorphous cellulose hydrolysed immediately to glucose and was taken as the initial glucose fraction. The kinetic parameters for hydrolysis of pure cellulose (Solka Floc) in a plug flow reactor were determined over the following range of independent variables : temperature from 180° to 240°C, sulphuric acid concentrations from 5 to 20 g/ℓ and slurry concentrations from 50 to 135 g/ℓ. Activation energies of 42 500 cal/gmole and 32 700 cal/gmole were obtained for the crystalline cellulose hydrolysis and glucose decomposition respectively.

Grethlein[118] points out that to minimise acid usage concentration must be kept low (5 to 20 g/ℓ). However, at these concentrations the hydrolysis of crystalline cellulose does not proceed at useful rates until a temperature greater than 180°C. He also claims that the reaction rate is not a function of particle size when the cellulose is present in chips of 50 mm or less.

In a paper comparing the economics of acid and enzymatic hydrolysis of newsprint, Grethlein[145] has developed a kinetic model for a cellulosic substrate consisting of

hemicelluloses and amorphous and crystalline cellulose. The hemicellulose and amorphous cellulose hydrolyse so rapidly above 180°C that they are converted to sugars immediately and are thus taken as initial xylose and glucose concentrations respectively. The expression derived from maximum glucose production indicates that glucose yields increase with increasing values of k_1/k_2 which increases with increasing temperature. Thus the highest glucose yield would be achieved by operating at the highest practical isothermal temperature. Activation energies of 45 100, 32 800 and 33 560 cal/gmole were obtained for cellulose hydrolysis, glucose decomposition and xylose decomposition respectively.

A 38 mm diameter isothermal plug flow pipe reactor fed by a high-solids twin-ram pump was used by Church and Wooldridge[136] to study the continuous acid hydrolysis of unpretreated lignocellulosic and starchy biomass. Coarse oak sawdust, newspaper, straw and bagasse at 35% input solids were hydrolysed at 200° to 225°C and the kinetic parameters for net glucose production were derived using the simple consecutive reaction model: cellulose $\xrightarrow{k_1}$ glucose $\xrightarrow{k_2}$ decomposition products. The kinetic parameters previously derived by Saeman[148] and Thompson and Grethlein[144] did not fit their data and a search was made for new values which would allow a fit to the kinetic model, without departing any further than necessary from the previously determined ranges. Activating energies of 42 900 cal/gmole and 30 000 cal/gmole were obtained for the crystalline cellulose hydrolysis and glucose decomposition respectively.

2.8.3 Kinetics of Xylose and Glucose Disappearance

Saeman[148] investigated the relative decomposition rates of sugars in aqueous sulphuric acid solutions. In all the sugars tested, including xylose and glucose, straight lines were produced when the logarithm of residual sugar concentration was plotted as a function of time. This is the criterion of a first-order reaction. In such a reaction, the rate is directly proportional to the concentration of the reacting substances. The activation energies obtained

for both xylose and glucose decomposition were about 32 000 cal/gmole.

Dunlop[153], working in the Quaker Oats Company laboratories in Chicago, also noted that the rate of pentose disappearance in an aqueous, acidic medium followed a first order rate expression. The rate of pentose disappearance was proportional to the pentose and hydrogen ion concentrations. Thus the reaction is at least second order, but it can be reduced to first order relation for any single set of conditions. The rate also approximately doubled for each 10°C increase in temperature.

Data for the rate of xylose disappearance was collected by Root et al[154] at an initial xylose concentration of 0,666 gram moles/litre over the temperature range 160° to 280°C with sulphuric acid concentrations from 0,00625 N to 0,8 N. Xylose disappearance was first order and the Arrhenius plot gave an activation energy of 33 560 calories/gmole. They also developed a general rate equation including the effects of liquid expansion, acid : water ratio and acid concentration (rate not exactly proportional to acid concentration).

2.8.4 Kinetics of Furfural Formation

If xylose and furfural disappearance were the two rate phenomena which determined the rate of furfural formation the reaction mechanism would be : Xylose $\overset{k_1}{\rightarrow}$ Furfural $\overset{k_2}{\rightarrow}$ Destruction Products.

Since k_2 is much smaller than k_1 over the temperature range 100° to 210°C and from 0 to 160 g/ℓ sulphuric acid[154] high furfural yields would be expected. However, furfural yields are much lower than those predicted by this simple two-step mechanism[70].

Root et al[154] have developed a correlation to fit their experimental data for furfural formation over the temperature range 160° - 210°C and sulphuric acid concentrations from 0,00625N to 0,8N. Furfural yields from xylose increased with increasing temperature and decreasing xylose concentrations. However, the acid concentration was found to have no effect on furfural yield when above 0,1N H_2SO_4.

Saad et al[155] also investigated the kinetics of formation of furfural from Egyptian bagasse using three boiling acid solutions viz. HCl, H_2SO_4 and H_3PO_4 at concentrations of 1,5N to 5,5N and times ranging from 30 minutes to 4 hours. For any given acid concentration hydrochloric acid had the highest reaction rate constant, followed by sulphuric acid, whilst phosphoric acid showed the lowest value.

2.8.5. Kinetics of Furfural Destruction

Williams and Dunlop[156] found that the destruction of furfural in dilute aqueous solutions of mineral acids (HCl and H_2SO_4) is a pseudo-unimolecular reaction, the rate of which is directly proportional to furfural concentration and to the hydrogen ion concentration. Their tests covered the ranges 150° to 210°C and 0,05 to 0,1 N H_2SO_4. An increase in temperature accelerated the reaction and the Arrhenius equation relating reaction velocity and temperature was derived giving an activation energy of 21 970 cal/gmole.

Root et al[154] also investigated the disappearance of furfural in dilute aqueous sulphuric acid solutions (0,00625N to 0,8N) over the wide temperature range of 160° to 280°C. They found that furfural decomposition was a first-order disappearance with an activation energy of 22 070 cal/gmole.

2.8.6. Overall Reaction and Mechanism

The overall reaction sequence for the pentosan fraction of bagasse in the presence of a dilute mineral acid can be

summarised[70, 157]:

Transformation of pentosans to pentoses (hydrolysis)

$$(C_5H_8O_4)_n + nH_2O \xrightarrow[H_2O]{\text{Temp H ion conc}} n\,C_5H_{10}O_5$$

Transformation of pentoses to furfural (dehydration)

$$(C_5H_{10}O_5)_n \xrightarrow[\text{H ion conc}]{\text{Temp Pressure}} n\,C_5H_4O_2 + 3nH_2O$$

Possible reaction for furfural destruction[95]

$$C_5H_4O_2 \xrightarrow[H_2O]{\text{H ion conc}} \begin{matrix} H_2C & - & CH_2 \\ | & & | \\ OHC & & CHO \end{matrix} + HCOOH$$

The reaction rates of the above reactions are temperature sensitive and the Arrhenius equation gives the relationship between rate k and temperature T, i.e. $k = k_o e^{-E/RT}$ where E is the activation energy and R the Universal Gas Constant. Thus the sensitivity of a reaction rate to temperature is primarily determined by the activation energy E of the reaction and when comparing two reactions with different activation energies that reaction with the lower activation energy will show the greater proportional increase in rate (k/k_o) with increasing temperature.

The accepted reaction mechanism for the reaction of pentosans in dilute mineral acids which accounts for the poor furfural yield is[70, 154]:

Pentosans (Polymeric) → Pentoses (Monomeric) → Intermediate Product → Furfural → Destruction Product

Furfural → Condensation Products

During the thermo-chemical treatment of bagasse acetic and formic acids are also formed[70, 157] from the acetyl and formic groups of the fibrous raw material which can catalyse the hydrolysis of pentosans and dehydration of the pentose sugars.

2.9 Proposed Process

The proposed process for the utilisation of bagasse for the production of C_5- and C_6- sugars can be subdivided into three sections each of which deals primarily with one of the constituents of the bagasse viz. hemicellulose, lignin and cellulose.

2.9.1 Dilute Acid Hydrolysis

Hydrolysis of the hemicellulose to mono- and oligo-saccharides is comparatively an easy process which can be accomplished with acids under moderate conditions. The first stage of the proposed process thus consists of a dilute mineral acid hydrolysis under moderate conditions which will yield pentose sugars, mainly xylose and a small amount of sugar decomposition products such as furfural.

2.9.2 Lignin Removal

The second stage of the process will probably involve the delignification of the bagasse residue from the dilute acid hydrolysis by the conventional soda pulping process. This process step is mainly a pretreatment stage for the enzymatic saccharification of the cellulose fraction in the residue although the lignin could be recovered from the soda pulping liquor if a market exists for these lignin derivatives.

2.9.3 Enzymatic Saccharification

This final stage will convert the cellulose rich , delignified bagasse residue to hexose sugars using Trichoderma reesei cellulase.

2.9.4 Advantages of the Process

The proposed process has the following advantages :

(a) All three main constituents of the bagasse can be utilised.

(b) The hemicellulose fraction is recovered as xylose rather than furfural and xylose can be used as raw material for a wider range of products through chemical and biochemical conversion than furfural.

(c) The sugar solutions from the hemicellulose and cellulose hydrolyses would be suitable for fermentation.

(d) The delignification process - soda pulping - is extensively used in the pulp and paper industry and thus utilises proven technology.

(e) Enzymatic hydrolysis of cellulose to glucose does not produce any of the byproducts and sugar decomposition products that would be produced by high temperature dilute acid hydrolysis - the enzymes are specific in their action.

(f) The enzymatic hydrolysis of cellulose can be carried out at atmospheric pressure and moderate temperatures.

2.10. Proposed Research

2.10.1 Batch Dilute Acid Hydrolysis

Batch dilute mineral acid hydrolysis of bagasse will be investigated with the object of optimising xylose yield from the hemicellulose fraction.

The effect of solid to liquid ratio on xylose production will first be determined. The solid to liquid ratio which gives optimum xylose yield for a given set of hydrolysis conditions (3 hours at 100°C and 20 g/ℓ H_2SO_4) will then be used for the entire batch acid hydrolysis test programme.

Acid hydrolysis temperatures from 80° to 160°C at 10°C intervals will be used. The choice of the lower temperature limit is based on the fact that after sugar extraction the bagasse residue leaves the diffusion process at between 80° and 90°C. The aim of the dilute acid hydrolysis is the removal of the hemicellulose fraction only and thus the acid hydrolysis temperature must be kept below 180°C, the

temperature at which hydrolysis of cellulose proceeds at a useful rate[118]. The acid hydrolysis temperature must also be mild to avoid the formation of furfural from xylose. Funk[146] maintains that the acid hydrolysis temperature should even be kept below 135°C to avoid xylose decomposition. Hence the selection of the upper temperature limit of 160°C.

The sulphuric acid concentration range that will be investigated is from 0 to 40 g/ℓ sulphuric acid. The higher acid concentrations will be most effective at the lower temperatures but at the upper end of the temperature range acid concentrations of between 0 and 20 g/ℓ should be sufficient for hemicellulose hydrolysis due to the effect of temperature on reaction rate.

All acid hydrolyses at atmospheric pressure, i.e. 80°, 90° and 100°C will be performed in two litre laboratory glassware reactors in an ethylene glycol/water bath. At higher temperatures, the hemicellulose hydrolysis will be conducted in a teflon lined, steam jacketed reaction vessel.

During each dilute sulphuric acid hydrolysis, sugar, acetic acid and furfural production and mineral acid consumption, will be monitored. A kinetic model for the dilute acid hydrolysis of the hemicellulose fraction will then be developed to obtain expressions for maximum xylose concentration and optimum residence time for a given set of reaction conditions.

2.10.2 Continuous Dilute Acid Hydrolysis

If encouraging xylose yields are obtained from the batch hemicellulose hydrolyses a continuous plug flow hydrolysis reactor will be designed and constructed to process significant quantities of bagasse at low liquid to solid ratios.

The reactor will then be commissioned and a limited amount of experiments performed to determine whether xylose yields similar to the batch hydrolysis yields could be obtained at the continuous hydrolysis conditions tested.

2.10.3 Secondary Investigations

Once the dilute acid hydrolysis tests have been completed two further investigations will be performed.

The effect of dilute acid hemicellulose hydrolysis conditions on the enzymatic susceptibility of prehydrolysed bagasse after attritor milling will be determined and this susceptibility will be compared with other pretreated bagasse substrates.

Secondly, the amount of calcium ions remaining in the hydrolysate produced by the continuous reactor after neutralisation at room temperature to various pH values with $CaCO_3$ and $Ca(OH)_2$ will be determined and the neutralisation conditions producing a minimum calcium ion concentration obtained. This is necessary as an excessive amount of calcium ions will interfere with any subsequent fermentation of the hydrolysate.

3. ANALYTICAL METHODS

3.1 Bagasse Composition

The following methods of analysis were used to determine the composition of raw bagasse and hydrolysis residues:

Lignin	Tappi T13m-54[158]
Pentosan	Tappi T19m-50[158]
Ash	Tappi T15m-58[158]
Hot water solubles	Tappi T1m-59[158]
Alcohol-benzene solubles	Tappi T6m-59[158]
Cellulose	Seifert method[159]

The values obtained for bagasse components often vary according to the actual analytical procedure. This can be attributed to the complex structure of lignocellulosic materials and the close association between the carbohydrates and lignin. Thus each analytical procedure must be adhered to rigidly in order to obtain consistent results.

This is particularly significant for the pentosan determination where there are various methods in the literature[160, 161, 162, 163]. These usually involve determining the amount of furfural produced from the pentosan fraction by acid hydrolysis and dehydration but produce wide differences in the pentosan value. The present pentosan determination is based on the gravimetric determination of the furfural produced and has been used since the inception of the bagasse project at the Department of Chemical Engineering.

The precision of the lignin, cellulose and pentosan analyses were determined using a hammermilled bagasse obtained from the Tongaat sugar mill. The percent relative deviation for lignin, cellulose and pentosan was 1,34%, 2,45% and 1,32% respectively (See Appendix A).

3.2 Quantitative Sugar Analyses

Acid hydrolysis of bagasse hemicellulose produces a mixture of sugars, the principle constituents being xylose, arabinose and glucose[16, 17].

In recent years high pressure liquid chromatography(HPLC) has been used for the rapid determination of sugars in solution[164, 165, 166, 167]. The column packing is an amino bonded silica and the extent of separation is dependent on the ratio of water to acetonitrile in the eluant. These columns do age as the amino groups are destroyed and the proportion of acetonitrile in the eluant needs to be increased to maintain a given separation. Eventually this is no longer possible and the column needs to be replaced.

This problem has recently been overcome[168] and it is now possible to get a constant separation with the same eluant for an indefinite period by the simple process of adding a small amount of amine (0,01% tetraethylene - pentamine) to the eluant. Old columns can also be restored by this method. (In this work several carbohydrate columns were used as the amine was only obtained after the completion of this acid hydrolysis investigation).

The sugars were separated on a 30 cm μ Bondapak carbohydrate column (Waters Associates) and the sugars detected by a Waters R401 differential refractometer. The carrier solvent used was approximately 80% acetonitrile and 20% water and was pumped at 2mℓ/min using a 1082A Hewlett Packard liquid chromatography unit. The amounts of sugars were determined by peak height measurements and a sample chromatogram is shown in Figure 3.1.

The precision of the xylose and glucose analyses were determined using 1% standard solutions and peak heights. The percent relative deviation for xylose and glucose was 0,84% and 0,69% respectively (See Appendix A).

3.3 Thin Layer Chromatography

Thin layer chromatography was used for the qualitative determination of the sugar composition of the hydrolysates. The method of De Stefanis and Ponte[169] was used to produce the chromatograms.

3.4 Acetic Acid Analyses

The amount of acetic acid in the hydrolysates was determined by gas chromatography using a Varian 2700 G.C. fitted with a 1,4 m by 1,6 mm I.D. stainless steel column packed with Chromosorb 101. The injector and detector temperatures were set at 250°C and the nitrogen carrier gas flowrate was 30mℓ/min. The column temperature was set at 135°C and an injection volume of 1 µℓ was used.

After each analysis the column temperature was raised to 220°C for five minutes to remove any other organics such as furfural which would interfere with the following analysis. A sample chromatograph from a typical acid hydrolysate is shown in Figure 3.2.

3.5 Furfural Analyses

The amount of furfural in the hydrolysates from the batch hydrolyses was determined by gas chromatography using the same conditions as those used for the acetic acid analyses (section 3.4) except that the column temperature was set at 160°C. A sample chromatogram is shown in Figure 3.3.

However, Uobe et al[170] recently have found that furfural can be produced by sugar degradation (from xylose, arabinose and glucose) during G.C. analyses of neutralised sugar solutions. Thus all the furfural analyses on the hydrolysates produced by continuous hydrolysis were performed according to the colourimetric method of Duncan[171] which relies on the reaction of furfural with aniline acetate and is specific to furfural.

3.6 Titrimetric Analyses

On completion of each batch hydrolysis, the hydrolysate was sampled and a 25 mℓ aliquot titrated against 0,5N NaOH using a Radiometer titrator TTT2 and an auto burette ABU13 connected to a Servograph pen drive.

A 25 mℓ aliquot of the original dilute sulphuric acid was also titrated against 0,5N NaOH using the same automatic titration system. These two titration curves were then compared by overlaying the hydrolysate curve on the original acid curve. By this method the amounts of sulphuric acid consumed and acidic organics produced were determined as shown in Figure 3.4.

The sulphuric acid concentrations of the hydrolysates produced by continuous hydrolysis were determined in a different manner. It was found that the high acetic acid concentration (due to the low liquid to solid ratio) made the overlay method unsatisfactory. The total acidity of the hydrolysate, expressed in g/ℓ H_2SO_4, was determined by neutralisation of a 25 mℓ aliquot with 0,5N NaOH to pH7. The sulphuric acid concentration of the solution was then determined by subtracting the sulphuric acid equivalent of the acetic acid present determined by G.C. from the value obtained for the total acidity of the hydrolysate.

3.7 Metal Cation Analysis

The metal cation analyses on the hydrolysate produced by continuous hydrolysis were performed by atomic absorption spectrophotometry using a Perkin-Elmer atomic absorption spectrophotometer (model No. 303) fitted with the appropriate Perkin-Elmer lamps.

3.8 Enzyme Assay

The enzyme assay on the cellulase solution used for enzymatic hydrolysis was carried out according to the filter paper activity (FPA) method of Mandels et al[172] and the results are expressed as international units (IU). One IU is the

amount of enzyme which will produce one micromole of reducing sugar as glucose per minute (under spare specified fixed conditions).

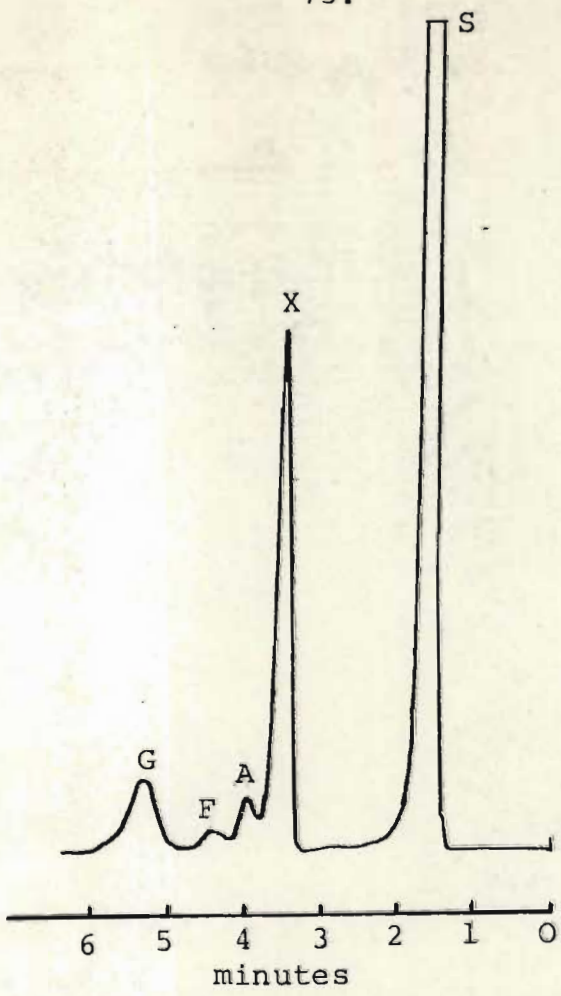


Figure 3.1. H.P.L.C. analysis of hydrolysate:
S = solvent, X = xylose,
A = arabinose, F = fructose and
G = glucose

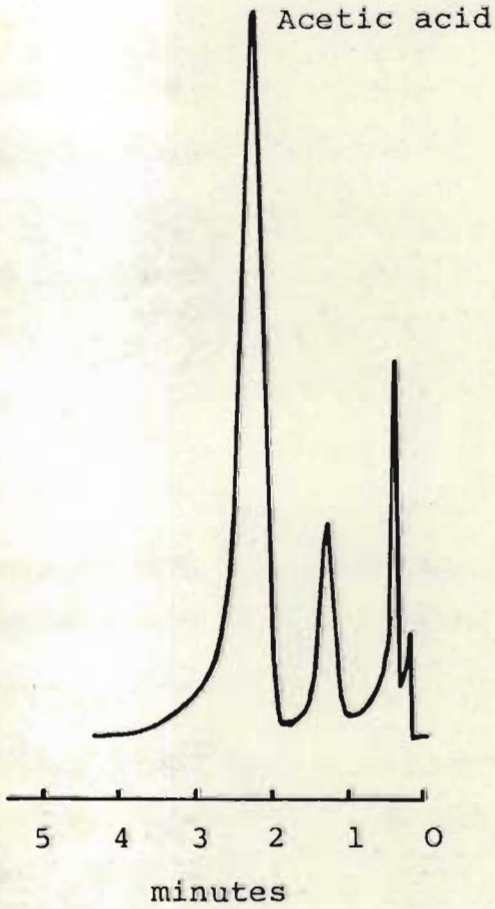


Figure 3.2. G.C. analysis of acetic acid in hydrolysate

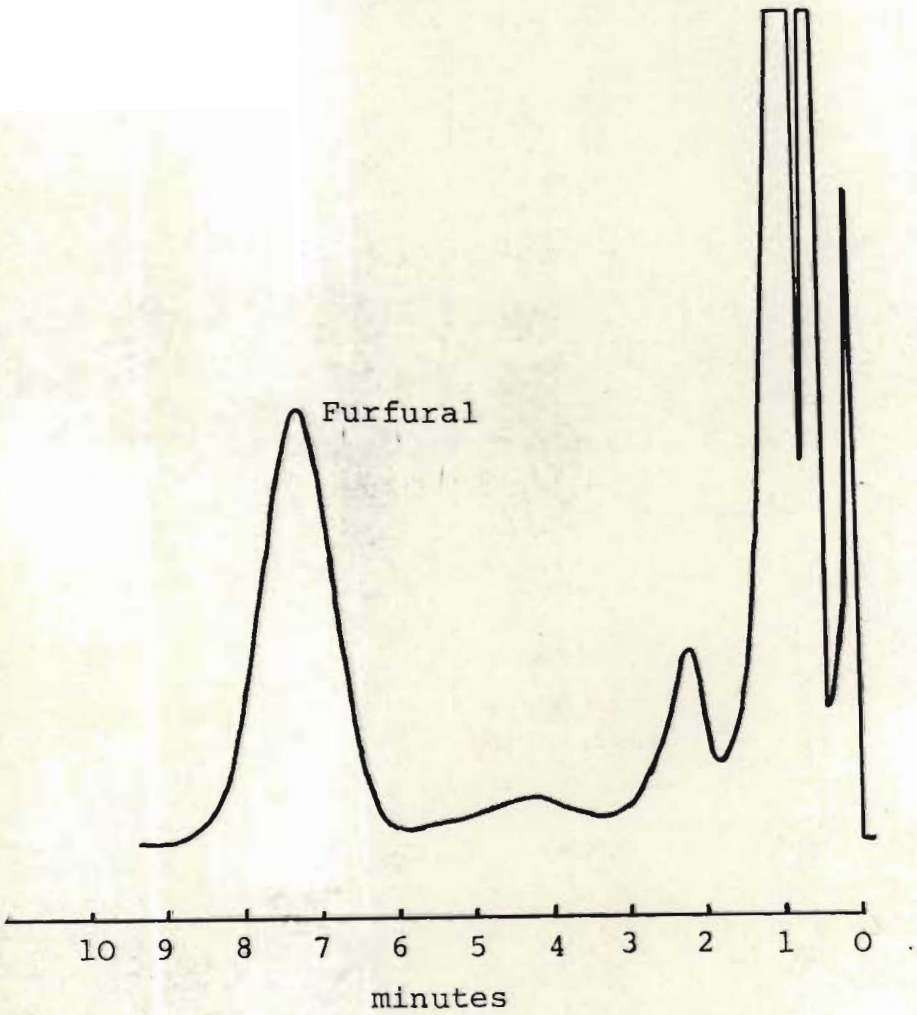


Figure 3.3. G.C. analysis of furfural in hydrolysate

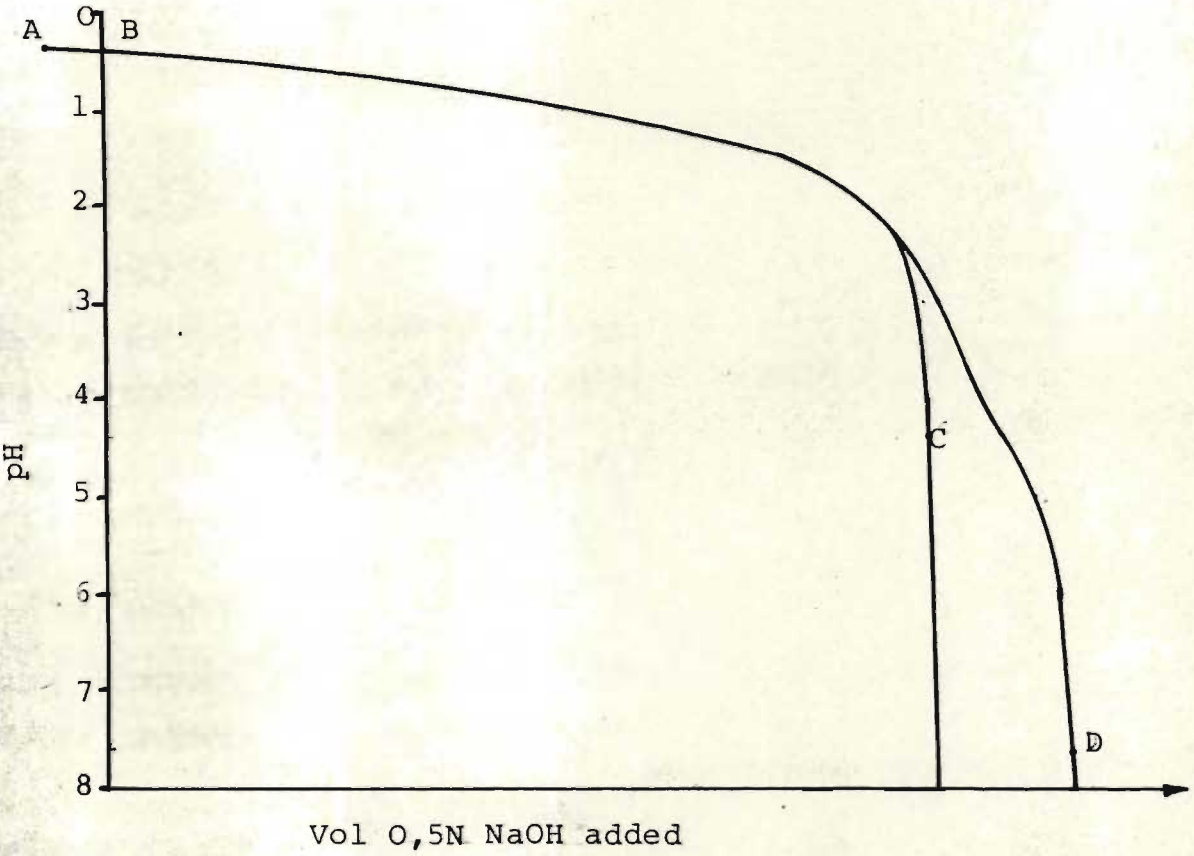


Figure 3.4. Titrimetric analysis of batch hydrolysate

Curve ABC: Titration curve for original dilute sulphuric acid before acid hydrolysis

Curve BD : Titration curve for acid hydrolysate

Curve AB : Sulphuric acid consumption

Curve AC : Initial sulphuric acid concentration

Curve BC : Final sulphuric acid concentration

Curve CD : Acidic organic formation during hydrolysis

4. REACTOR SYSTEMS AND EXPERIMENTAL PROCEDURE

This section covers the various dilute acid hydrolysis reactors developed to investigate bagasse hemicellulose hydrolysis - the batch atmospheric hydrolysis reactor, the batch pressure hydrolysis reactor and the continuous pressure hydrolysis reactor.

4.1 Batch Atmospheric Hydrolysis Reactor

4.1.1 Reactor System

Dilute acid hydrolyses at atmospheric pressure were performed in a batch reactor system consisting of two main parts - an acid preheat vessel and the hydrolysis reaction vessel.

The reactor system consisted mainly of Quickfit laboratory glassware. Both the reactor and preheat vessels were 2ℓ wide neck reaction flasks fitted with 100 mm multisocket flat flange lids secured by spring wire clips. Each vessel was also fitted with a water-cooled Graham coil condenser (Heat transfer area = $20 \times 10^{-2} m^2$). The system is illustrated in Figures 4.1 and 4.2.

The reactor system was immersed in a Labotec 30ℓ water bath fitted with a heater/temperature control unit and filled with an ethylene glycol/water mixture (60 : 40 by volume).

Agitation was provided in the hydrolysis reactor by a stainless steel two-paddle stirrer powered by a Heidolph variable speed drive (speed set at 80 rpm). This stirrer, which entered the reactor through a PTFE stuffing box gland, was spray coated with teflon before each hydrolysis to ensure that any possible corrosion products did not interfere with the hydrolysis.

A sample point was also provided in the hydrolysis vessel. This consisted of a blanked-off glass tube with small holes

in its circumference which allowed hydrolysate to flow up the tube to a 30 cm Liebig condenser where it could be cooled and collected.

4.1.2. Start-Up Procedure

1,5ℓ of acid solution were charged to the preheater and the waterbath set at the required reaction temperature. When the acid had reached the set temperature the hydrolysis reactor was charged with bagasse.

An air overpressure of 25kPa was then applied to the acid solution which was transferred to the hydrolysis vessel via a silicon rubber tube. The reaction time was taken from the instant that the acid was completely transferred to the reactor (Transfer time ±30 seconds).

4.1.3 Sampling Procedure

An acid hydrolysate sample was withdrawn from the reactor by applying a slight vacuum (15 kPa) using a Vac Torr PV35 vacuum pump. The sample passed through the Liebig condenser and was collected in a 100 mℓ Buchner flask. 15 mℓ of hydrolysate were used for analyses and the remainder immediately returned to the reaction vessel by pouring the excess hydrolysate down the condenser.

4.1.4 Shut-Down Procedure

On completion of the hydrolysis the bagasse residue was vacuum filtered using a Buchner funnel and a hydrolysate sample taken for titrimetric analysis. The residue was then washed with four litres of hot followed by four litres of cold water before air drying, weighing and analysis.

4.1.5 Sample Preparation

Each 15mℓ hydrolysate sample was divided into three fractions - 6mℓ was neutralised immediately with $BaCO_3$ and of the

remaining 9mℓ, 6mℓ was subjected to further hydrolysis and 3mℓ retained for the acetic acid analysis.

Further hydrolysis to convert all the oligosaccharides to their monomeric forms was performed at 100°C using 20 g/ℓ H_2SO_4 for one hour[151]. In this work those hydrolysates produced with 40g/ℓ H_2SO_4 were treated at 100°C for half an hour and all other hydrolysates were further hydrolysed using 20g/ℓ H_2SO_4 for one hour. Those hydrolysate samples which did not contain 20g/ℓ acid were processed in the following manner - 4 mℓ of hydrolysate was mixed with 0,5 mℓ of sulphuric acid solution of the required concentration to produce the desired 2% acid concentration (total sugar analysis results were multiplied by 1,125 to compensate for this dilution). After further hydrolysis the samples were also neutralised with $BaCO_3$.

Both sets of neutralised samples were then centrifuged at approximately 1 000 rpm and filtered through Whatman No. 1 filter paper. The hydrolysates were then stored overnight in the freezer, defrosted the following day and again filtered through Whatman No. 1 filter paper before analysis by HPLC for free and total sugars.

4.2 Batch Pressure Hydrolysis Reactor

4.2.1. Reactor System

Dilute acid hydrolyses at temperatures above 100°C were performed in a batch reactor system consisting of two main parts - an acid preheat vessel and a hydrolysis reactor.

Both vessels in the reactor system were constructed from 100 mm Schedule 40 316L stainless steel pipe with 16 mm flanges at each end. The design calculations and reactor drawings are available in Appendix B. Each 2 litre vessel was also teflon-lined and all metal valves and fittings in contact with the hydrolysate were machined from Hastelloy C to ensure that corrosion products which might interfere with the hydrolyses were minimised.

The preheat and hydrolysis vessels were steam jacketed. Temperature control (±1°C) was achieved by regulating the steam pressure and using a solenoid valve on the steam inlet connected to a Eurotherm temperature controller. The steam jacket was also used to cool the reactor during shut-down by closing the steam supply and allowing water to flow through the jacket.

Agitation was provided in the hydrolysis reactor by a teflon three-paddle stirrer powered by a variable speed D.C. drive (set at 80 rpm).

A sample point was also provided in the hydrolysis vessel. It consisted of a piece of thick-walled teflon tubing with small holes drilled in its circumference which allowed hydrolysate to flow up the tube to a 30 cm stainless steel water-cooled condenser where it was cooled and collected. (The system is illustrated in Figures 4.3 and 4.4.).

4.2.2. Start-Up Procedure

A known volume of acid (approximately 1,5ℓ) was charged to the preheater and steam heating commenced. When this dilute sulphuric acid had reached the required temperature the hydrolysis reactor was charged with bagasse and preheated for five minutes.

A nitrogen over-pressure of 2 bar above the vapour pressure of the dilute acid at the set temperature was then applied to the solution in the preheater and was transferred to the hydrolysis vessel via a teflon-lined hydraulic hose (transfer time ± 30 seconds). The reaction time was taken from the instant that the temperature reached 30°C below the set temperature. (Only about 15 seconds required to reach final temperature).

4.2.3. Sampling Procedure

Prior to sample withdrawal a nitrogen overpressure was applied to the sample point for 1 minute to force all the hydrolysate in the sample leg back into the reactor. The hydrolysate sample was then withdrawn from the reactor by opening the leg to atmospheric pressure. The sample passed through the condenser, the first 3 mℓ was discarded and 13 mℓ of hydrolysate was collected for analysis.

4.2.4. Shut-Down Procedure

The hydrolysis reactor was shut-down by closing the steam supply and allowing water to flow through the steam jacket (cooling rates of approximately 20°C/minute were achieved). The vessel was then depressurised, a hydrolysate sample taken for titrimetric analysis and the bagasse residue collected. This residue was vacuum filtered using a Buchner funnel and washed with 4 litres of hot followed by 4 litres of cold water before air drying, weighing and subsequent analysis.

4.2.5. Sample Preparation

Each hydrolysate sample was divided into three fractions - 5,5 mℓ was neutralised immediately with $BaCO_3$ and of the remaining 7,5 mℓ, 5,5 mℓ was subjected to further hydrolysis and 2,0 mℓ retained for the acetic acid analysis.

Further hydrolysis and sample preparation was performed according to the method given in section 4.1.5.

4.3 Continuous Pressure Hydrolysis Reactor

4.3.1. Reactor Design

4.3.1.1. Design Criteria

The following design criteria were adopted for the design of the hydrolysis reactor :

1. continuous operation
2. plug flow reactor
3. operation up to 1000 kPa steam (185°C)
4. materials of construction suitable for acid or alkali pulping
5. high degree of automation
6. operation by single person

The continuous hydrolysis reactor design was based on the plug flow digesters used for the alkali pulping of bagasse in the pulp and paper industry. (See Appendix C for design calculations).

4.3.1.2. Materials of Construction

904L stainless steel was used for the construction of the main components of the reactor. This stainless steel (similar to Carpenter 20Cb3 alloy) had the following composition :

C 0,02% max, Cr 19,0-21,0%, Ni 24,0-26,0%, Mo 4,0-5,0% Cu 1,0-2,0% and Fe balance.

All other metal components were constructed from 316L stainless steel, except the steam jacket which was made of 304 stainless steel.

4.3.1.3. Reactor Specifications

A complete collection of reactor drawings is presented in Appendix C. The hydrolysis reactor is shown in Figures 4.5, 4.6, 4.7 and 4.8.

4.3.2. Reactor System

4.3.2.1. Bagasse Feed System

Bagasse is fed into the reactor by a pneumatically operated piston. This is synchronised with a pneumatic 316L stainless steel slide valve by the feed control system (see Appendix C) which initiates bagasse feed

This batch feed system was preferred to a continuous screw feeder which is used in industrial digesters. These conical screw feeders compress the bagasse to a high density plug which acts as a moving seal against the reactor pressure. The batch feed system is relatively simple to construct and operate on this laboratory scale when compared to a screw feeder.

4.3.2.2. Plug Flow Reactor

The bagasse flows from the feed system into a T-pipe where it drops into the plug flow screw reactor.

This consists of a 1,6 m steam jacketed 100 mm schedule 40 pipe with a 75 mm inlet and outlet for the bagasse. The bagasse is transported by a screw (45 mm pitch) driven by a speed controlled 0,75 kW D.C. drive (17 rpm max. speed) through a 5 : 1 reduction box and connected by a shear coupling to the screw shaft.

4.3.2.3. Bagasse Discharge System

The hydrolysed bagasse is discharged through two 316L stainless steel slide valves in series. These are connected to a pneumatic control system (see Appendix C) which ensures that discharge is achieved safely and without depressurisation of the reactor.

The bagasse is then discharged into an expansion vessel where it flashes to atmospheric pressure and is collected in a large bucket.

4.3.3. Reactor Operation

The complete operating procedure for the continuous acid hydrolysis reactor is available in Appendix D.

The dilute acid hydrolyses performed can be divided into two groups; those performed with acid injection and continuous steam addition and the other group performed using acid impregnated bagasse and controlled steam injection.

During the first group of hydrolysis tests the following were monitored to obtain a mass balance :

i) volume and concentration of acid added.
ii) mass and moisture content (± 50%) of bagasse added.
iii) mass of discharged residue and hydrolysate.
iv) dry mass of residue washed from the reactor after completion of the hydrolysis.
v) mass and moisture content of the air dried washed residue.

In an attempt to decrease the liquid to solid ratio bagasse was impregnated with an acid solution before charging to the reactor. Acid impregnation was achieved by hand mixing air dried bagasse with an acid solution for half an hour to obtain an approximate liquid content (acid and water) of 50%. The amount of acid/kg bagasse was then determined by contacting 10g of the acid impregnated bagasse with 150 ml of water and titrating the supernatant liquid against 0,05 N NaOH.

The following were monitored during the dilute acid hydrolysis performed with acid impregnated bagasse :

i) mass of acid impregnated bagasse added.
ii) mass of discharged residue and hydrolysate.
iii) dry mass of residue washed from the reactor after completion of the hydrolysis.
iv) mass and moisture content of the air dried washed residue.

The residence times of the bagasse in the continuous hydrolysis reactor were obtained by placing a number of 3mm glass beads in the bagasse feed. These were detected in the residue discharge and the residence time obtained.

4.3.4. Sample Preparation

The bagasse residue collected after hydrolysis was vacuum filtered on a Buchner funnel to obtain a hydrolysate sample for analysis. The residue was then thoroughly washed with

hot followed by cold water before filtration, air drying and weighing.

A fraction of the hydrolysate sample was processed according to the method given in section 4.1.5. and the remainder was stored to obtain a composite acid hydrolysate for neutralisation tests.

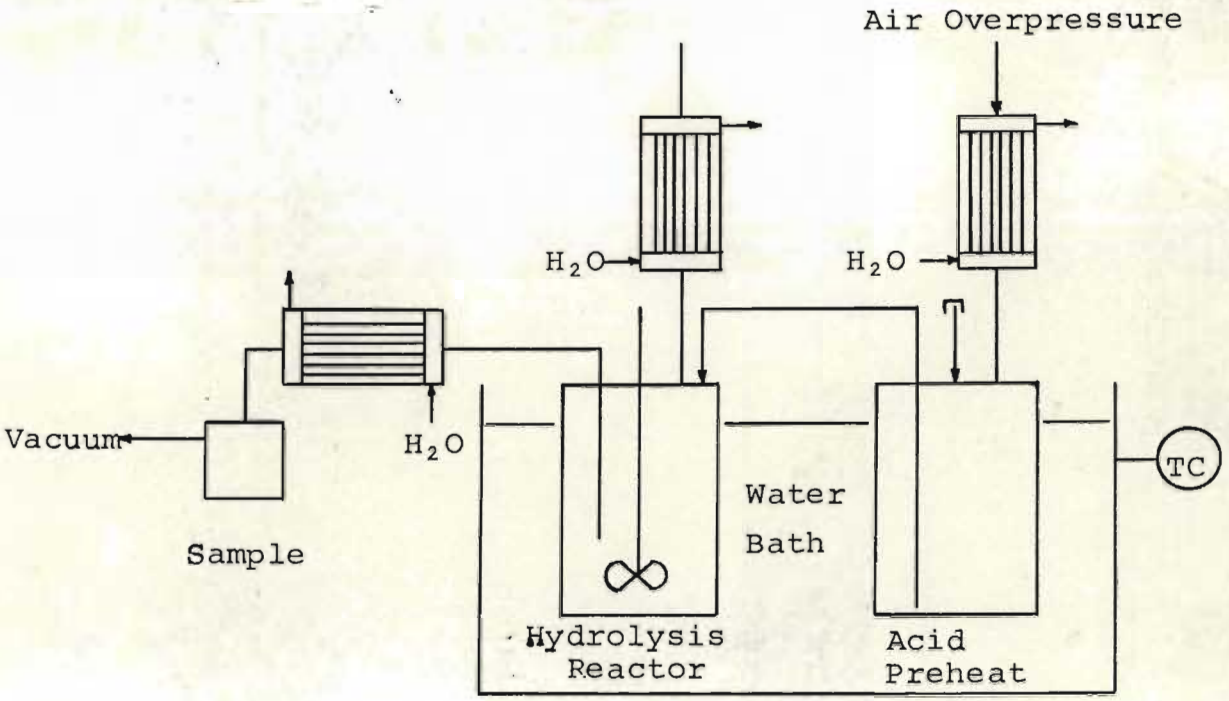


Figure 4.1. Diagram of batch atmospheric acid hydrolysis reactor system

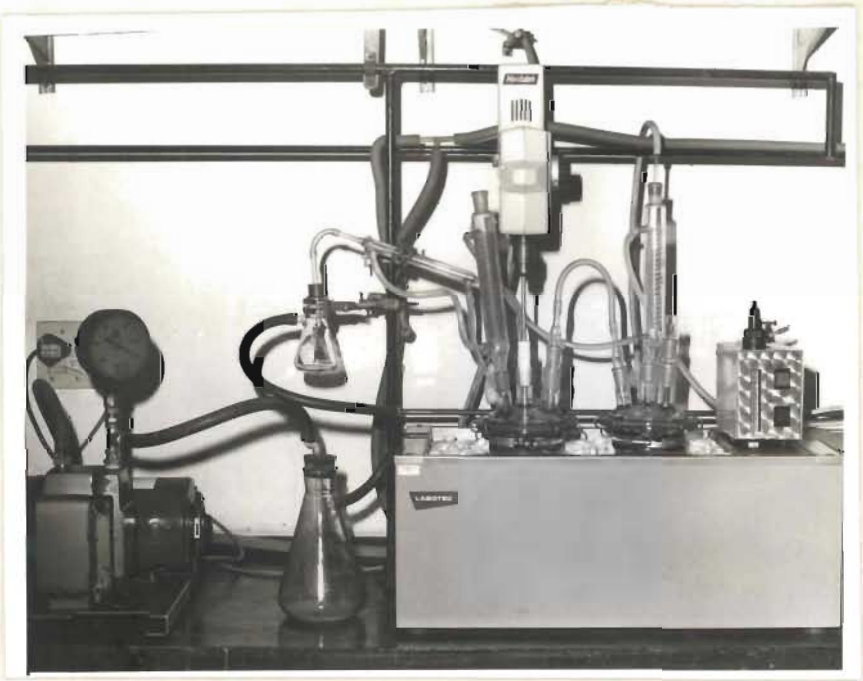


Figure 4.2. View of batch atmospheric hydrolysis reactor system

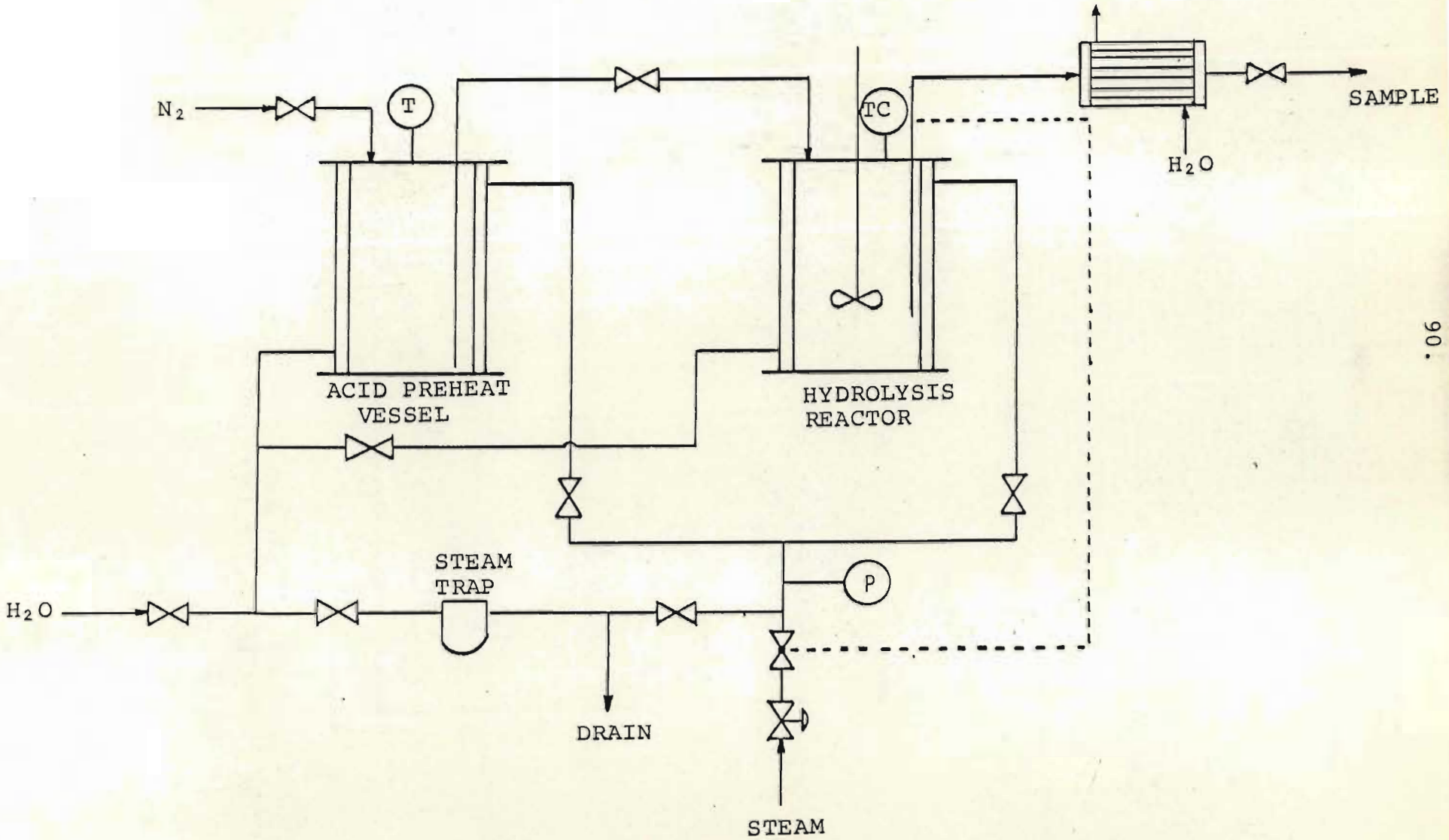


Figure 4.3. Diagram of batch pressurised acid hydrolysis reactor system

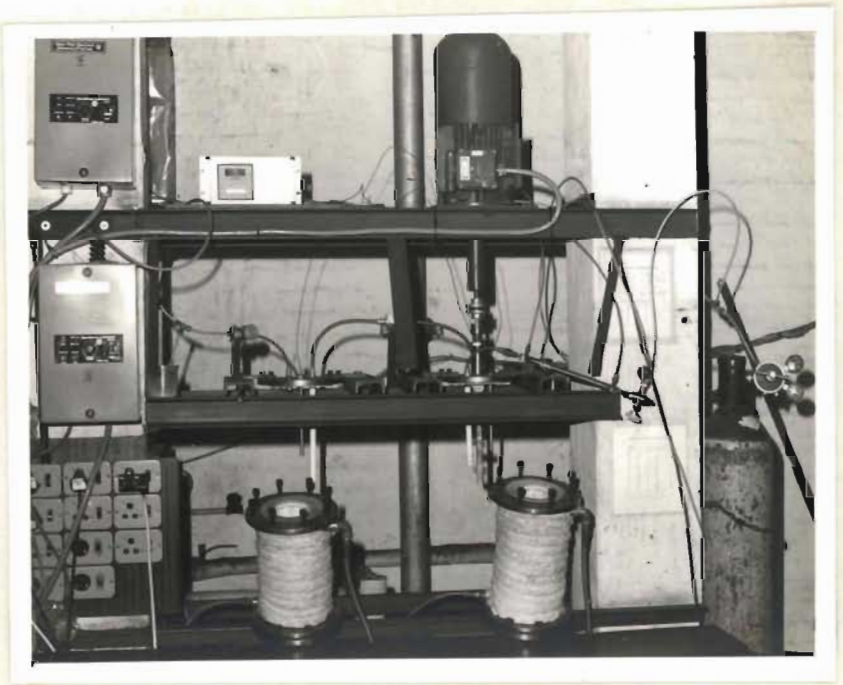


Figure 4.4. View of batch pressurised hydrolysis reactor system

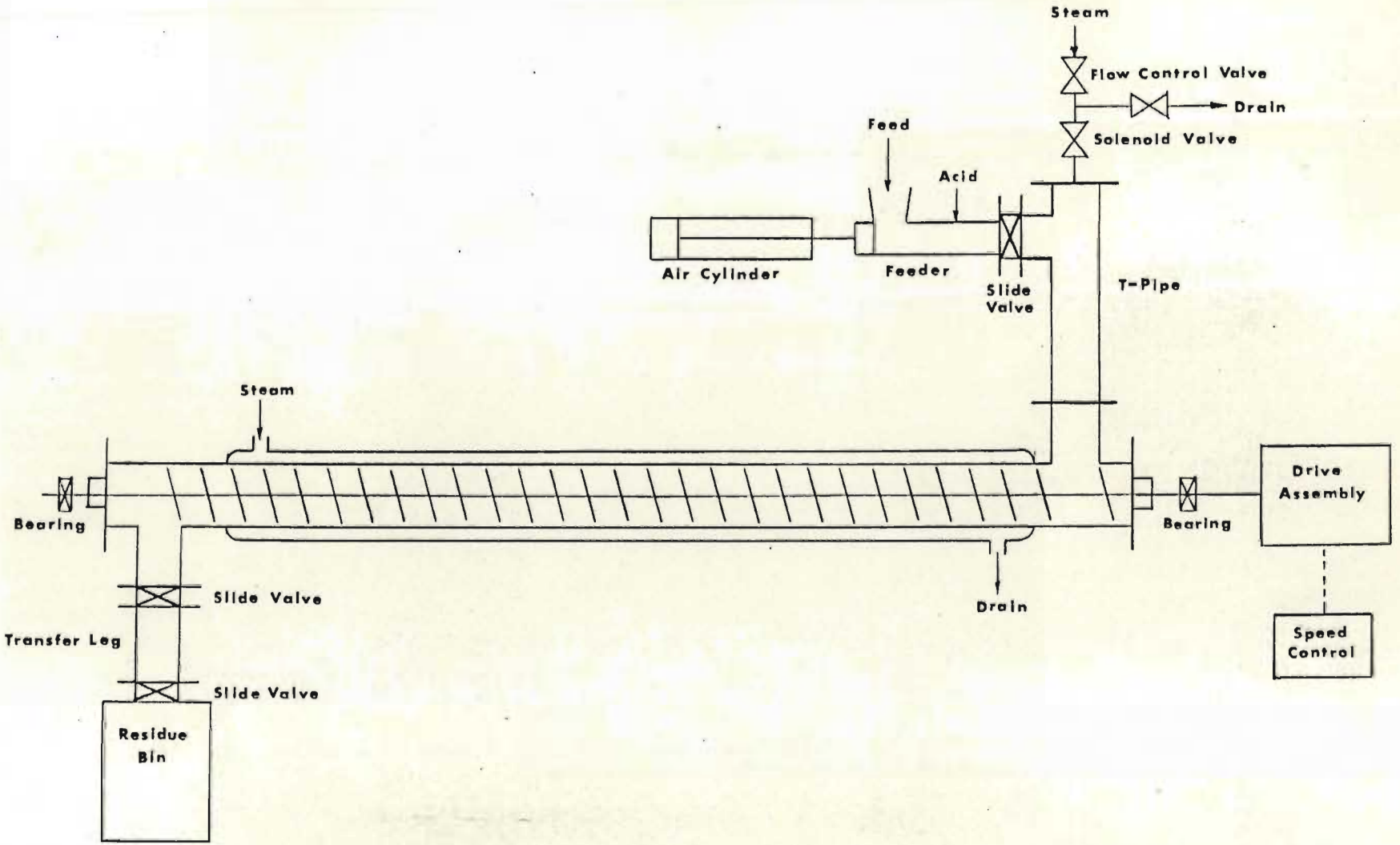


Figure 4.5. Diagram of continuous acid hydrolysis reactor

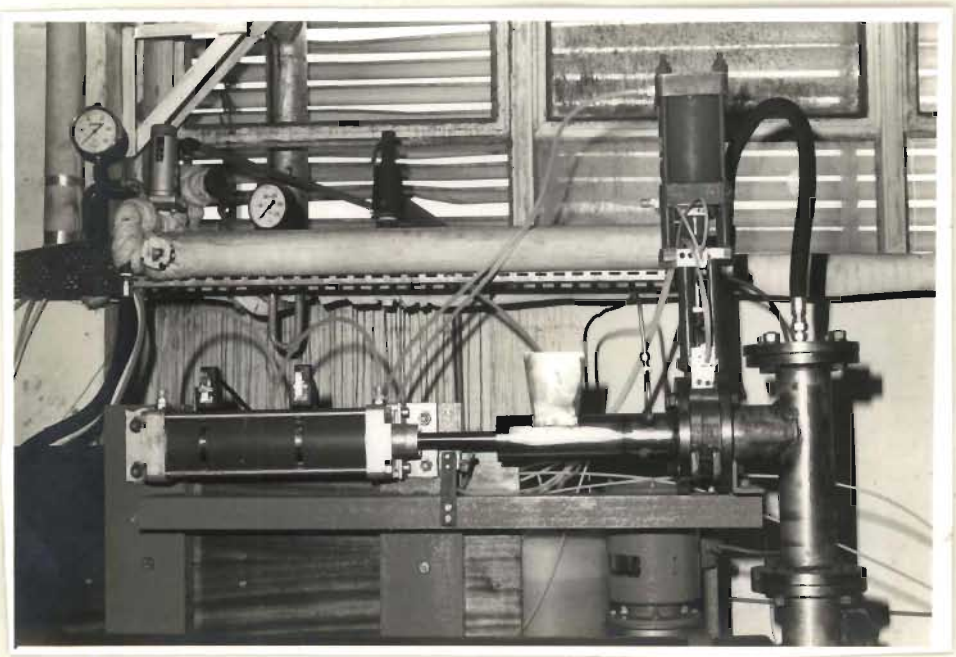


Figure 4.6. View of bagasse feed system

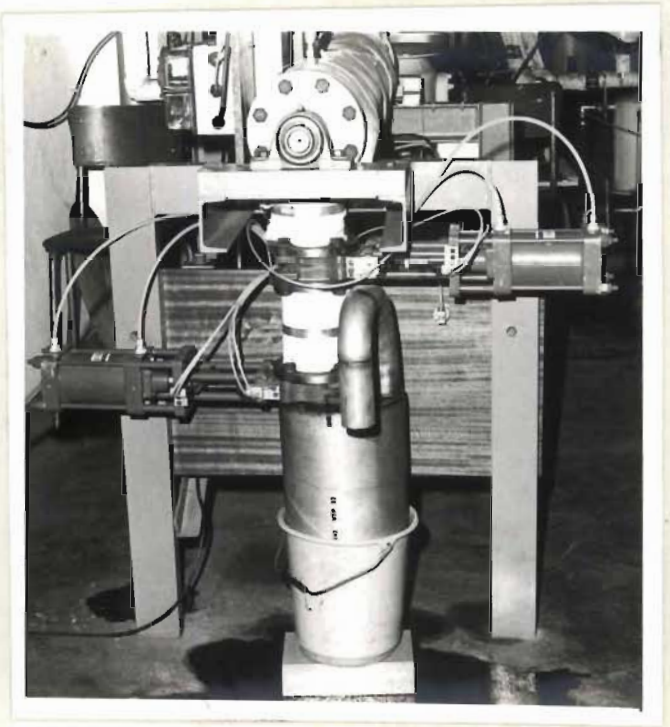


Figure 4.7. View of bagasse discharge system

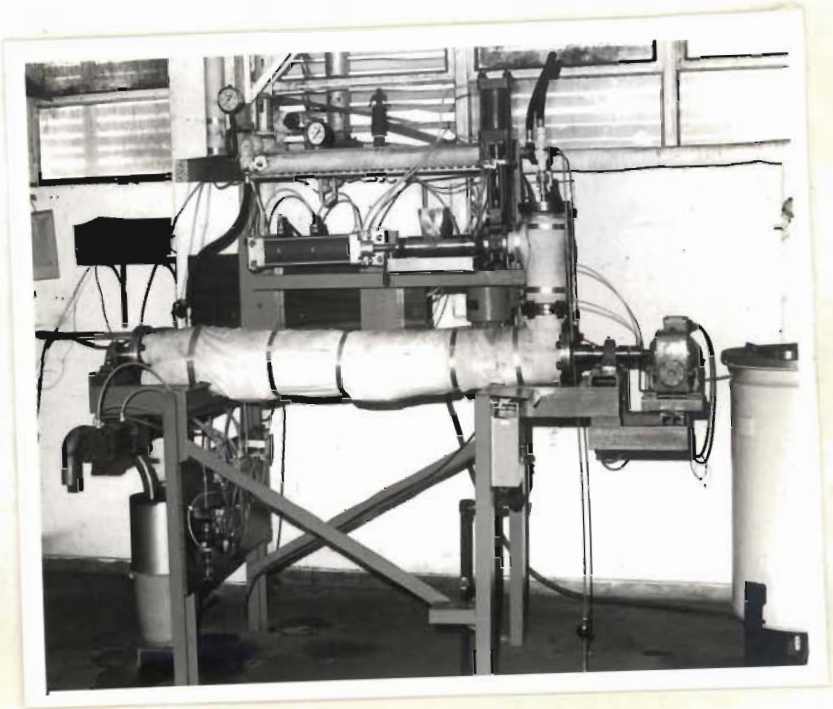


Figure 4.8. View of continuous acid hydrolysis reactor

5. COMPLETE HYDROLYSIS OF RAW BAGASSE

5.1 Objective

The raw bagasse was completely hydrolysed to determine the actual amount of pentose sugars in bagasse hemicelluloses and to compare the conventional bagasse analyses with those obtained by complete hydrolysis.

5.2 Bagasse Sample

The bagasse sample, obtained from the Sugar Milling Research Institute (SMRI), was a 'standard' bagasse which had been dried immediately after sugar extraction

The SMRI 'standard' bagasse was passed over a 6 mm screen. The +6 mm fraction was batch hammer milled for 6 minutes and then combined with the -6 mm fraction. This bagasse was then analysed by the conventional analysis methods and its composition is shown in Table 5.1.

Table 5.1. : Composition of bagasse obtained from SMRI

Analysis	%
Cellulose	38,5
Lignin	22,2
Pentosan	31,4
Ash	3,1
Hot Water Solubles	4,7
Alcohol-Benzene Solubles	6,6

5.3 Method of Complete Hydrolysis

This method of complete hydrolysis was adapted from the work of Timell[176] and Saeman[177].

5.3.1 Sample Preparation

The bagasse sample was Wiley milled to <40 mesh, extracted with alcohol-benzene solvent according to TAPPI T6m - 59 and then extracted with hot water according to TAPPI T1m-59. This soluble-free bagasse sample was then oven dried at 105°C before complete hydrolysis.

5.3.2 Complete Hydrolysis

Approximately 1g of the dry bagasse was accurately weighed into a broad test-tube. 9 mℓ of 72% H_2SO_4 was added and the test-tube sealed with a rubber bung. After thorough mixing on a vortex mixer, the test tube was placed in a water bath at 30°C for 1 hour.

The mixture was then quantitatively transferred into a 500 mℓ conical flask and made up to 250 mℓ with distilled water. The flask was covered with aluminium foil and placed in an autoclave at 121°C (100 kPa) for 1 hour. The mixture was then allowed to cool, filtered through Whatman filter paper No. 540 and 5 mℓ of clear filtrate was taken for acetic acid and furfural analysis. The residue was also weighed and ashed and reported as lignin.

The remaining filtrate was neutralised to pH 4 with barium hydroxide crystals and then centrifuged at 5 000 rpm for 10 minutes. The clear supernatant in the centrifuge tube was quantitatively decanted and placed in a rotary evaporator. Concentration of the solution under vacuum was performed at 50°C ± 5°C until approximately 5 mℓ remained. This concentrate was quantitatively washed through a filter paper into a volumetric flask and made up to the mark with distilled water before analysis by HPLC.

A control sample, containing xylose, glucose and arabinose in the approximate ratio that they occur in bagasse, was hydrolysed in the same manner as the bagasse to produce correction factors that would account for sugar degradation during the complete acid hydrolysis.

5.4 Results

The complete hydrolysis of raw bagasse was performed in duplicate - samples A and B.

Table 5.2 : Furfural, acetic acid and lignin analyses

Sample	Sample Mass (g)	Acetic Acid (mg/ℓ)	Furfural (mg/ℓ)	Mass of Lignin Residue (g)
SMRI Bagasse A	0,9500	210	40	0,1827
SMRI Bagasse B	1,096	235	40	0,2145

Table 5.3 : H.P.L.C. analyses of sugar concentrates

Sample	Vol of Conc. (mℓ)	Xylulose (g/100 mℓ)	Xylose (g/ 100 mℓ)	Arabinose (g/100 mℓ)	Glucose (g/ 100 mℓ)	Cellobios (g/100 mℓ
SMRI Bagasse A	20	0,02	1,13	0,20	1,74	0,04
SMRI Bagasse B	25	0,02	1,02	0,19	1,64	0,04
Control	20	0,08	1,30	0,32	1,87	-

5.5 Discussion

After complete acid hydrolysis of the SMRI bagasse the following sugars were detected by HPLC : xylose, arabinose, glucose, cellobiose and a sugar which eluted before xylose. This sugar has been tentatively identified as xylulose as its presence in hemicellulose acid hydrolysates has been reported[14, 17] and it had the same retention time by HPLC as a sample of pure xylulose.

These sugars detected by H.P.L.C. were then converted back to the quantities of pentosan and cellulose in the bagasse (taking the conversion factors which allow for the sugar degradation into account as shown in Appendix E). There was good agreement between these results and those obtained for bagasse composition by the conventional analytical methods as shown in Table 5.4.

Table 5.4 : Bagasse composition by complete hydrolysis

Analysis	Complete Hydrolysis %	Conventional Analyses %
Acetic Acid	5,5	-
Pentosan	30,2	31,4
Cellulose	36,4	38,5
Lignin	19,4	22,2

The maximum yields of xylose and arabinose which should be obtainable from bagasse hemicellulose (considering the sugar degradation conversion factors) are 271 and 66 mg/g dry bagasse respectively (average values). The actual values obtained in the complete hydrolysis were 240 and 44 mg/g of dry bagasse for the xylose and arabinose respectively. (See Appendix E).

A sample of the acidic and neutralised hydrolysate from the complete hydrolysis was also subjected to thin layer chromatography. The following sugars were detected in the neutralised hydrolysate by TLC : xylose, arabinose, glucose, cellobiose and xylulose.

There was no trace of glucuronic acid in the acidic hydrolysate sample. This could possibly be accounted for by the fact that the linkage between the glucuronic acid and xylose in bagasse xylan is especially acid resistant[18]. All attempts using TLC to detect the aldobiuronic acid {2-O-(4-O-methyl α D glucopyranosyluronic acid)-D-xylose} in the hydrolysate were also unsuccessful.

6. EFFECT OF LIQUID TO SOLID RATIO ON FREE XYLOSE YIELD

6.1 Objective

Batch dilute acid hydrolysis of bagasse was investigated at a single hydrolysis condition to determine the effect of liquid to solid ratio on free xylose yield over the range 5 → 20 : 1.

6.2 Hydrolysis Conditions

The dilute acid hydrolyses were performed at 100°C using 20 g/ℓ sulphuric acid in a 2 litre laboratory glassware reactor according to the method in section 4.1.

6.3 Bagasse Sample

Bagasse obtained from Tongaat sugar mill was air dried and batch hammermilled for 5 minutes. This treatment effectively reduced the fibre length and increased the bulk density, producing a bagasse sample which could easily be processed in the batch reactor. The bagasse composition is shown in Table 6.1.

Table 6.1 : Composition of bagasse used to study effect of liquid to solid ratio

Analysis	%
Cellulose	37,1
Lignin	24,8
Pentosans	31,3
Ash	2,7
Moisture	9,9

This substrate was only used to determine the effect of liquid to solid ratio on free xylose yield during dilute acid hydrolysis as it has a higher lignin content than normal and it was suspected that there might have been some microbial degradation of the carbohydrate fraction during wet storage.

6.4 Results

Table 6.2 : Effect of liquid to solid ratio on free xylose yield.

L/S Ratio	Time (minutes)	mg free xylose/g bagasse
5 : 1	60	79,9
	90	127,8
	120	154,2
	150	163,8
	180	167,0
10 : 1	60	70,2
	90	117,2
	120	149,2
	150	156,5
	180	167,7
15 : 1	60	97,8
	90	146,2
	120	168,0
	150	177,5
	180	198,9
20 : 1	60	92,8
	90	141,7
	120	172,6
	150	185,7
	180	202,6

6.5 Discussion

Maximum xylose yield after three hours of dilute acid hydrolysis (100°C, 20 g/ℓ H_2SO_4) was obtained at liquid to solid ratios of 15 : 1 and above.

In the case of the two liquid to solid ratios tested below 15 : 1 there was an evident lower xylose yield after 3 hours. This could possibly be attributed to a mass transfer effect as it was noticeable, especially at liquid to solid = 5 : 1, that the initial contact of acid and bagasse in the batch reactor resulted in

uneven wetting of the bagasse. The stirring of these lower liquid to solid ratio mixtures was also difficult.

It was decided to conduct all further acid hydrolyses at a liquid to solid ratio of 15 : 1.

7. AUTOHYDROLYSIS OF BAGASSE

7.1 Objective

Xylose production during the autohydrolysis of bagasse at a liquid to solid ratio of 15 : 1 was monitored over the temperature range 100° to 160°C to determine the xylose yields from the hemicellulose fraction by this hydrolysis method.

7.2 Hydrolysis Conditions

The batch authohydrolyses were performed at 100°, 120°, 140° and 160°C using a solid : liquid ratio of 1 : 15 in a stirred 2 litre pressurised teflon-lined reactor system (Reactor system and experimental procedure in Section 4.2).

The initial water volume was set at 1,5 litres in all the hydrolyses and the mass of bagasse was adjusted to obtain the required solid : liquid ratio (mass basis).

7.3 Bagasse Sample

The bagasse sample was obtained from the SMRI and was prepared according to the method in Section 5.2. Its composition is found in Table 5.1.

7.4. Results

7.4.1 Hydrolysate Analyses

The actual results obtained from the autohydrolyses are shown in Appendix F. These results were corrected for the gradual change in hydrolysate volume during the hydrolyses due to sample withdrawal by the method in Appendix F and the corrected yield data expressed in mg/g of dry bagasse are shown in Table 7.1.

Table 7.1 : Autohydrolysis yield data

Temp	Time (min)	Free Xylose (mg/g)	Total Xylose (mg/g)	Total Glucose (mg/g)	Acetic Acid (mg/g)	Furfural (mg/g)
100°C	120	no trace	no trace	14,92	not analysed	not analysed
	255			16,40		
	485			16,40		
	730			16,40		
	1290		2,86	14,97		
	1565		7,10	16,38	6,38	
120°C	30	no trace	no	8,98	not analysed	not analysed
	60		trace	10,46		
	120		2,93	13,39		
	165		4,38	13,39		
	243		5,81	13,39		
	300		7,23	13,39		
	360		10,03	13,39	5,07	
140°C	60	no trace	7,30	7,30	1,61	1,90
	120		26,06	10,18	3,63	2,20
	180		40,34	14,47	5,20	2,71
	240	<2,80	55,87	14,47	6,33	3,08
	300	<2,80	69,84	13,07	9,40	3,26
	360	<2,80	79,51	14,45	10,92	3,62
160°C	30	no	28,09	13,31	3,99	1,48
	60	trace	67,60	13,31	5,31	2,00
	90	4,34	97,20	17,65	9,80	2,67
	125	8,64	112,25	17,65	11,66	6,31
	165	18,55	124,99	17,65	16,90	19,45

7.4.2 Residue Analyses

Table 7.2 : Autohydrolysis residue analyses

Temperature (°C)	100°	120°	140°	160°
Autohydrolysis Time (min)	1565	360	360	165
Initial dry mass (g)	100,51	100,25	102,46	102,46
Residue dry mass (g)	91,49	91,80	77,38	68,03
Mass loss (%)	8,97	8,43	24,48	33,60
Residue Analysis (%)				
Cellulose	39,42	41,11	47,03	50,68
Pentosan	32,34	29,77	22,34	15,97
Lignin	24,28	24,24	27,46	29,50
Ash	4,02	4,35	4,00	5,00
Loss (%)				
Cellulose	6,9	2,3	7,8	12,7
Pentosan	6,1	13,1	46,2	66,2
Lignin	0,6	0,0	6,8	11,9

7.5 Discussion

Acids, especially acetic acid, are liberated from the lignocelluloses by steaming at elevated temperatures[70]. In the acid medium the hemicelluloses are hydrolysed and become soluble - this phenomenon is termed autohydrolysis.

The autohydrolyses covered by this investigation were performed at a liquid : solid ratio of 15 : 1 and the acetic

acid liberated from the bagasse at the elevated temperatures was extensively diluted by this high liquid to solid ratio. Autohydrolyses performed at lower liquid : solid ratios would thus have a higher acetic acid concentration in the hydrolysate to catalyse the hemicellulose hydrolysis and might prove more effective. However, the liquid : solid ratio of 15 : 1 was the highest solid concentration that could be processed in the batch reactor.

The autohydrolyses performed at 100° and 120°C indicated that these temperatures had little or no effect on the bagasse hemicellulose fraction even though there was some acetic acid formation. There was no evidence of free xylose in the hydrolysates and even after further hydrolysis (100°C and 20g/ℓ H_2SO_4 for 1 hour) only small amounts of xylose were detected. Glucose production was also monitored but the majority of it was generated by conversion of residual sucrose to glucose and fructose (±10 mg sucrose/g dry bagasse feed).

The ineffectiveness of the autohydrolyses at 100° and 120°C was reflected by the bagasse residue analyses which confirmed the low hemicellulose extraction at these temperatures.

Significant hemicellulose extraction occurred during the autohydrolyses performed at 140° and 160°C. In both cases, however, very little of the xylan extracted from the bagasse was in its monomeric form - xylose.

Acetic acid formation was significantly higher than at temperatures below 140°C but did not approach the acetic acid yield obtained during complete hydrolysis of bagasse. Furfural formation was also monitored at these higher temperatures and it is obvious that xylose losses due to furfural formation would become prohibitive during autohydrolyses above 140°C if a monomeric xylose product was required. The glucose content of the autohydrolysate could be attributed to sucrose in the bagasse feed, removal of hexose side-chains from the hemicellulose fraction and hydrolysis of amorphous cellulose.

Significant amounts of hemicellulose have been extracted from lignocelluloses by autohydrolysis. Puls et al[71, 72, 73, 74] have treated agricultural residues with saturated steam at 170° to 200°C for some minutes followed by hot stage defibration. Under these conditions an average of 20% carbohydrates (based on raw material) consisting mainly of xylans were extracted by a water wash after the steam treatment.

A continuous high pressure steaming process for the autohydrolysis of lignocellulose materials has been developed by Stake Technology[79, 80]. This process incorporates a plug flow reactor operating between 200° and 220°C with a residence time of less than 5 minutes and it is claimed that 85% of the hemicellulose fraction could be extracted by a water wash after processing.

Both these processes have a major disadvantage; the hemicellulose extracts consist mainly of oligomers which require dilute mineral acid hydrolysis for conversion to their monomeric forms. However, large quantities of polymeric sugars can also be produced during very dilute acid hemicellulose hydrolyses. Limbaugh et al[140] hydrolysed oak hardwood hemicellulose over the temperature range 140° to 180°C using 0,05% H_2SO_4 at a liquid : solid ratio of 10 : 1 and observed substantial amounts of xylose oligosaccharides over this experimental range.

It would thus appear that the single-stage production of xylose from bagasse hemicellulose by autohydrolysis is not practical and can only be achieved by dilute mineral acid hydrolysis. The extent to which this acid concentration can be reduced without significant oligomer formation and maintaining xylose yield will be determined in investigations using dilute acid for hydrolysis (See Sections 8 and 9.).

8. BATCH ATMOSPHERIC DILUTE ACID HYDROLYSIS OF BAGASSE

8.1 Objective

Atmospheric dilute acid hydrolysis of bagasse hemicellulose was investigated to obtain xylose, furfural, glucose and acetic acid yields and sulphuric acid consumption during the hydrolyses.

8.2 Hydrolysis Conditions

The dilute acid hydrolyses were performed at 80°, 90° and 100°C using sulphuric acid concentrations in the range 10 to 40 g/ℓ in a stirred 2 litre laboratory glassware reactor at a solid : liquid ratio of 1 : 15. (Experimental procedure in Section 4.1.).

The initial acid volume was set at 1,5 litres in all the hydrolyses and the mass of bagasse was adjusted to obtain the required solid : liquid ratio (mass basis).

8.3 Bagasse Sample

The bagasse was obtained from the SMRI and was prepared according to the method in Section 5.2. Its composition is available in Table 5.1.

8.4 Results

8.4.1 Acid Hydrolysate Analyses

The actual results from the dilute acid hydrolyses are shown in Appendix G. These results were corrected for the gradual change in hydrolysate volume during the hydrolyses due to sample withdrawal by the method in Appendix F and the corrected yield data expressed in mg/g of dry bagasse are shown in Tables 8.1, 8.2 and 8.3.

Table 8.1 : Batch acid hydrolysis yield data at 80°C

Acid Conc. (g/ℓ)	Time (min)	Free Xylose (mg/g)	Total Xylose (mg/g)	Free Glucose (mg/g)	Total Glucose (mg/g)	Acetic Acid (mg/g)	Furfural (mg/g)	Acidic Organics (mg/g)	Acid Consumption (mg/g)
20	100	10,36	48,84	14,80	19,24	21,90	-		
	180	25,01	98,65	20,66	23,63	35,38	0,44		
	260	49,66	120,40	23,56	20,73	42,63	0,60		
	420	101,34	150,55	26,43	23,17	45,65	1,10		
	745	169,53	177,54	27,85	27,85	47,07	1,61		
	1025	183,59	187,38	29,26	30,66	47,77	2,26		
	1320	193,33	195,73	33,43	34,84	47,77	2,68	60,38	6,56
40	50	16,17	63,23	11,76	19,12	21,32	-		
	90	33,64	105,45	16,13	20,57	34,28	0,45		
	130	53,82	129,94	20,45	22,01	36,58	0,65		
	180	93,76	154,19	21,88	22,01	39,58	0,88		
	300	155,87	183,83	26,12	27,66	41,98	1,32		
	420	182,41	188,03	27,51	30,45	44,49	1,58		
	540	190,76	193,55	28,89	30,45	45,05	2,01		
	660	193,44	199,02	35,73	34,56	45,32	2,30	65,88	7,96

Table 8.2 : Batch acid hydrolysis yield data at 90°C

Acid Conc. (g/ℓ)	Time (min)	Free Xylose (mg/g)	Total Xylose (mg/g)	Free Glucose (mg/g)	Total Glucose (mg/g)	Acetic Acid (mg/g)	Furfural (mg/g)	Acidic Organics (mg/g)	Acid Consumption (mg/g)
10	90	10,44	56,66	14,91	17,89	23,56	-		
	180	51,77	105,37	19,34	19,37	27,10	0,77		
	260	76,61	131,67	20,80	19,37	30,90	1,06		
	480	140,25	162,05	25,14	25,15	34,52	1,73		
	720	171,74	182,09	26,57	29,45	38,38	2,74		
	955	185,90	192,00	29,40	32,28	40,93	3,54		
	1200	197,12	200,41	35,01	35,08	41,91	4,49	60,09	6,90
20	50	14,84	68,27	17,81	19,29	29,98	-		
	90	36,88	105,00	20,75	22,23	38,35	0,88		
	130	67,42	134,09	22,20	22,23	40,97	1,39		
	245	152,36	170,08	23,64	25,11	44,57	2,01		
	360	180,85	185,75	26,49	29,39	44,43	2,69		
	480	193,54	196,30	32,13	35,03	44,71	3,17		
	600	197,73	201,22	37,71	42,00	46,25	3,87	62,71	9,06
40	25	14,70	61,76	16,17	19,12	16,91	-		
	45	38,00	111,25	17,63	19,12	28,70	0,77		
	68	76,90	138,63	23,39	24,88	31,37	1,45		
	122	155,35	180,00	27,67	29,16	35,86	2,62		
	180	180,76	188,47	29,09	34,81	39,96	2,87		
	240	187,75	194,05	33,28	39,00	41,22	3,43		
	300	193,28	198,20	36,04	37,61	42,32	3,71	68,95	21,04

Table 8.3 : Batch acid hydrolysis yield data at 100°C

Acid Conc. (g/ℓ)	Time (min)	Free Xylose (mg/g)	Total Xylose (mg/g)	Free Glucose (mg/g)	Total Glucose (mg/g)	Acetic Acid (mg/g)	Furfural (mg/g)	Acidic Organics (mg/g)	Acid Consumption (mg/g)
	38	14,87	66,91	22,30	26,76	24,98	0,59		
	80	47,25	115,48	26,72	28,23	30,28	1,13		
	120	88,05	146,07	29,63	31,15	37,56	1,40		
10	240	165,42	180,69	36,84	34,03	42,18	2,18		
	375	193,04	196,39	38,27	39,74	46,46	3,15		
	480	201,52	203,45	39,68	38,33	47,02	3,42		
	600	205,71	210,44	45,27	45,32	47,44	4,02	61,99	8,35
	20	13,32	66,59	17,76	22,20	22,20	0,67		
	40	47,02	113,48	26,55	25,13	29,52	1,62		
	60	86,17	145,38	26,55	25,13	39,10	1,94		
20	120	175,17	184,14	29,42	32,31	41,10	3,01		
	180	187,96	194,09	32,26	39,41	42,81	3,65		
	240	196,39	201,11	36,48	42,22	45,20	4,47		
	300	206,13	209,46	42,04	40,83	45,90	4,80	58,90	8,90
	10	16,13	61,60	16,13	17,60	21,12	0,65		
	20	61,14	112,41	19,04	19,05	31,28	1,17		
	30	104,26	142,59	21,91	23,36	39,62	1,84		
40	60	172,54	181,00	27,60	33,32	43,60	2,33		
	90	190,84	193,67	36,05	38,95	44,59	3,19		
	120	195,02	197,85	36,05	36,17	45,42	3,76		
	150	199,16	203,37	41,56	40,30	46,39	4,10	66,91	13,37

8.4.2 Atmospheric Hydrolysis Residues

Table 8.4. : Atmospheric hydrolysis residue analyses

Temp (°C)	Acid Conc. (g/ℓ)	Bagasse Dry Mass (g)	Residue Dry Mass (g)	Mass Loss %	Residue Analysis (%)			
					Cellulose	Pentosan	Lignin	Ash
80	20	101,36	65,99	34,90	52,23	12,11	30,69	4,08
	40	102,01	63,50	37,75	52,38	12,61	32,77	2,15
90	10	100,60	62,97	37,41	52,08	10,75	30,43	4,06
	20	101,07	62,42	38,24	52,22	10,74	31,86	4,01
	40	102,01	63,90	37,36	52,11	11,06	31,06	4,56
100	10	100,89	63,29	37,27	51,56	10,61	31,82	5,26
	20	101,36	63,47	37,38	52,12	11,01	30,76	4,74
	40	102,28	63,52	37,90	52,34	9,82	32,07	3,29

These results were then processed to yield cellulose, pentosan and lignin losses during the various acid hydrolyses, which are reproduced in Table 8.5.

Table 8.5. : Bagasse component losses during atmospheric hydrolyses

Temp (°C)	Acid Conc. (g/ℓ)	Cellulose			Pentosan			Lignin		
		Initial Mass (g)	Final Mass (g)	% Loss	Initial Mass (g)	Final Mass (g)	% Loss	Initial Mass (g)	Final Mass (g)	% Loss
80	20	39,02	34,47	11,67	31,83	7,99	74,89	22,50	20,25	9,99
	40	39,27	33,26	15,30	32,03	8,01	78,00	22,65	20,81	8,13
90	10	38,73	32,79	15,32	31,59	6,77	78,57	22,33	19,16	14,19
	20	38,91	32,60	16,32	31,74	6,70	78,88	22,44	19,89	11,38
	40	39,27	33,30	15,21	32,03	7,07	77,94	22,65	19,85	12,37
100	10	38,84	32,63	15,98	31,68	6,72	78,80	22,40	20,14	10,09
	20	39,02	33,08	15,22	31,83	6,99	78,05	22,50	19,52	13,32
	40	39,38	33,25	15,58	32,12	6,24	80,58	22,71	20,35	10,30

8.5 Discussion

8.5.1 Acetic Acid Formation

The production of acetic acid during the dilute acid hydrolysis of bagasse at atmospheric pressure approaches a limiting maximum value and at this point all the acetyl groups in the hemicellulose fraction should have been converted to acetic acid. A typical curve is shown in Figure 8.1. From the results the bagasse hydrolysed would appear to have an acetyl content of 45,4 mg/g of dry bagasse (average value).

At each hydrolysis temperature i.e. 80°, 90° and $100^{\circ}C$ the amount of acidic organics (expressed as acetic acid) increases with increasing acid concentration. Since the amount of acetic acid at the end of each hydrolysis remains constant it would appear that additional organic acids (or alkali consuming compounds) are being produced as the hydrolysis conditions increase in severity.

8.5.2 Furfural Formation

Furfural formation is an important parameter that must be monitored during dilute acid hydrolyses of bagasse especially if the xylose solution is to be fermented as the furfural produced is toxic to micro-organisms.

Furfural formation increases with increasing temperature for a given acid concentration during the dilute acid hydrolysis of bagasse at atmospheric pressure. A typical furfural yield curve is shown in Figure 8.2. However, furfural formation does not exceed 4,8 mg/g of dry bagasse during these acid hydrolyses.

8.5.3 Glucose Formation

The amount of glucose produced in the dilute acid hydrolyses increases with increasing hydrolysis time and acid concentration at any given temperature. Some of this glucose can

be accounted for in the bagasse feed which contains approximately 1% sucrose. In the presence of dilute acid this sucrose will then be broken down into equal amounts of glucose and fructose. The balance of the glucose is due to the removal of hexose side chains from the hemicellulose fraction and hydrolysis of amorphous cellulose.

8.5.4. Acid Consumption

At each hydrolysis temperature i.e. 80°, 90° and 100°C the sulphuric acid consumption increases slightly with increasing acid concentration.

Bagasse contains an amount of ash, usually termed 'active ash', which will react with and neutralise sulphuric acid. However, it appears that the consumption of acid is not entirely due to the 'active ash' and that the additional acid is consumed by reactions which occur as the hydrolysis conditions become more severe.

8.5.5. Xylose Formation

The amounts of free and total xylose produced in the dilute acid hydrolyses increase with increasing hydrolysis time and acid concentration at any given hydrolysis temperature. A maximum yield of approximately 210 mg xylose/g of dry bagasse is obtained at 100°C which corresponds to a xylose yield of 58,9% based on initial pentosan in the bagasse.

The total xylose analyses are also higher than the free xylose analyses during the initial stages of acid hydrolysis at all the hydrolysis conditions tested as shown in Figure 8.3. This is indicative of the acid hydrolysis reaction pathway of bagasse xylan i.e. xylan → soluble xylan (oligomers) → xylose (monomer). However, as the times approach at which maximum xylose yields occur the total and free xylose yields converge.

8.5.6 Residue Analyses

At all the atmospheric hydrolysis conditions tested the amounts of lignin and cellulose lost in the hydrolyses are similar. On average 15,1% of the initial cellulose and 11,2% of the initial lignin appear to be solubilised at the hydrolysis end.

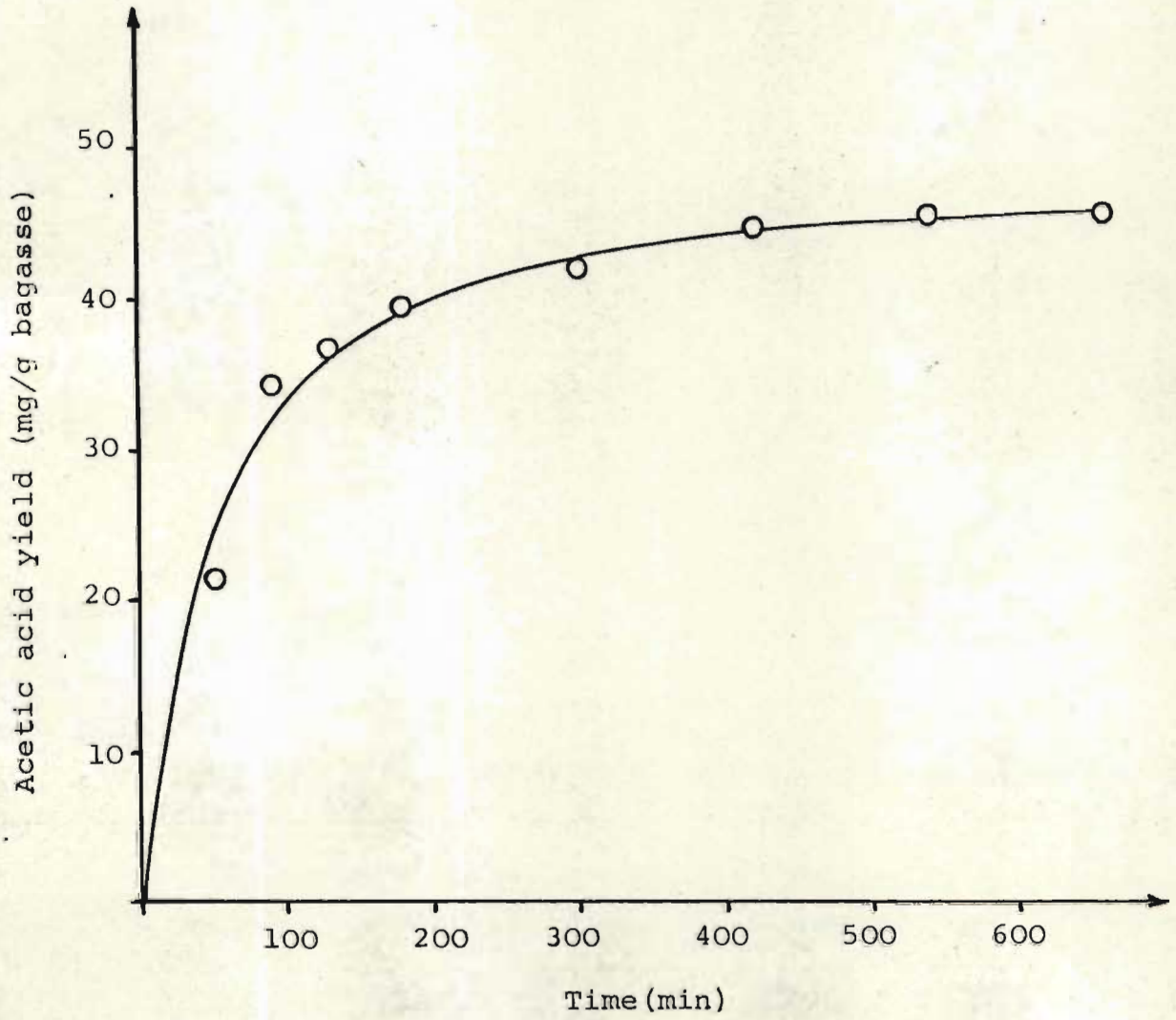


Figure 8.1. Acetic acid formation at 80°C and 40 g/ℓ sulphuric acid

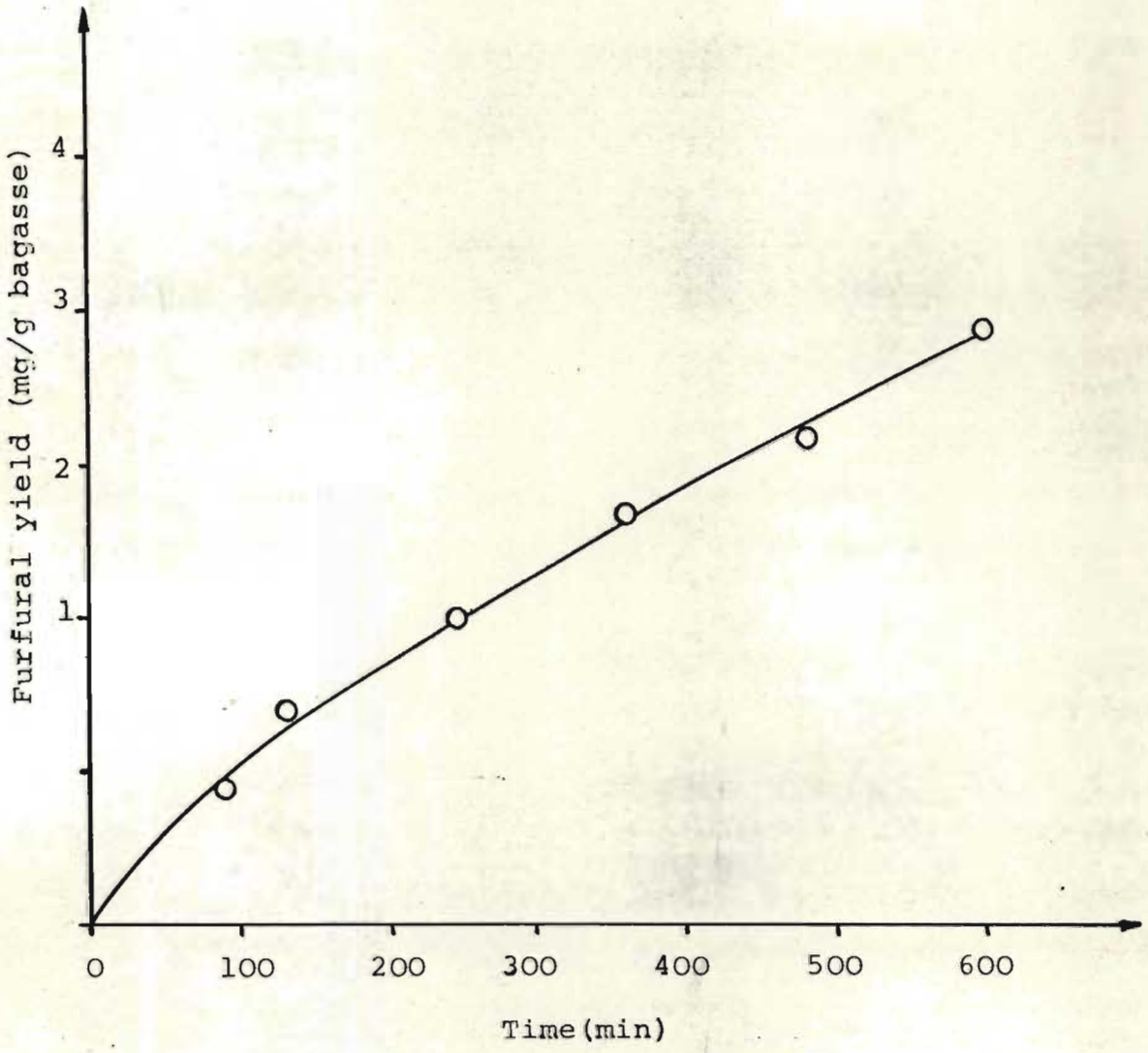


Figure 8.2. Furfural formation at 90°C and 20 g/ℓ sulphuric acid

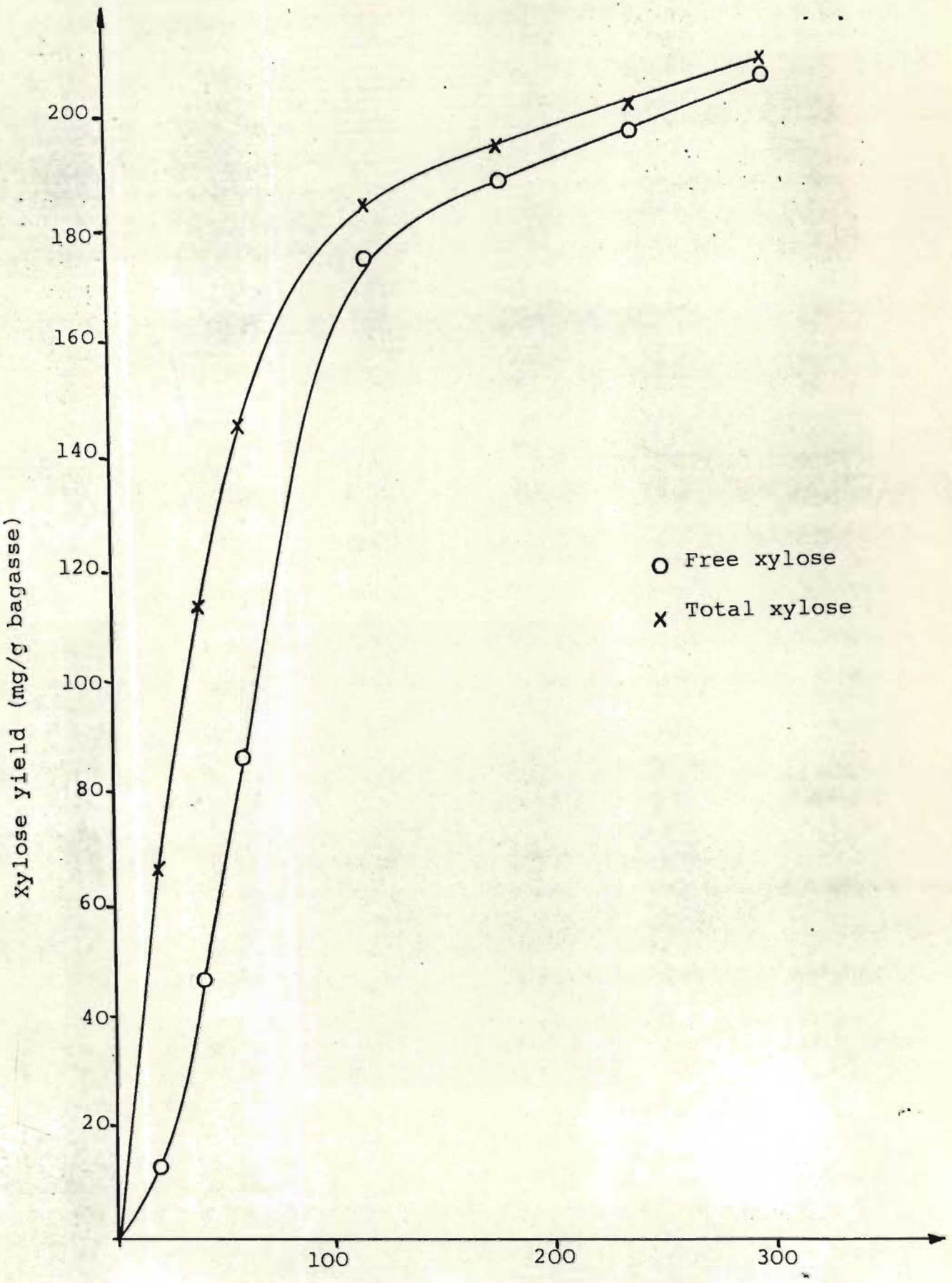


Figure 8.3. Xylose yields at 100°C and 20 g/ℓ sulphuric acid

9. BATCH PRESSURE DILUTE ACID HYDROLYSIS OF BAGASSE

9.1 Objective

Pressure dilute acid hydrolysis of bagasse hemicellulose was investigated to obtain xylose, furfural, glucose and acetic acid yields and sulphuric acid consumption during the hydrolysis.

9.2 Hydrolysis Conditions

The acid hydrolyses were performed at 110°, 120°, 130°, 140° and 150°C using sulphuric acid concentrations in the range 3 to 40 g/ℓ in a stirred 2 litre pressurised teflon-lined reactor system at a solid : liquid ratio of 1 : 15 (Reactor system and experimental procedure in Section 4.2).

At the upper end of the temperature range acid concentrations below 20 g/ℓ were used in the hydrolyses due to the effect of temperature on reaction rate.

9.3 Bagasse Sample

The bagasse was obtained from the SMRI and was prepared according to the method in Section 5.2. Its composition is available in Table 5.1.

9.4. Results

9.4.1 Acid Hydrolysate Analyses

The actual results from the batch pressure dilute acid hydrolyses are shown in Appendix H. These results were corrected for the gradual change in hydrolysate volume during the hydrolyses due to sample withdrawal (see Appendix F) and the corrected yield data expressed in mg/g dry bagasse are shown in Tables 9.1, 9.2, 9.3, 9.4 and 9.5.

Table 9.1. : Batch acid hydrolysis yield data at 110°C

Acid Conc. (g/ℓ)	Time (min)	Free Xylose (mg/g)	Total Xylose (mg/g)	Free Glucose (mg/g)	Total Glucose (mg/g)	Acetic Acid (mg/g)	Furfural (mg/g)	Acidic Organics (as acetic acid) (mg/g)	H_2SO_4 Consumption (mg/g)
	15	13,33	60,72	10,37	11,85	17,18	1,41		
	30	45,56	110,53	14,76	19,17	25,38	2,15		
	50	91,94	135,17	16,21	22,07	28,43	2,88		
10	120	156,44	162,40	23,38	26,37	36,31	3,75		
	200	173,45	179,42	33,30	33,46	38,01	5,64		
	300	184,66	186,42	37,51	39,09	40,25	6,42		
	400	190,21	193,35	38,89	40,45	41,92	7,17		
	510	192,95	196,09	40,26	40,45	41,92	8,54	60,42	12,19
	8	10,29	32,35	8,82	11,76	7,94	0,63		
	16	51,02	94,89	16,09	17,58	24,42	1,53		
	25	95,62	126,54	18,97	19,02	29,41	2,44		
20	60	156,80	162,12	28,93	27,56	37,38	3,61		
	110	179,32	181,82	35,97	37,41	42,02	4,80		
	160	183,49	184,60	40,14	40,19	42,02	6,75		
	200	189,00	191,48	41,52	42,94	42,72	8,33		
	260	191,72	192,84	42,88	42,94	42,72	9,69	57,83	11,24
	4	18,21	53,22	11,20	12,60	17,93	1,54		
	8	54,21	97,54	15,36	16,76	27,62	2,79		
	12	98,02	127,66	16,73	20,87	32,28	3,33		
40	30	162,99	168,26	26,20	28,99	42,43	4,82		
	50	180,38	182,97	35,57	34,34	44,17	5,56		
	71	186,99	189,58	39,53	39,63	44,17	6,55		
	95	189,60	192,20	40,84	40,93	44,17	7,40		
	120	192,18	194,78	40,84	42,22	44,17	8,75	62,52	12,63

Table 9.2. : Batch acid hydrolysis yield data at 120°C

Acid Conc. (g/ℓ)	Time (min)	Free Xylose (mg/g)	Total Xylose (mg/g)	Free Glucose (mg/g)	Total Glucose (mg/g)	Acetic Acid (mg/g)	Furfural (mg/g)	Acidic Organics (as acetic acid) (mg/g)	H_2SO_4 Consumption (mg/g)
	15	26,70	80,09	13,35	16,31	18,54	0,53		
	30	56,04	102,10	14,82	16,31	25,73	1,30		
	46	93,78	136,93	17,72	20,67	29,21	2,31		
5	121	161,25	169,95	27,77	30,72	38,11	3,42		
	200	179,70	184,14	34,87	36,40	43,08	4,20		
	280	186,72	188,36	39,08	39,20	43,78	6,02		
	370	190,88	193,91	41,85	41,98	43,78	8,18		
	460	196,37	198,02	43,22	44,72	43,78	11,02	55,02	10,17
	7,75	34,27	82,82	15,71	18,56	23,27	1,07		
	15,75	82,28	130,82	18,53	22,80	27,23	2,41		
	25	132,54	165,73	24,12	25,59	32,39	2,90		
10	60	194,65	203,00	33,78	39,39	38,47	4,14		
	100	211,03	212,55	40,60	40,76	39,56	6,71		
	143	216,39	219,26	45,98	46,14	42,80	11,09		
	240	219,03	223,21	48,61	48,77	43,59	14,64	54,12	10,64
	4	38,91	89,90	12,08	13,42	15,83	1,01		
	8	93,28	136,31	14,73	17,40	23,66	1,40		
	12	144,37	170,37	19,97	21,33	31,52	2,71		
20	30	202,62	210,50	30,32	32,98	38,90	4,36		
	50	217,96	219,45	38,00	38,09	41,32	6,76		
	70	221,75	223,45	39,26	40,62	42,21	7,90		
	90	223,00	224,48	41,75	41,86	42,21	11,02		
	110	224,23	225,71	42,98	44,33	42,21	13,85	60,38	11,18

Table 9.3. : Batch acid hydrolysis yield data at 130°C

Acid Conc. (g/ℓ)	Time (min)	Free Xylose (mg/g)	Total Xylose (mg/g)	Free Glucose (mg/g)	Total Glucose (mg/g)	Acetic Acid (mg/g)	Furfural (mg/g)	Acidic Organics (As acetic acid) (mg/g)	H_2SO_4 Consumption (mg/g)
	6	23,94	63,34	11,97	14,96	15,41	1,44		
	15	75,76	114,68	14,93	19,41	24,00	2,22		
	30	134,35	157,16	22,25	23,80	28,25	4,11		
	70	176,36	177,44	32,40	33,94	36,22	5,94		
5	120	184,96	187,47	36,70	39,67	37,65	8,83		
	160	184,96	187,47	39,53	41,09	38,92	11,55		
	200	183,56	186,07	40,93	42,49	39,34	14,54		
	240	182,17	183,30	40,93	43,49	39,62	16,74	53,42	9,68
	4	37,47	82,44	14,99	16,49	18,59	2,55		
	8	93,83	126,94	17,96	20,94	26,30	2,99		
	16	148,13	162,16	25,29	28,28	30,70	3,36		
10	30	182,97	191,19	35,46	36,99	38,25	4,67		
	55	201,64	201,24	39,76	39,86	39,69	8,83		
	80	203,06	202,66	41,18	44,12	42,24	12,06		
	105	198,85	199,85	43,99	45,52	42,24	14,75		
	130	194,68	198,46	43,99	46,91	42,24	16,96	56,76	5,73
	2,75	65,79	105,83	18,59	18,59	23,45	2,86		
	5,75	122,36	144,02	21,42	21,42	29,68	3,40		
	10	158,73	166,40	22,82	28,41	31,92	4,01		
20	20	183,62	185,76	31,12	35,33	36,06	6,45		
	30	193,20	195,33	36,59	38,06	37,98	8,83		
	40	191,84	193,98	39,29	39,42	40,41	11,69		
	50	190,51	191,31	39,29	40,75	40,41	15,73		
	60	189,19	188,67	41,93	42,07	40,41	17,76	60,92	11,47

Table 9.4. : Batch acid hydrolysis yield data at 140 C

Acid Conc. (g/ℓ)	Time (mins)	Free Xylose (mg/g)	Total Xylose (mg/g)	Free Glucose (mg/g)	Total Glucose (mg/g)	Acetic Acid (mg/g)	Furfural (mg/g)	Acidic Organics (as acetic acid) (mg/g)	H_2SO_4 Consumption (mg/g)
	4	16,5	66,36	9,05	12,07	15,68	1,21		
	8	49,41	102,17	12,03	15,05	23,00	3,15		
	16	96,65	137,59	14,98	20,95	27,72	6,69		
3	35	150,66	162,41	22,28	28,25	32,10	14,43		
	60	166,54	169,63	29,80	29,70	33,69	21,21		
	80	170,83	172,48	33,79	35,41	36,40	26,21		
	110	172,24	175,31	35,20	36,82	38,66	31,15		
	140	173,63	178,10	36,59	38,21	39,22	36,73	52,03	9,61
	4,5	43,70	102,48	12,06	16,58	15,67	2,28		
	9	88,43	135,28	18,02	24,03	21,19	4,38		
	16	126,78	155,93	22,45	28,46	25,02	4,81		
5	30	164,72	176,36	32,66	35,75	28,96	6,73		
	55	183,48	185,01	38,43	38,64	35,03	11,90		
	80	179,20	179,31	41,29	38,64	37,74	18,09		
	105	174,97	175,07	41,29	40,05	37,74	29,01		
	130	159,62	161,12	41,29	41,45	38,16	34,27	53,80	10,49
	3	80,13	120,96	13,61	16,63	24,49	2,71		
	6	128,01	149,38	18,10	22,62	28,23	3,78		
	10	154,65	165,66	25,50	28,54	32,08	5,03		
10	20	176,61	175,91	31,35	34,39	34,13	7,47		
	30	180,95	181,70	35,70	35,84	36,88	11,79		
	40	179,52	180,27	37,13	40,14	37,46	16,99		
	50	179,52	178,86	39,96	41,55	38,59	22,13		
	60	178,12	177,46	41,36	42,95	38,87	28,64	54,28	9,48
	3	130,94	145,99	21,07	24,08	34,47	3,19		
	6	160,72	165,35	30,01	31,53	34,91	4,95		
	10	176,93	175,66	34,43	35,95	37,56	7,38		
20	15	179,85	178,58	37,34	35,95	38,73	10,32		
	20	182,73	181,46	40,22	38,83	38,73	14,56		
	25	179,88	180,04	40,22	41,68	39,02	25,28		
	30	179,88	180,04	44,45	44,50	39,02	28,38		
	35	178,48	178,64	47,24	47,29	39,02	31,96	61,41	13.83

Table 9.5. : Batch acid hydrolysis yield data at 150°C

Acid Conc. (g/ℓ)	Time (min)	Free Xylose (mg/g)	Total Xylose (mg/g)	Free Glucose (mg/g)	Total Glucose (mg/g)	Acetic Acid (mg/g)	Furfural (mg/g)	Acidic Organics (as acetic acid) (mg/g)	H_2SO_4 Consumption (mg/g)
	3	33,40	80,46	10,63	13,66	14,73	1,37		
	8	88,98	130,03	16,64	21,17	21,04	3,02		
	15	135,81	153,81	21,09	28,60	28,91	6,73		
3	31	160,03	161,16	29,91	39,95	33,03	14,82		
	47	161,49	165,52	34,28	37,41	34,34	25,00		
	60	162,93	162,65	35,71	38,85	37,21	39,30		
	80	157,24	158,38	35,71	37,42	37,	39,30		
	100	148,81	149,95	37,12	38,83	37,21	42,81	51,28	9,16
	3	82,61	114,15	16,52	18,02	16,37	3,76		
	6	122,73	145,36	20,98	26,94	24,25	4,32		
	10	152,13	160,06	28,33	31,35	28,95	9,70		
	20	168,13	168,78	34,15	37,17	32,01	12,64		
5	30	172,44	171,66	39,90	38,61	33,16	15,26		
	40	171,02	170,24	41,32	41,45	34,72	26,21		
	50	165,40	163,21	45,54	42,86	34,72	29,72		
	60	161,23	159,04	45,54	45,64	34,72	35,60	51,97	9,57
	3	127,43	142,59	21,24	22,75	24,58	4,96		
	6	160,45	163,61	28,74	30,26	29,83	6,06		
	10	169,36	169,55	34,68	34,71	30,57	9,03		
10	15	170,83	171,02	36,15	36,18	32,04	14,37		
	20	169,37	168,11	39,06	42,00	34,07	23,35		
	25	163,62	163,80	40,05	43,43	34,07	34,59		
	29	159,36	160,96	41,92	44,85	34,79	40,79		
	34	157,96	158,15	46,13	47,66	34,50	43,35	56,89	10,11

9.4.2. Hydrolysis Residues

Table 9.6. : Pressure acid hydrolysis residue analyses

Temp (°C)	Acid Conc. (g/ℓ)	Dry Mass (g)	Residue Dry Mass (g)	Residue Analysis (%)				Mass Loss %
				Cellulose	Pentosan	Lignin	Ash	
110	10	100,88	61,43	56,23	6,75	32,33	6,37	39,11
	20	101,34	61,52	55,12	7,02	32,18	5,49	39,29
	40	101,89	61,43	57,70	7,28	30,79	5,89	39,71
120	5	100,80	61,23	53,37	9,24	30,43	7,10	39,26
	10	100,99	61,10	55,18	6,14	32,81	6,35	39,50
	20	101,36	60,71	55,99	5,33	32,04	5,18	40,10
130	5	100,91	59,31	60,08	4,84	31,74	4,48	41,22
	10	101,74	60,21	57,62	5,45	32,53	6,33	40,82
	20	102,02	59,93	60,03	5,30	32,55	2,65	41,26
140	5	100,20	59,45	56,11	3,81	33,41	5,23	40,67
	10	100,20	59,68	55,26	4,03	33,54	6,34	40,44
	20	100,66	57,03	57,98	3,63	32,53	6,05	43,34
150	5	100,20	59,43	57,13	5,87	32,40	4,15	40,69
	10	100,20	57,68	58,94	4,30	32,68	5,24	42,44

These results were then further processed to yield cellulose, pentosan and lignin losses during the various acid hydrolyses, which are reproduced in the following table.

Table 9.7 : Bagasse component losses during pressure acid hydrolysis

Temp (°C)	Acid Conc. (g/ℓ)	Cellulose			Pentosan			Lignin		
		Initial Mass (g)	Final Mass (g)	% Loss (g)	Initial Mass (g)	Final Mass (g)	% Loss (g)	Initial Mass (g)	Final Mass (g)	% Los (g)
110	10	38,77	34,54	11,14	31,64	4,15	86,88	22,44	19,68	11,50
	20	39,05	33,91	13,16	31,78	4,32	86,41	22,54	19,80	12,16
	40	39,26	35,45	9,70	31,95	4,47	86,01	22,66	18,91	16,53
120	5	38,84	32,68	15,86	31,61	5,66	82,09	22,42	18,63	16,90
	10	38,91	33,71	13,36	31,67	3,75	88,16	22,46	20,05	10,73
	20	39,05	33,99	12,96	31,79	3,24	89,81	22,54	19,45	13,71
130	5	38,88	35,63	8,36	31,65	2,87	90,93	22,44	18,82	16,13
	10	39,20	34,69	11,51	31,91	3,28	89,72	22,63	19,59	13,43
	20	39,31	35,98	8,47	31,99	3,18	90,06	22,69	19,51	14,01
140	5	38,61	33,36	13,60	31,42	2,27	92,78	22,28	19,86	10,85
	10	38,61	32,98	14,58	31,42	2,41	92,33	22,28	20,02	10,16
	20	38,78	33,07	14,72	31,57	2,07	93,44	22,39	18,55	17,15
150	5	38,61	33,95	12,07	31,42	3,49	88,89	22,28	19,26	13,55
	10	38,61	34,00	11,94	31,42	2,48	92,11	22,28	18,85	15,39

9.5 Discussion

9.5.1 Acetic Acid Formation

The production of acetic acid during the dilute acid hydrolysis of bagasse hemicellulose at temperatures above 100°C approaches a maximum value and at this point all the acetyl groups in the hemicellulose fraction should have been converted to acetic acid. For the temperatures at or below 120°C an average of 43,1 mg acetic acid/g of dry bagasse is produced. However, at temperatures above 120°C there is a gradual decrease in the maximum acetic acid production. This is probably due to the fact that acetic acid boils at 118,1°C at normal atmosphere pressure and an equilibrium is set up between the vapour space and the hydrolysate in the hydrolysis reactor which accounts for the lower acetic acid analyses from the liquid phase at the higher temperatures.

At each hydrolysis temperature the amount of acidic organics (i.e. NaOH consuming organics) expressed as acetic acid tends to increase with increasing acid concentration. Since the amount of acetic acid at the end of each hydrolysis remains constant at a given temperature it would seem that additional organic acids are being produced as the hydrolysis conditions increase in severity.

9.5.2 Furfural Formation

Furfural is an important parameter that must be monitored during dilute acid hydrolyses of bagasse especially if the xylose solution is to be fermented. It represents a loss in pentose production and the furfural is also toxic to many micro-organisms - a furfural concentration of 0,740 g/ℓ results in a 25% inhibition of alcohol production from glucose by yeast[120].

Furfural formation increases with increasing temperature for a given acid concentration during the dilute acid hydrolysis of bagasse at temperatures above 100°C as shown in Figure 9.1.

At a constant temperature furfural formation also appears to be comparable at the different acid concentrations (at conditions of maximum xylose yield).

However, there may have been some sugar degradation in the G.C. injection port during the furfural analyses which could account for some of this furfural formation. In a recent publication Uobe et al[170] have found that furfural can be produced by sugar degradation (from xylose, arabinose and glucose) during G.C. analyses of neutralised sugar solutions. Since these furfural results have all been produced using the same G.C. conditions they should still be suitable for comparative purposes. For example, furfural formation at the hydrolysis end at 150°C and 10 g/ℓ H_2SO_4 is approximately five times greater than at 110°C and 10 g/ℓ H_2SO_4.

9.5.3 Glucose Formation

The amount of glucose produced in the dilute acid hydrolyses increases with increasing hydrolysis time at any given acid hydrolysis condition. Some of this glucose can be accounted for in the bagasse feed which contained approximately 1% sucrose for these acid hydrolyses. In the presence of dilute acid this sucrose will be broken down into equal amounts of glucose and fructose. The balance of the glucose is due to the removal of hexose side chains from the hemicellulose fraction and hydrolysis of amorphous cellulose.

The maximum total glucose production during the acid hydrolyses is similar for all the acid hydrolysis conditions and ranged from 40 to 47 mg glucose/g dry bagasse.

9.5.4 Acid Consumption

Bagasse contains an amount of ash, usually termed 'active ash', which will react with and neutralise sulphuric acid. At each hydrolysis temperature the sulphuric acid consumption tends to be slightly higher at the highest acid concentration. However, there were small differences and the average

sulphuric acid consumption was 10,60 mg H_2SO_4/g of dry bagasse.

9.5.5 Xylose Formation

The amounts of free and total xylose produced in the dilute acid hydrolyses increase with increasing hydrolysis time until a maximum value and then begin to decrease as a result of sugar degradation in the acidic medium. A maximum yield of approximately 220 mg xylose/g of dry bagasse is obtained at 120°C which corresponds to a xylose yield of 61,8% based on initial pentosan in the bagasse.

This maximum value of xylose yield decreases at temperatures above 120°C and at 150°C it reaches a value of approximately 170 mg/g of dry bagasse as shown in Figure 9.2. This decrease in yield can be attributed to the increase in sugar degradation at the higher temperatures.

The total xylose analyses are also higher than the free xylose analyses during the initial stages of acid hydrolysis at all the hydrolysis conditions tested. This is indicative of acid hydrolysis reaction pathway of bagasse xylan, i.e. xylose → soluble xylan (oligomers) → xylose (monomer). However, as the times approach at which maximum xylose yields occur, the total and free xylose analyses converge.

9.5.6 Hydrolysate Composition

Apart from xylose and glucose there is also some arabinose formation from the hemicellulose fraction during the dilute acid hydrolysis of bagasse.

A typical dilute acid hydrolysate from a bagasse prehydrolysis gave the following approximate composition under conditions at which there is not excessive furfural formation.

Table 9.8 Approximate acid hydrolysate composition

Compounds	Yield mg/g dry bagasse
Xylose	220
Arabinose	40
Glucose*	35
Acetic Acid	45

*This excludes any glucose produced from residual sucrose in the bagasse.

9.5.7 Residue Analyses

At all the acid hydrolysis conditions tested above 100°C it would appear that the amounts of lignin and cellulose lost in the hydrolyses are very similar. On average 12,3% of the initial cellulose and 13,7% of the initial lignin appear to be solubilised during the dilute acid bagasse hemicellulose hydrolyses.

The pentosan analyses of bagasse feed and residue, however, indicate a trend of increasing pentosan extraction with increasing severity of hydrolysis conditions. This pentosan loss could also not be solely accounted for by the xylose in the hydrolysate. At 120°C there was approximately 90% pentosan extraction and only 60% of it could be accounted for by the xylose analyses.

This anomaly can be explained in terms of the composition of bagasse hemicellulose and the characteristics of the TAPPI pentosan determination.

The hemicelluloses of bagasse consist mainly of xylans[14] which have a straight chain backbone of xylose monomers connected by β(1:4) bonds. Attached to these chains are

substituents of the hydroxyl groups such as arabinose, acetate and 4-O-methylglucuronic acid groups. Thus while the bulk of the hydrolysis product is xylose there will also be some arabinose, uronic acid and uronide formation.

The pentosan content of the bagasse is determined by analytical method TAPPI T19m-50[158], which is essentially a hydrochloric acid hydrolysis of the hemicellulose fraction followed by gravimetric analysis of the furfural produced. This furfural is formed by pentose sugar (xylose and arabinose) degradation and breakdown of the uronic acids and uronides.

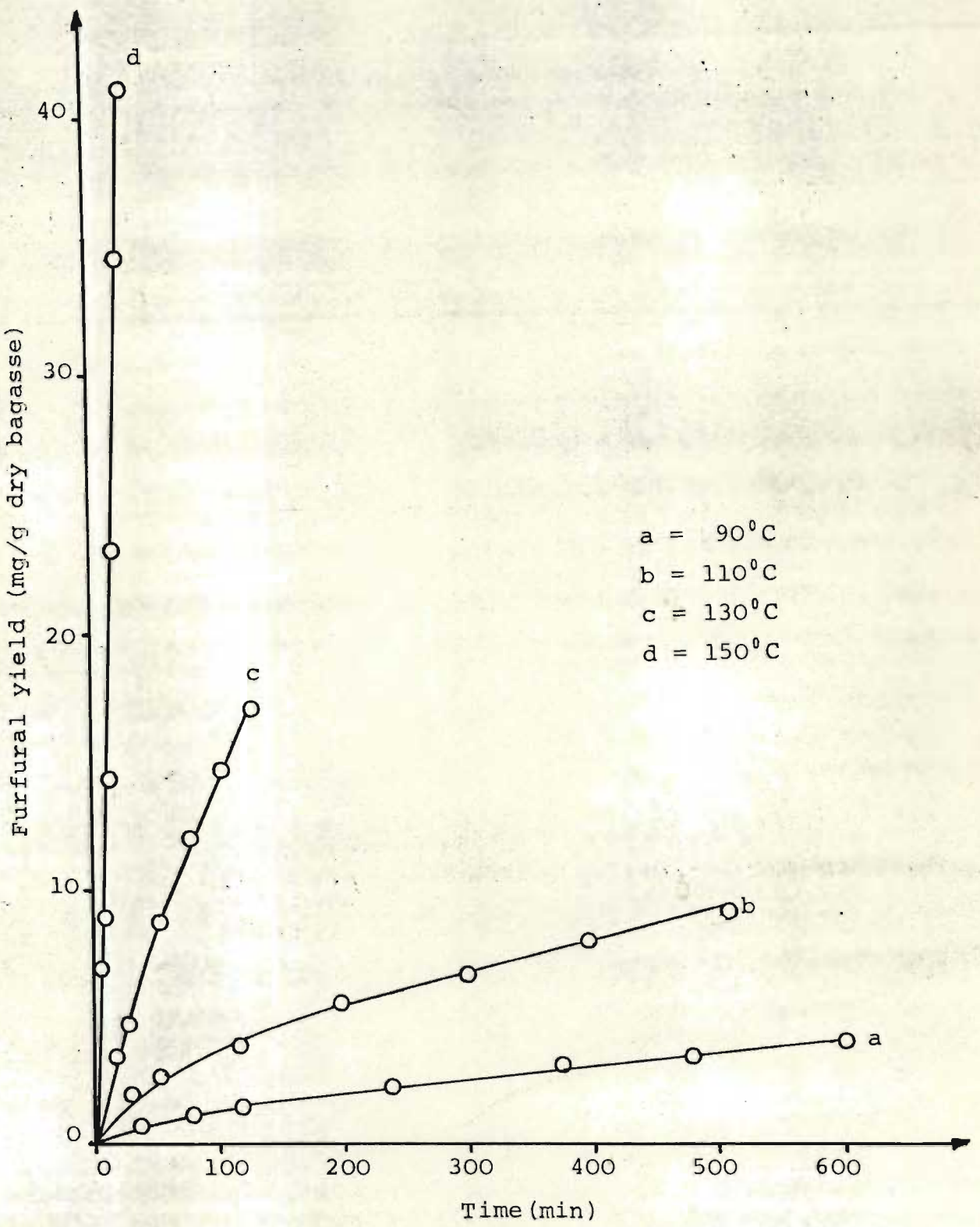


Figure 9.1. Furfural formation during acid hydrolyses at 10 g/ℓ H_2SO_4

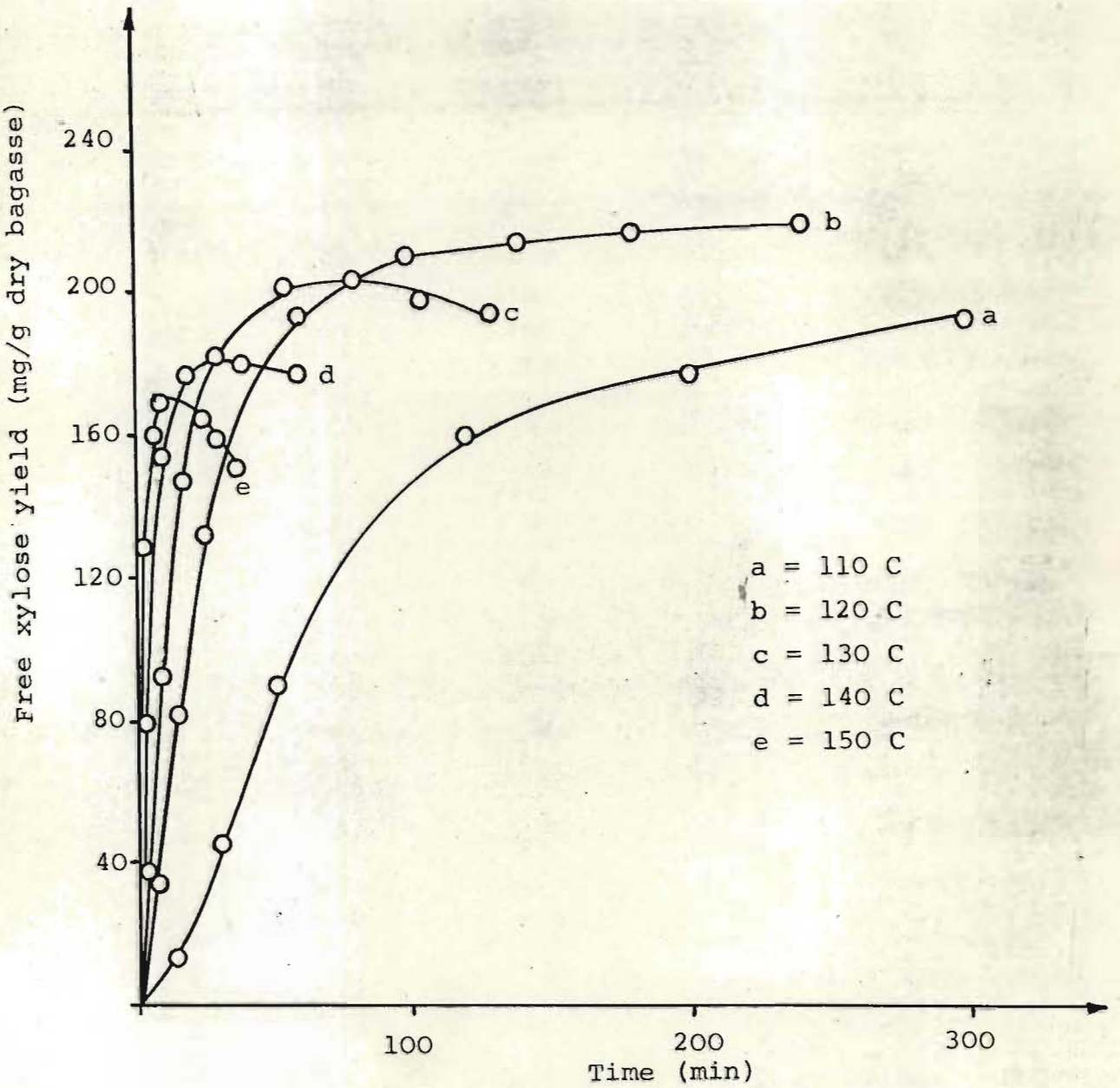


Figure 9.2. Free xylose yield during acid hydrolyses at 10 g/ℓ H_2SO_4

10. ENZYME HYDROLYSIS OF WHOLE AND PRETREATED BAGASSE

10.1 Objective

The effect of dilute acid hemicellulose hydrolysis conditions on the susceptibility of bagasse cellulose after attritor milling to enzymatic saccharification by Trichoderma viride cellulase was determined. This susceptibility was also compared with that of other pretreated bagasse substrates.

10.2 Substrates for Enzymatic Hydrolysis

10.2.1 Whole Bagasse

The whole bagasse was obtained from the SMRI and was hammermilled according to the method in Section 5.2. Its composition is available in Table 5.1.

10.2.2 Acid Treated Bagasse

All the dilute acid treatments to remove bagasse hemicellulose were performed using a liquid to solid ratio of 15 : 1 in 2 litre reactors (Section 4.1 and 4.2) with SMRI bagasse as substrate.

A prehydrolysed bagasse sample was selected (110°C, 40 g/ℓ H_2SO_4 and 120 min) to indicate the typical enhancement in enzymatic digestibility of the cellulose fraction after dilute acid hydrolysis. The composition of this residue after air drying is shown in Table 10.1.

Table 10.1 : Composition of prehydrolysed bagasse (110°C, 40 g/ℓ H_2SO_4 and 120 min)

Analysis	%
Cellulose	57,7
Pentosan	7,3
Lignin	29,8
Ash	5,9
Moisture	9,9

10.2.3 Attritor Milled Prehydrolysed Bagasse

Two groups of prehydrolysed bagasse samples were selected to determine the influence of acid hydrolysis conditions on the enzymatic susceptibility of the cellulose fraction after attritor milling.

(i) Constant temperature 130°C. Acid concentration 5, 10 and 20 g/ℓ H_2SO_4.

(ii) Constant acid conc. 20 g/ℓ H_2SO_4. Temperature 100°, 110°, 120° and 130°C.

These acid hydrolysis residues were milled in a type 1S pilot model attritor made by Torrance and Sons, England. This machine was powered by a 0,37 kw motor and 10 mm steatite balls were used as the grinding medium. Each residue was milled for 30 minutes at 5% consistency and 240 rpm (75g prehydrolysed bagasse in 1,5ℓ water). These slurries were then filtered and air dried to approximately 70% moisture content before enzymatic hydrolysis.

Table 10.2 : Composition of attritor milled prehydrolysed bagasse.

Analysis (%)	20 g/ℓ H_2SO_4			130°C		
	100°C 300 min	110°C 260 min	120°C 110 min	5g/ℓ H_2SO_4 240 min	10g/ℓ H_2SO_4 130 min	20g/ℓ H_2SO_4 65 min
Cellulose	52,12	55,12	55,99	60,08	57,62	60,03
Pentosan	11,01	7,02	5,33	4,84	5,45	5,30
Lignin	30,76	32,18	32,04	31,74	32,53	32,55
Ash	4,74	5,49	5,18	4,48	6,33	2,65
Moisture	72,17	68,57	64,20	71,74	71,00	67,68

10.2.4 Bleached Bagasse Pulp

This sample was obtained from Stanger Pulp and Paper (now SAPPI Fine Papers) and is used for the manufacture of high quality paper.

Depithed bagasse was treated with caustic soda for 18-20 minutes at 174°C (820 kPa) and then washed on a rotary

vacuum filter. The pulp was further treated with chlorine to reduce the lignin content and then finally bleached with hypochlorite to yield high quality pulp. The composition of the pulp is given in Table 10.3.

Table 10.3. : Composition of bleached bagasse pulp

Analysis	%
Cellulose	72,4
Pentosan	25,2
Lignin	0,5
Ash	0,1
Moisture	8,6

10.2.5 Acid Extracted/Caustic Treated Bagasse

Prehydrolysed bagasse (100°C, 1N H_2SO_4 for 2 h) was soda pulped under the following conditions

Temperature	170°C
NaOH concentration	0,32g NaOH/g bagasse
Time	24 min heat up and 15 min at temperature

This treatment produced a bagasse pulp with a low lignin content but there was a significant cellulose loss during the pulping (±30% loss based on cellulose in original whole bagasse). The composition of this pulp is shown in Table 10.4.

Table 10.4 : Composition of acid extracted/caustic treated bagasse

Analysis	%
Cellulose	83,5
Pentosan	2,6
Lignin	3,0
Ash	9,0
Moisture	24,2

10.3 Method of Enzymatic Hydrolysis

The cellulase was obtained from Trichoderma reesei QM9414 cultures grown in batch fermenters on commercial bagasse pulp (soda cooked) according to the method of Sternberg and Dorval[178]. The cellulase solution was then concentrated by ultrafiltration and had the following enzyme assay :

Cellulase (FPA)	=	1,09 IU/mℓ
β glucosidase	=	0,24 IU/mℓ

(FPA = filter paper activity)

In the enzymatic hydrolysis tests 2,5 g cellulose equivalent of each substrate was used in a 100 mℓ cellulase solution and the hydrolyses were performed in 150 mℓ round bottom stirred flasks maintained at 48 °C. The pH was buffered at 4,8 with acetate buffer.

The enzymatic hydrolyses were performed at two enzyme concentrations: 20 IU and 40 IU (FPA)/g cellulose. Supplementary β glucosidase (BDH product No. 39049) was also added to the cellulase enzyme solutions in the following proportions :

100 IU β glucosidase/100 mℓ enzyme for 40 IU/g cellulose hydrolyses

50 IU β glucosidase/100 mℓ enzyme for 20 IU/g cellulose hydrolyses.

The enzyme solutions were preserved with 20 ppm of tetracycline hydrochloride and enzyme dilutions for the different cellulase concentrations were made up with 0,05 M acetate buffer.

Five 3 mℓ samples were withdrawn from each hydrolysis over the 48 hour reaction time, filtered through glass fibre filter paper and then analysed for sugars by HPLC.

10.4 Results

The following substrates were treated with 40 IU/g cellulose :

1. Whole bagasse.
2. Acid treated bagasse (110°C, 40 g/ℓ H_2SO_4, 120 min)
3. Attritor milled acid treated bagasse (100°C 20 g/ℓ H_2SO_4, 300 min)
4. Attritor milled acid treated bagasse (110°C 20 g/ℓ H_2SO_4, 260 min)
5. Attritor milled acid treated bagasse (120°C 20 g/ℓ H_2SO_4, 110 min)
6. Attritor milled acid treated bagasse (130°C 20 g/ℓ H_2SO_4, 65 min)
7. Attritor milled acid treated bagasse (130°C 10 g/ℓ H_2SO_4, 130 min)
8. Attritor milled acid treated bagasse (130°C 5 g/ℓ H_2SO_4, 240 min)
9. Bleached bagasse pulp.
10. Acid extracted/caustic treated bagasse.

The following substrates were treated with 20 IU/g cellulose :

11. Whole bagasse.
12. Acid treated bagasse (110°C, 40 g/ℓ H_2SO_4, 120 min)
13. Attritor milled acid treated bagasse (130°C, 10 g/ℓ H_2SO_4, 130 min)
14. Bleached bagasse pulp.
15. Acid extracted/caustic treated bagasse.

The enzymatic hydrolysis results are presented in Tables 10.5 and 10.6.

Table 10.5. : Enzymatic hydrolysis results

Code	Hydrolysis Time (h)	Xylose (g/100 mℓ)	Glucose (g/100 mℓ)	Cellobiose (g/100 mℓ)	% Cellulose Hydrolysed
1	2,0	N.D.	0,17	0,18	12,8
	5,0	N.D.	0,26	0,25	18,8
	24,0	0,07	0,46	0,28	26,8
	26,0	0,10	0,49	0,27	28,0
	48,0	0,12	0,56	0,22	28,4
2	2,0	N.D.	0,17	0,23	14,8
	5,0	N.D.	0,29	0,56	31,6
	24,0	0,04	0,70	0,52	44,8
	26,0	0,06	0,73	0,47	44,0
	48,0	0,10	1,05	0,45	54,8
3	2,25	0,03	0,50	0,31	29,6
	6,0	0,05	1,03	0,27	47,6
	24,0	0,09	2,12	0,12	80,9
	26,25	0,10	2,18	0,12	83,1
	47,5	0,12	2,37	0,10	88,8
4	2,25	0,03	0,55	0,30	31,2
	6,0	0,05	1,02	0,41	52,4
	24,0	0,12	2,16	0,09	80,9
	26,25	0,10	1,90	0,08	79,3
	47,5	0,15	2,39	0,10	89,6
5	2,25	0,02	0,50	0,32	30,0
	6,0	0,02	1,03	0,26	47,2
	24,0	0,09	2,07	0,09	77,6
	26,25	0,09	2,20	0,11	83,2
	47,5	0,10	2,33	Trace	84,0
6	2,25	N.D.	0,51	0,37	32,4
	6,0	N.D.	1,02	0,25	46,4
	24,0	0,06	2,06	0,13	79,0
	26,25	0,08	2,08	0,10	78,7
	47,5	0,12	2,53	Trace	91,2
7	2,25	0,04	0,50	0,27	28,4
	6,0	0,06	0,98	0,33	47,6
	24,0	0,04	2,11	0,18	82,9
	26,5	0,05	2,13	0,16	82,6
	47,5	0,04	2,39	Trace	86,0
8	2,25	N.D.	0,49	0,34	30,4
	6,0	0,05	1,06	0,29	48,8
	24,0	0,06	2,16	0,13	82,4
	26,25	0,08	2,21	0,10	83,1
	47,5	0,11	2,58	Trace	92,8

N.D. = not detected

Table 10.6 Enzymatic hydrolysis results

Code	Hydrolysis Time (h)	Xylose (g/100 m)	Glucose (g/100 m)	Cellobiose (g/100 m)	% Cellulose Hydrolysed
9	2,0	0,12	0,59	0,28	31,6
	5,0	0,16	0,81	0,28	39,6
	24,0	0,31	1,82	0,20	73,0
	26,0	0,34	1,88	0,19	75,0
	48,0	0,41	2,30	0,14	88,0
10	2,0	N.D.	0,43	0,49	34,0
	6,0	0,03	1,04	0,57	59,0
	24,0	0,07	2,12	0,32	88,5
	26,0	0,05	2,29	0,31	94,2
	48,0	0,05	2,80	N.D.	100,8
11	2,0	N.D.	0,24	0,17	15,2
	5,0	N.D.	0,32	0,15	17,2
	24,0	0,10	0,49	N.D.	17,6
	26,0	0,11	0,52	N.D.	18,8
	48,0	0,15	0,62	N.D.	22,4
12	2,0	N.D.	0,22	0,19	15,2
	5,0	N.D.	0,35	0,19	20,0
	24,0	0,08	0,64	0,09	26,4
	26,0	0,10	0,67	0,09	27,2
	48,0	0,13	0,90	0,18	39,2
13	3,0	0,06	0,29	0,20	18,0
	5,0	0,07	0,49	0,25	27,2
	24,0	N.D.	1,13	0,43	57,2
	26,0	N.D.	1,23	0,36	58,0
	48,0	0,10	1,41	0,30	62,0
14	3,0	0,05	0,34	0,12	16,8
	5,0	0,14	0,61	0,35	35,2
	24,0	0,19	1,34	0,44	65,2
	26,0	0,17	1,47	0,43	69,2
	48,0	0,26	1,70	0,35	74,4
15 *	3,0	N.D.	0,31	0,30	25,8
	5,0	N.D.	0,52	0,54	45,1
	24,0	N.D.	1,33	1,51	77,3
	26,0	N.D.	1,39	0,53	80,5
	48,0	N.D.	1,62	0,47	87,4

* 23 IU/g cellulose and 2,17 g cellulose/100 mℓ enzyme (due to error in moisture)

N.D. = Not detected

10.5 Discussion

The enzymatic hydrolyses performed at 40 IU/g cellulose on the attritor milled bagasse samples which had been prehydrolysed under different conditions indicated that there was little or no difference in enzymatic susceptibility (see Table 10.5). On average 80% of the cellulose in these substrates was hydrolysed in 24 hours.

These hydrolyses were then compared with others performed on various bagasse-derived substrates under the same hydrolysis conditions (see Figure 10.1). The conversion of the acid extracted bagasse which was subsequently soda pulped under severe conditions was very good. The attritor milled acid extracted material gave good conversion comparable with that of commercial bleached bagasse pulp and the acid treated bagasse gave significantly higher sugar yields than the raw bagasse.

Attritor milling would thus appear to have considerable potential as a pretreatment of acid extracted bagasse for enzymatic hydrolysis. The enhanced susceptibility of prehydrolysed bagasse is comparable to that achieved with expensive commercial bleached bagasse pulp and a relatively low power input[5] is required for attritor milling.

A number of these enzymatic hydrolyses were repeated using a lower cellulase concentration - 20 IU/g cellulose (see Figure 10.1). With this enzyme concentration the conversion of attritor milled prehydrolysed bagasse was $\pm$ 12% lower than for commercial bleached bagasse pulp after 48 hours.

Enzymatic hydrolyses of identical substrates performed at different enzyme concentrations indicated that higher cellulose conversions were achieved at the higher enzyme concentrations. However, the efficiency of utilisation of the enzyme is also an important economic aspect and the Table 10.7 shows the efficiency of enzyme-utilisation after 24 hours hydrolysis for the hydrolyses described in this investigation

Table 10.7 : Efficiency of enzyme utilisation

Treatment Code	IU(FPA) Used	Glucose Produce mg/100 mℓ	mg Glucose/ IU(FPA)	Efficiency of Utilisation %*
1	2,5x40	744,4	74,4	2,9
2	2,5x40	1244,3	12,44	4,8
3	2,5x40	2247,0	22,47	8,7
4	2,5x40	2247,0	22,47	8,7
5	2,5x40	2155,3	21,55	8,3
6	2,5x40	2194,2	21,94	8,5
7	2,5x40	2302,5	23,03	8,9
8	2,5x40	2288,7	22,89	8,8
9	2,5x40	2027,6	20,28	7,8
10	2,5x40	2458,1	24,58	9,5
11	2,5x20	488,8	9,78	3,8
12	2,5x20	733,3	14,67	5,7
13	2,5x20	1588,7	31,77	12,3
14	2,5x20	1810,9	36,22	14,0
15	2,17x23	1863	37,27	14,4

* This value was determined from the ratio of the observed saccharification of the substrate to that of the predicted saccharification based on one IU(FPA) giving 10,8 mg glucose/h or 259,2 mg/24h.

From the table it can be seen that a dilute enzyme is more efficient than the concentrated enzyme, but the lower conversion then becomes an important aspect in the enzymatic hydrolysis process.

Mandels et al[179] have already highlighted these and other difficulties which will occur in the practical application of enzymatic hydrolysis of cellulose.

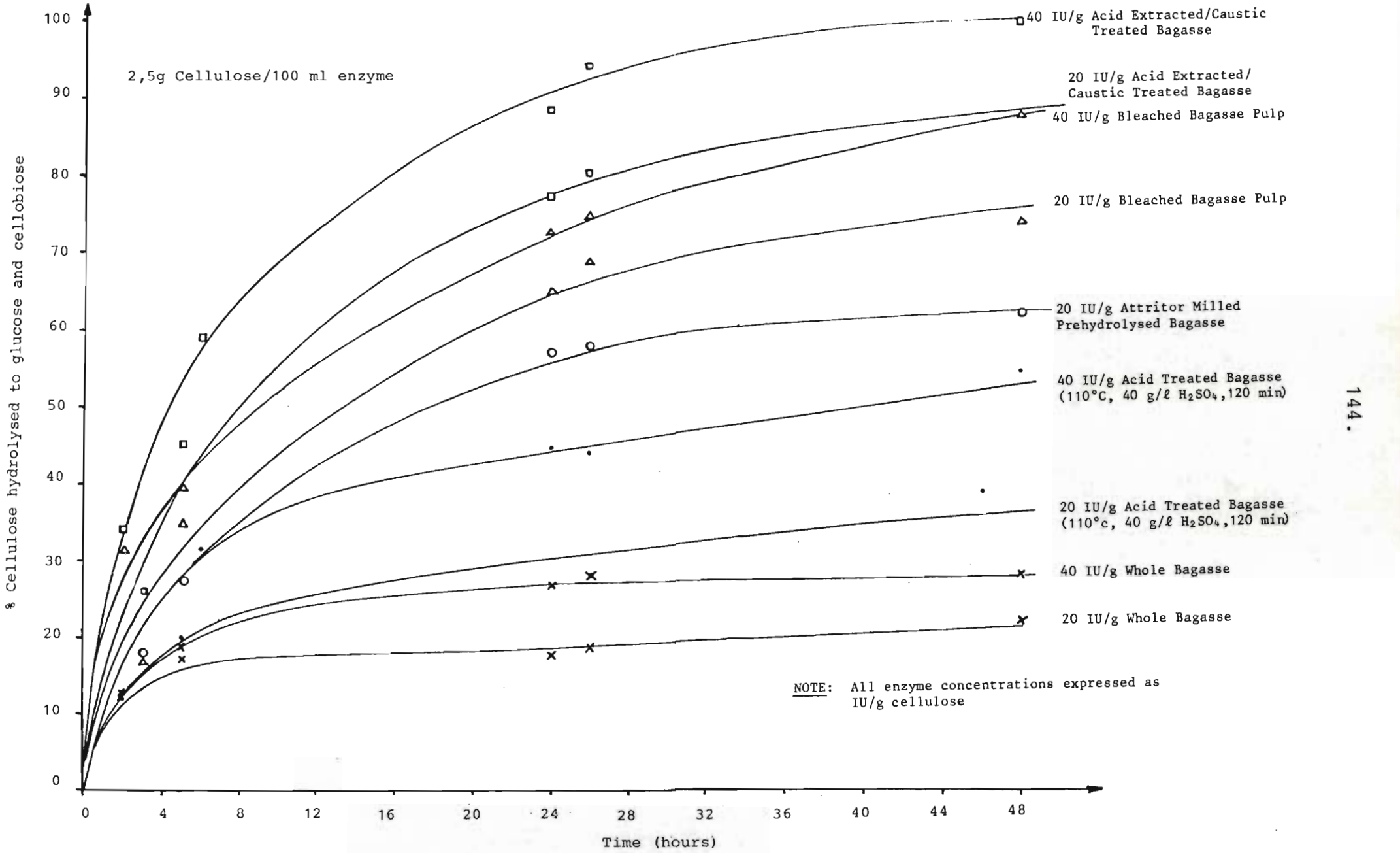


FIGURE: 10.1. Hydrolysis of bagasse substrates

11. KINETICS OF XYLOSE FORMATION

11.1 Objectives

There is extensive evidence in the literature that bagasse hemicellulose consists of two fractions, an easily hydrolysable portion and a more resistant fraction[147, 149, 151]. The relative amounts of xylose in these two fractions were thus determined.

A mathematical expression for total xylose yield over the entire range of acid hydrolysis conditions tested during the batch hydrolyses was also obtained by non-linear least squares fitting using the Nelder and Mead simplex procedure[180].

11.2 Hydrolysis Kinetics

Bagasse hemicellulose hydrolysis follows a complex reaction pathway involving several different oligosaccharide intermediates.

Saeman[148], however, found that a simple two-step reaction model adequately described the formation of glucose during wood hydrolysis. In Saeman's model each reaction step was assumed to be a first order, irreversible reaction. A similar model can be applied to hemicellulose hydrolysis assuming a single hemicellulose fraction :

$$\underset{\text{hemicellulose}}{A} \xrightarrow{k_1} \underset{\text{xylose}}{B} \xrightarrow{k_2} \underset{\text{decomposition products}}{C} \qquad \ldots\ (1)$$

The net rate of change in xylose concentration is given by :

$$\frac{d\{B\}}{dt} = k_1\{A\} - k_2\{B\} \qquad \ldots\ (2)$$

Expressing the polymer concentration in terms of potentially hydrolysable xylose, the rate of release of xylose is equal to the rate of decrease of polymer concentration :

$$\frac{d\{A\}}{dt} = -k_1\{A\} \qquad \ldots\ (3)$$

Defining the initial polymer and xylose concentration at time zero as $\{A_o\}$ and $\{B_o\}$ respectively the differential equations (2) and (3) can be solved to produce equation (4) which expresses xylose concentration as a function of time :

$$\{B\} = \frac{k_1 \{A_o\}}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) + \{B_o\} e^{-k_2 t} \quad .... (4)$$

The rate constants k_1 and k_2 are dependent on the acid concentration and temperature. Saeman[148] found that k_1 and k_2 follow the classical Arrhenius dependence on temperature and are proportional to the acid concentration to some power r.

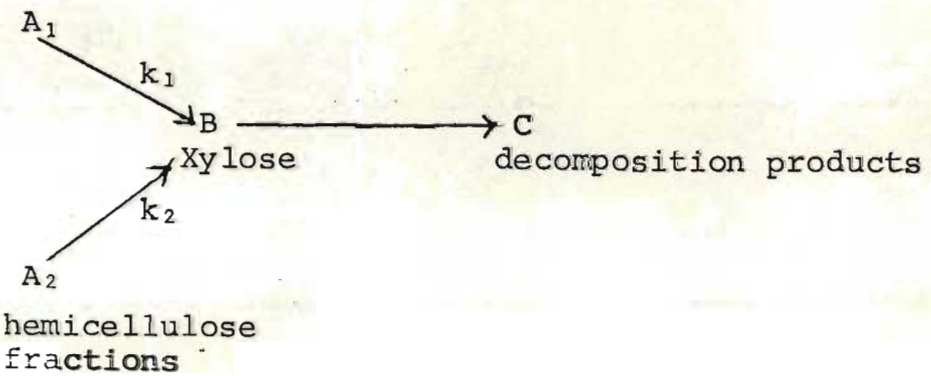
$$k = k' S^r \exp\left(\frac{-E}{RT}\right) \quad .... (5)$$

where S = sulphuric acid conc in g/100 mℓ
T = temperature in degrees K
E = activation energy in kcal/g mole
k = reaction rate in min^{-1}

For short preheat times during acid hydrolyses the value of $\{B_o\}$ is approximately zero. Equation (4) can thus be reduced to :

$$\{B\} = \frac{k_1 \{A_o\}}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \quad .... (6)$$

Since there is extensive evidence in the literature that bagasse hemicellulose consists of two fractions[147, 149, 151] some modification of the model assuming a single hemicellulose fraction is desirable. Saeman's model can be modified to account for two simultaneous first order reactions with a common product, xylose, which then decomposes according to a first order relation :



The sugar concentration can be expressed as a function of time using a similar procedure to that used to obtain equation (6) :

$$\{B\} = \frac{k_1\{A_1\}}{k_3 - k_1}(e^{-k_1t} - e^{-k_3t}) + \frac{k_2\{A_2\}}{k_3 - k_2}(e^{-k_2t} - e^{-k_3t}) \quad \ldots\ (7)$$

Equation (7) can be differentiated to obtain the time at which maximum xylose yield occurs for a given set of reaction conditions :

$$\frac{d\{B\}}{dt} = \frac{k_1\{A_1\}}{k_3 - k_1}(-k_1e^{-k_1t} + k_3e^{-k_3t}) + \frac{k_2\{A_2\}}{k_3 - k_2}(-k_2e^{-k_2t} + k_3e^{-k_3t}) = 0 \quad \ldots\ (8)$$

By substituting the values of k_1, k_2, k_3, A_1 and A_2 into equation (8) for a given set of reaction conditions, the value of t at which maximum xylose yield occurs can be determined. The value of the maximum xylose yield can then be determined from equation (7).

11.3 Hydrolysis Data

Batch dilute acid hydrolyses have been performed over the temperature range 80° to 150°C at acid concentrations at and below 40 g/ℓ sulphuric acid at a liquid to solid ratio of 15 : 1 (see sections 8.4 and 9.4).

A complete set of the xylose yield data (as used in the computer programme) is available in Appendix I (lines 5 to 189). The data are divided into five columns :

(i) Temperature (°C)
(ii) H_2SO_4 concentration (g/100 mℓ).
(iii) Time (min)
(iv) Free xylose yield (mg/g bagasse).
(v) Total xylose yield (mg/g bagasse)

11.4 Xylose Content of Hemicellulose Fractions

The total xylose yields from the batch dilute acid hemicellulose hydrolyses performed at atmospheric pressure at a liquid to solid ratio of 15 : 1 (80° to 100°C and 10 to 40 g/ℓ H_2SO_4) were used to determine the amounts of potential xylose in the two bagasse hemicellulose fractions. (See Appendix I - lines 5 to 61)

Only the acid hydrolyses performed under moderate conditions were used so that insignificant amounts of xylose would be lost by decomposition. Under more severe hydrolysis conditions the furfural formed cannot be converted to its xylose equivalent due to the inefficient xylose → furfural reaction[154] and the fact that furfural is also produced by dehydration of arabinose produced during the hydrolysis.

A xylose content of 270 mg/g dry bagasse was assumed (see Section 5.5) and the log of the xylose fraction in the residue was calculated for the various hydrolysis times at the range of hydrolysis conditions tested. These results are presented in Tables 11.1, 11.2 and 11.3.

The first order plots of xylose in the bagasse residue against time produced curves with slopes that show a break after a certain amount of xylose extraction. (See Figures 11.1 to 11.8.) This confirms that the bagasse hemicellulose consists of two fractions each having different reactivities.

The relative amount of xylose in the two hemicellulose fractions was obtained from the average y intercept of the lines representing the more resistant hemicellulose fractions in Figures 11.1 to 11.8. This average value of -0,41 corresponds to an easily hydrolysable fraction of 165 mg xylose/g bagasse and a resistant fraction containing 105 mg xylose/g bagasse.

11.5 Kinetics of Total Xylose Formation

11.5.1 Non-Linear Least Squares Curve Fitting

Various models for the kinetics of xylose formation were fitted to the xylose yield data. A simplex programme for

function minimisation[180] was used to minimise the sum of squares of errors (SSE); the sum of squares of the difference between model-predicted xylose yields and the measured values. The simplex program uses a trial and error method of finding the best values for the parameters in the model. The programme begins with several guesses for the parameter values and computes the SSE values corresponding to these guesses. Then by comparing these SSE values, it determines which direction to move for its next guess for the parameter values. This process continues until further guesses yield no decrease in the SSE.

This programme also uses a quadratic fit to obtain parameter variance estimates and faster convergence when applied to non-linear least squares fitting.

Table 11.1 : First order plot data for hydrolyses at 80°C

Acid Conc (g/ℓ)	Time (min)	$\frac{\text{Xylose in Residue}}{\text{Xylose in Bagasse}} = \frac{Cr}{Co}$	Log $\frac{Cr}{Co}$
20	100	0,819	-0,087
	180	0,635	-0,197
	260	0,554	-0,256
	420	0,442	-0,354
	745	0,342	-0,465
	1025	0,306	-0,514
	1320	0,275	-0,561
40	50	0,766	-0,116
	90	0,609	-0,215
	130	0,519	-0,285
	180	0,429	-0,368
	300	0,319	-0,496
	420	0,304	-0,518
	540	0,283	-0,548
	660	0,263	-0,580

Table 11.2 : First order plot data for hydrolyses at 90°C

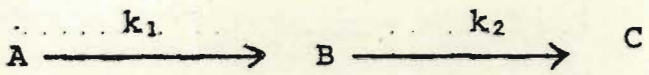
Acid Conc. (g/ℓ)	Time (min)	$\frac{\text{Xylose in Residue}}{\text{Xylose in Bagasse}} = \frac{Cr}{Co}$	Log $\frac{Cr}{Co}$
10	90	0,790	-0,102
	180	0,610	-0,215
	260	0,512	-0,290
	480	0,400	-0,398
	720	0,326	-0,487
	955	0,289	-0,539
	1200	0,258	-0,589
20	50	0,747	-0,129
	90	0,611	-0,214
	130	0,503	-0,298
	245	0,370	-0,432
	360	0,312	-0,506
	480	0,270	-0,564
	600	0,255	-0,594
40	25	0,771	-0,113
	45	0,588	-0,231
	68	0,487	-0,313
	122	0,333	-0,477
	180	0,302	-0,520
	240	0,281	-0,551
	300	0,266	-0,575

Table 11.3 : First order plot data for hydrolyses at 100°C

Acid Conc. (g/ℓ)	Time (min)	Xylose in Residue / Xylose in Bagasse = Cr/Co	Log Cr/Co
10	38	0,752	-0,124
	80	0,572	-0,242
	120	0,459	-0,338
	240	0,331	-0,480
	375	0,273	-0,564
	480	0,246	-0,608
	600	0,221	-0,656
20	20	0,753	-0,123
	40	0,580	-0,237
	60	0,462	-0,336
	120	0,318	-0,498
	180	0,281	-0,551
	240	0,255	-0,593
	300	0,244	-0,649
40	10	0,772	-0,112
	20	0,584	-0,234
	30	0,472	-0,326
	60	0,330	-0,482
	90	0,283	-0,549
	120	0,267	-0,573
	150	0,247	-0,608

11.5.2 Single Hemicellulose Fraction

Although the semilogarithmic time plots (Figures 11.1 to 11.8) indicate that the bagasse hemicellulose consists of two fractions which react at different rates, it was decided to fit the total xylose yield data to a simple consecutive first order system :

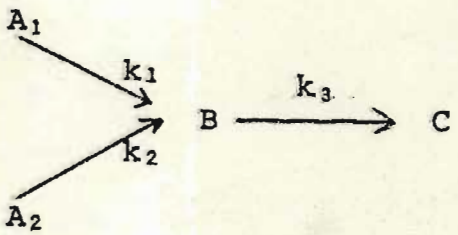


The two constants k_1 and k_2 were assumed to follow the Arrhenius dependence on temperature and to be proportional to acid concentration.

A poor fit was obtained using this first order consecutive reaction model. The SSE was $9,423 \times 10^4$ using the 185 data points (ND) and fitting four parameters (NP), k_1, E_1, k_2 and E_2. The standard deviation, defined as SQRT (SSE/(ND-NP)), was 22,82.

11.5.3 Two Hemicellulose Fractions

From the semilogarithmic time plots (Figures 11.1 to 11.8) it appears that the bagasse hemicelluloses consist of two fractions A_1 and A_2, containing 165 and 105 mg xylose/g bagasse respectively. The xylose yield data was thus fitted to a simple first order consecutive reaction system which includes two simultaneous reactions forming a common product :



The three rate constants k_1, k_2 and k_3 were assumed to follow the Arrhenius dependence on temperature and to be proportional to acid concentration. In the computer programme the Arrhenius expression includes a reference temperature (T_R) to ensure that the exponent does not become excessively large, i.e.

$$k = k_o \exp\left[\frac{-E}{R}\left(\frac{1}{T} - \frac{1}{T_R}\right)\right]$$

The best fit was obtained when the activation energy of xylose decomposition was set at 33,56 k cal/g mole[154] and the other parameters, k_1, k_2, k_3, E_1 and E_2 were allowed to vary. The SSE was 1,883 x 10^4 and the standard deviation was 10,23.

When the activation energy of decomposition, E_3, was also allowed to vary 2,777 x 10^4 and 12,46 were obtained for the SSE and standard deviation respectively.

A copy of the user supplied subroutines and the results obtained from the best model fit are presented in Appendix J. The hydrolysis conditions for each result are obtainable from Appendix I (results appear in the same order as the data).

11.6 Discussion

The amounts of xylose in the two hemicellulose fractions were obtained from Figures 11.1 to 11.8. The easily hydrolysable hemicellulose fraction contained 165 mg xylose/g bagasse and the more resistant fraction 105 mg xylose/g bagasse, representing a total xylose content of 270 mg/g bagasse. These values of xylose content of the hemicellulose fractions are average values but the two hemicellulose fraction reaction model was not susceptible to small changes in these amounts (± 10 mg/g) - these resulted in negligible changes in the standard deviation.

The values at 61% xylose in the easily hydrosable hemicellulose fraction and 39% in the more resistant fraction do seem to agree with the findings of Aleman et al[151] who found that 65% of the bagasse hemicellulose hydrolyses at a faster rate than the remainder.

The total xylose yields, rather than the free xylose yields, were used to find a suitable reaction model. At the beginning of the hemicellulose hydrolysis the free xylose yields are much lower than the total xylose yields due to oligomer formation but as the time approaches at which maximum xylose yield occurs total and

free xylose yields converge as shown in Figure 8.3. The reaction model will thus actually produce free xylose yield data as the maximum xylose yield is approached (which is in the region of interest).

The best fit for the experimental total xylose yield data was obtained using the two hemicellulose fraction first order reaction model with the activation energy of xylose decomposition set at 33,56 k cal/g mole[154]. A complete set of results is presented in Appendix J and some of the model predicted total xylose yields are plotted and compared with the experimental values in Figures 11.9 to 11.14.

An attempt to improve the fit using the two hemicellulose first order reaction model by allowing for sulphuric acid consumption of approximately 10 mg/g bagasse as found in Section 9.5.4. (assuming acid consumed at the start of the hydrolysis) was unsuccessful. In fact, the standard deviation was increased. The sulphuric acid consumption might be countered by the formation of organic acids, such as acetic acid, in the hydrolyses.

The two hemicellulose fraction first order reaction model developed expresses the total xylose yield as a function of the hydrolysis time at a given set of hydrolysis conditions :

Total xylose yield (mg/g) =

$$\frac{165\ k_1}{k_3 - k_1}\left(e^{-k_1 t} - e^{-k_3 t}\right) + \frac{105\ k_2}{k_3 - k_2}\left(e^{-k_2 t} - e^{-k_3 t}\right)$$

where $k_1 = 0,1224 \times 10^{-2} \times C \exp\left[\frac{-24,680 \times 10^3}{R}\left(\frac{1}{T} - \frac{1}{348,15}\right)\right]$

$k_2 = 0,1078 \times 10^{-3} \times C \exp\left[\frac{-22,343 \times 10^3}{R}\left(\frac{1}{T} - \frac{1}{348,15}\right)\right]$

$k_3 = 0,2793 \times 10^{-5} \times C \exp\left[\frac{-33,56 \times 10^3}{R}\left(\frac{1}{T} - \frac{1}{348,15}\right)\right]$

$R = 1,987$ cal g mole^{-1}K^{-1}

C = concentration of H_2SO_4 (g/100 mℓ)

and T = temperature in K

This reaction model is suitable for the design of an industrial continuous plug flow or batch hemicellulose hydrolysis reactor

provided that the kinetics are unaffected by the lower liquid to solid ratios which will be used in the industrial hydrolyses.

The reaction model also gives the effect of temperature on the reaction constants k_1, k_2 and k_3. The ratios of $^{k_1}/_{k_3}$ and $^{k_2}/_{k_3}$ are important as these effect the xylose yield - the higher the ratios the greater the maximum xylose yield. At constant acid concentration the ratios $^{k_1}/_{k_3}$ and $^{k_2}/_{k_3}$ decrease with increasing temperature as shown in Table 11.4. Thus to obtain high xylose yields the hydrolysis temperature should not exceed approximately 130°C and not be so low as to take an excessive time for hydrolysis.

Table 11.4 : Effect of temperature on $^{k_1}/_{k_3}$ and $^{k_2}/_{k_3}$

Temperature °C	$^{k_1}/_{k_3}$	$^{k_2}/_{k_3}$
80	365,4	30,7
100	185,4	13,0
120	100,8	6,0
140	58,2	3,0
160	35,3	1,6

This phenomenon of decreasing maximum xylose yield with increasing temperature has also been noted by Limbaugu et al [140] who monitored the xylose yield from the hydrolysis of oak hemicellulose.

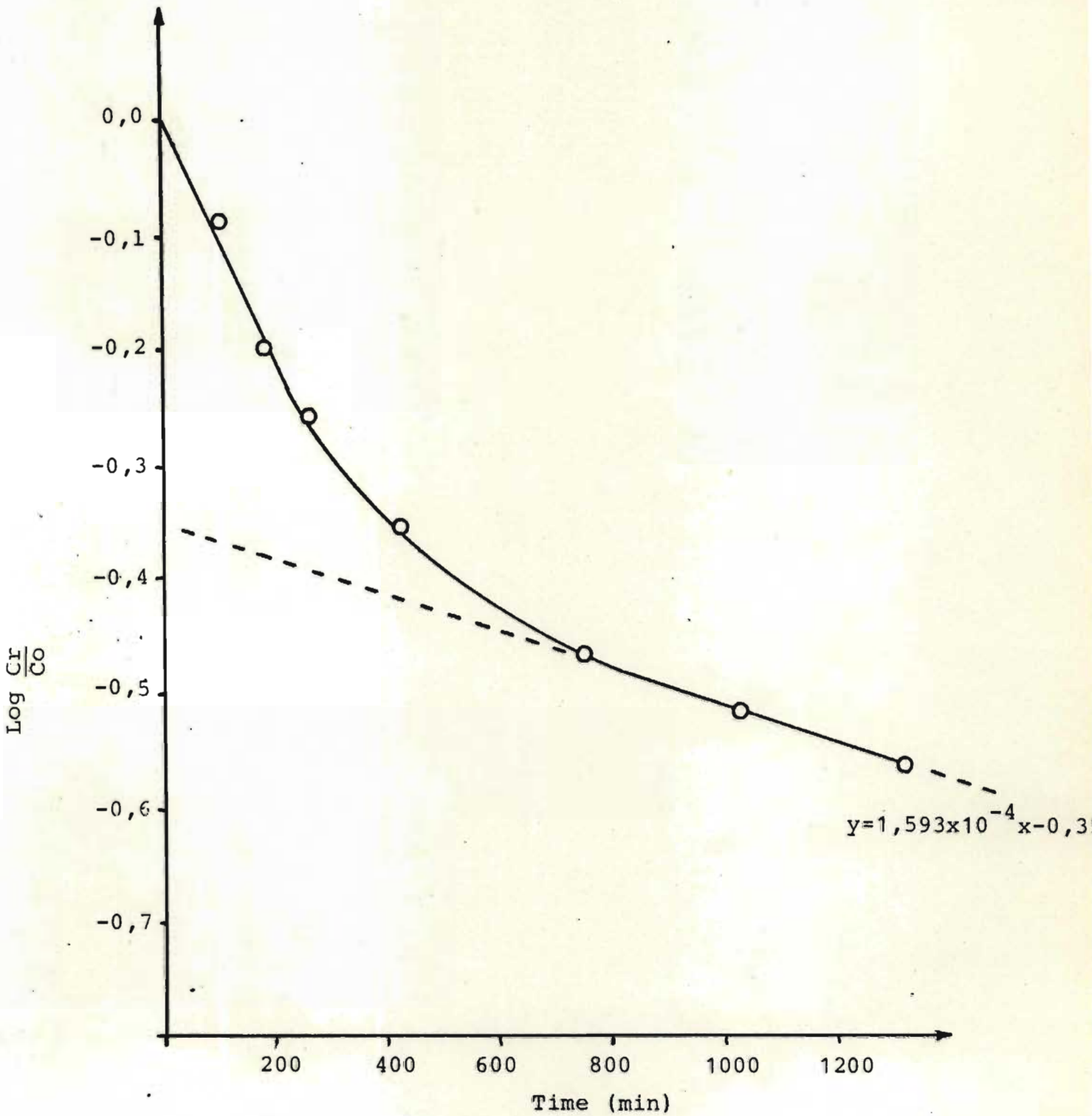


FIGURE 11.1 First order plot for hydrolysis at 80°C and 20 g/ℓ H_2SO_4

(Cr = concentration of xylose in residue, Co = concentration of xylose in bagasse)

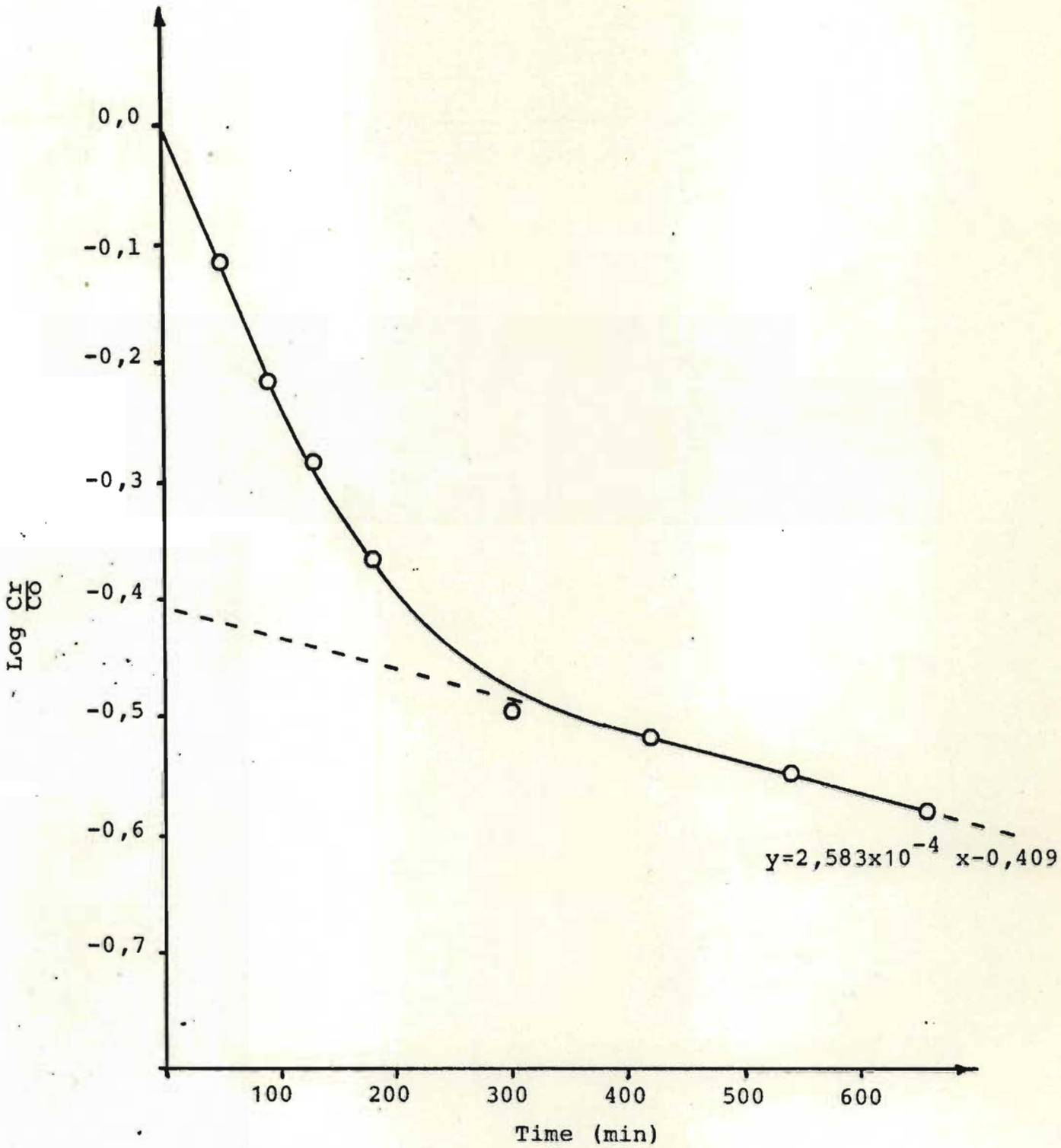


FIGURE 11.2 First order plot for hydrolysis at 80°C and 40 g/ℓ H_2SO_4

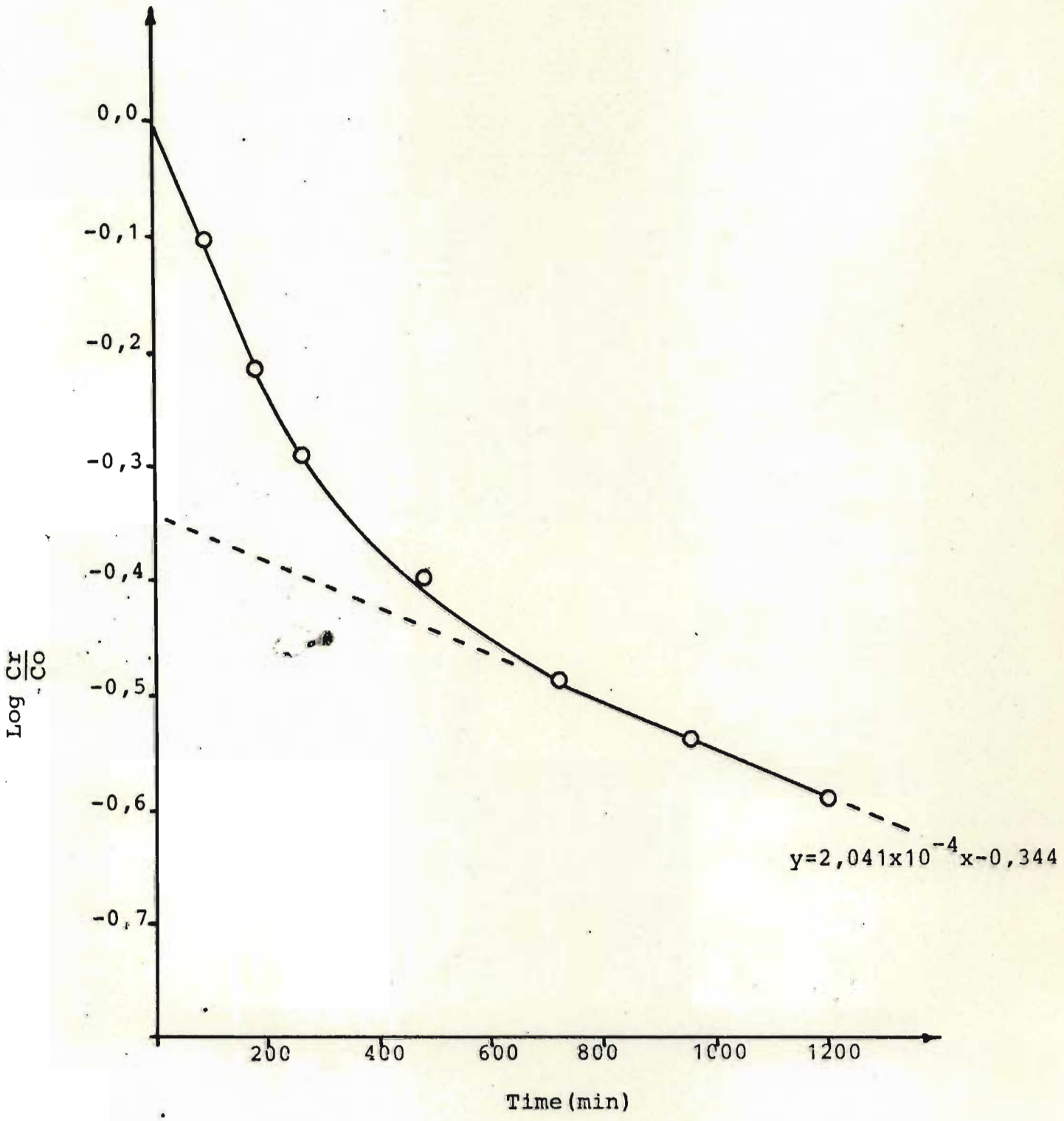


FIGURE 11.3 First order plot for hydrolysis at 90°C and 10g/ℓ H_2SO_4

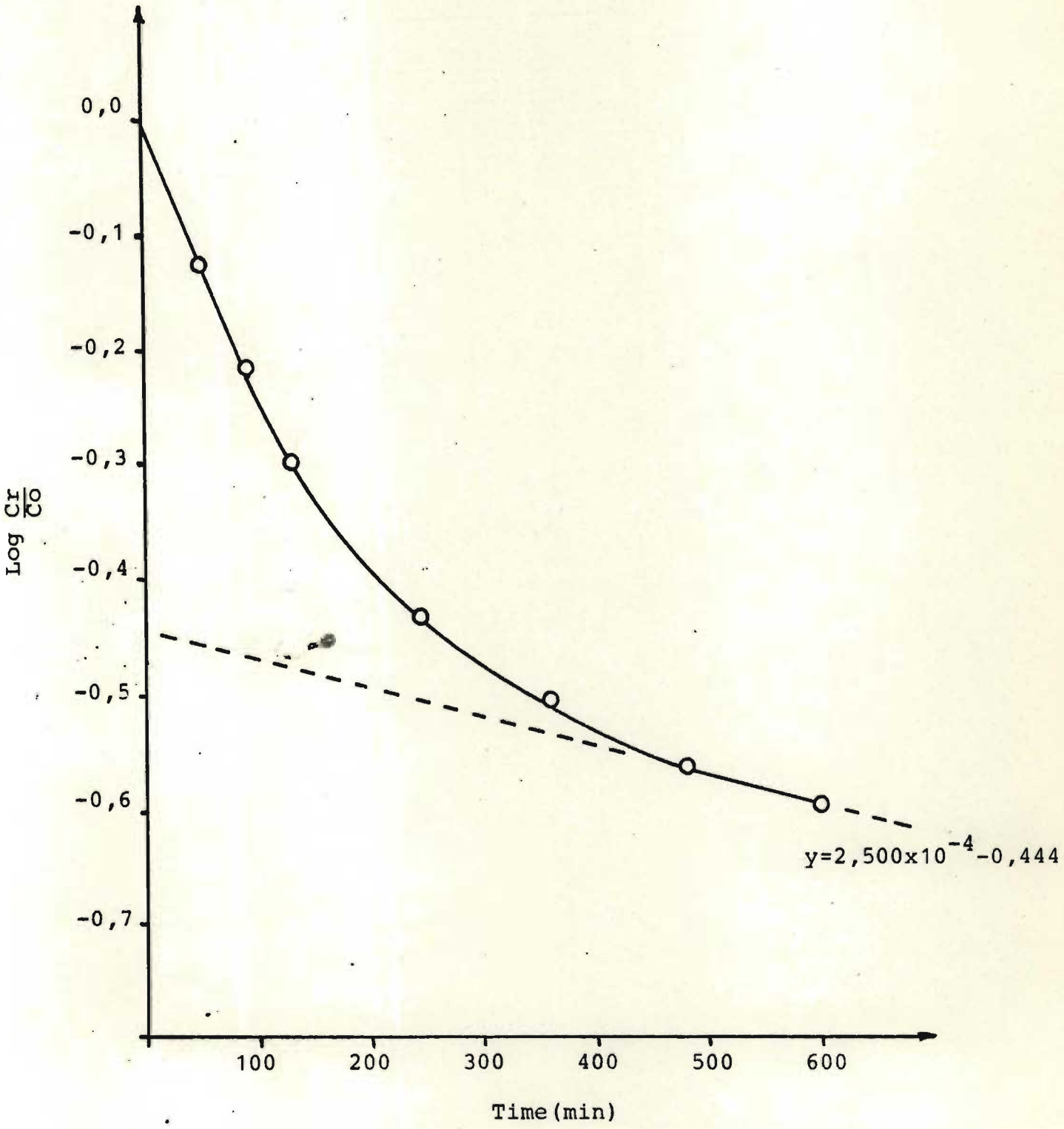


FIGURE 11.4 First order plot for hydrolysis at 90°C and 20 g/ℓ H_2SO_4

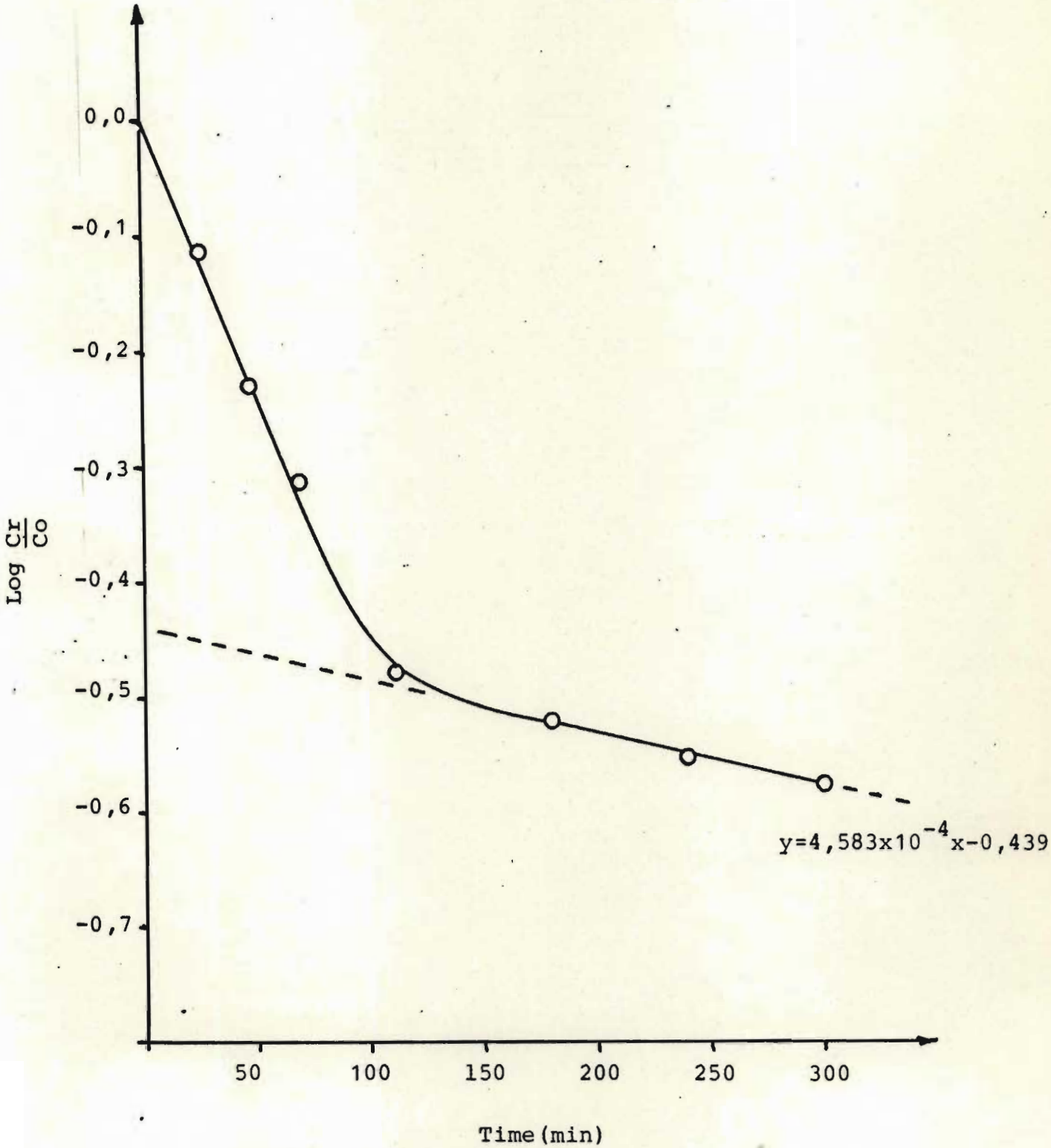


FIGURE 11.5 First order plot for hydrolysis at 90 °C and 40 g/ℓ H_2SO_4

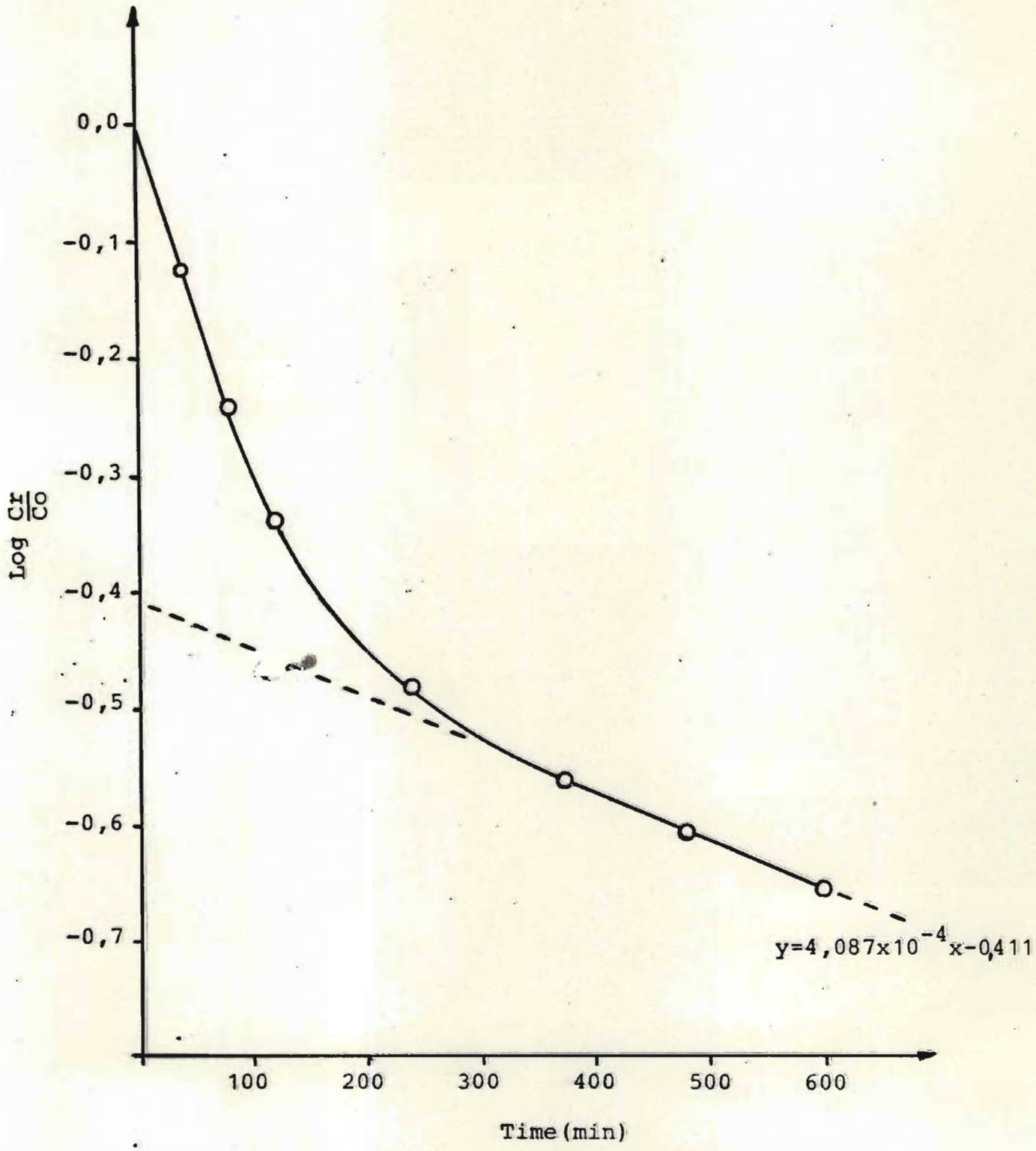


FIGURE 11.6 First order plot for hydrolysis at 100°C and 10 g/ℓ H_2SO_4

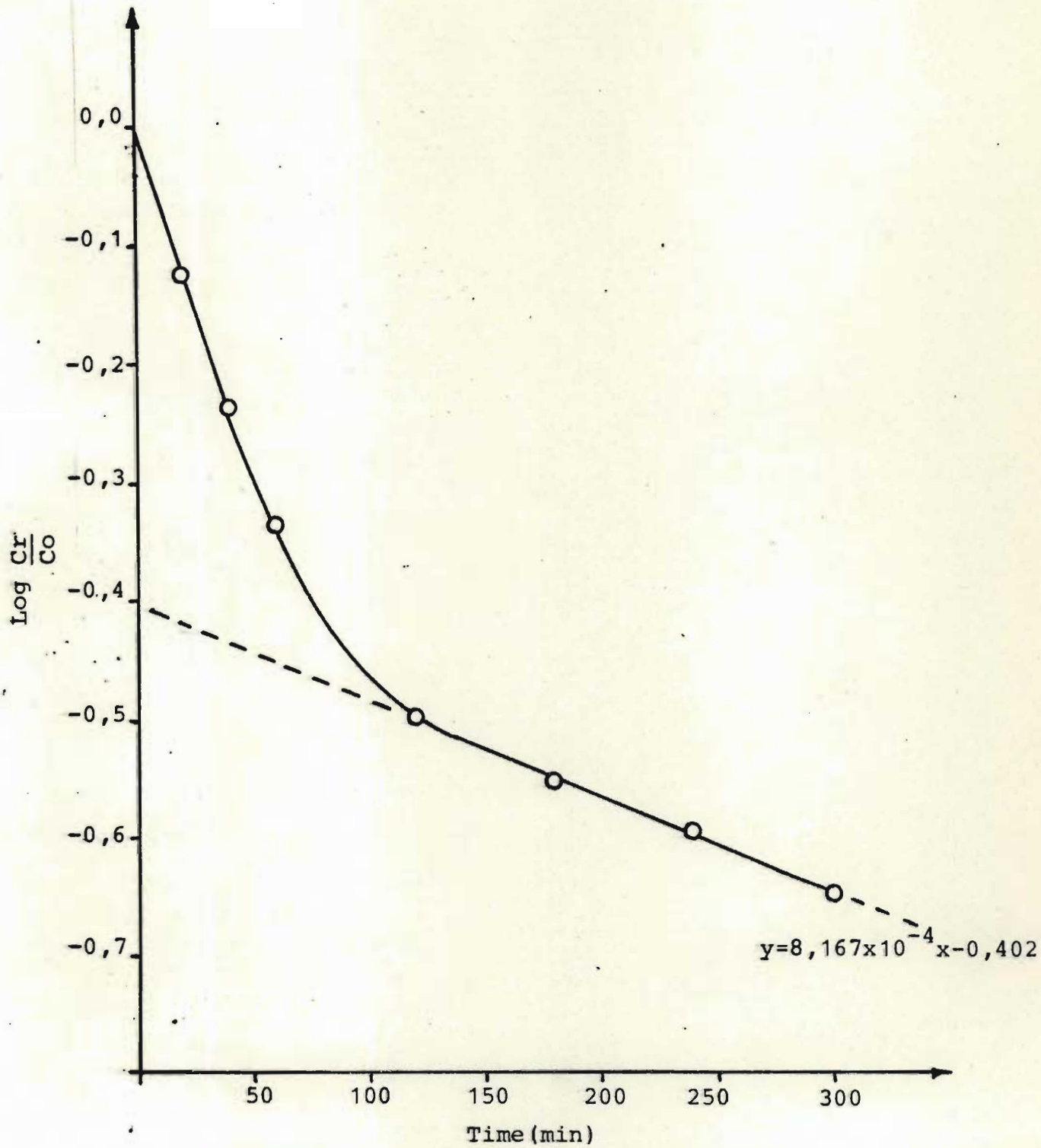


FIGURE 11.7 First order plot for hydrolysis at 100°C and 20 g/ℓ H_2SO_4

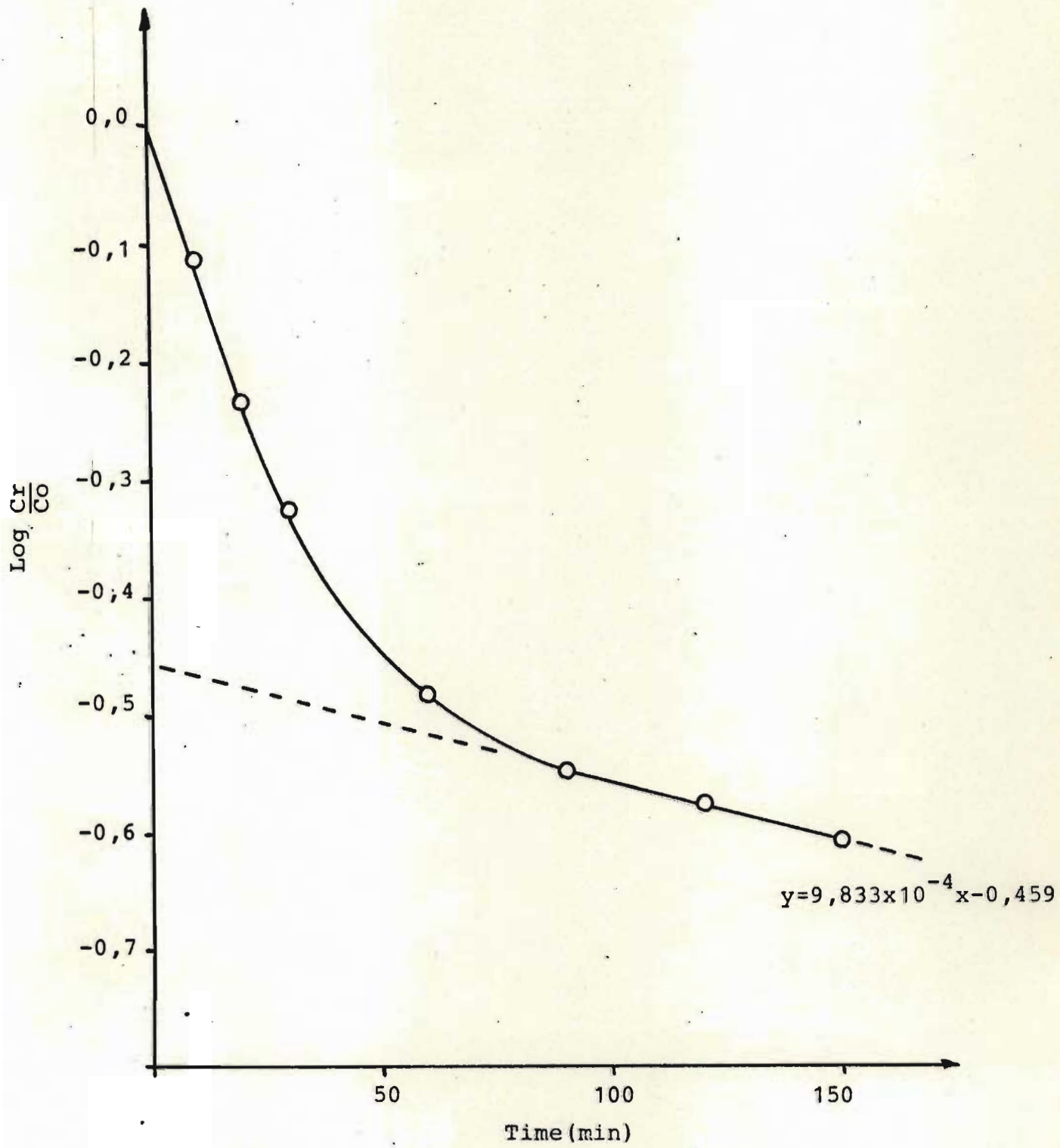


FIGURE 11.8 First order plot for hydrolysis at 100°C and 40 g/ℓ H_2SO_4

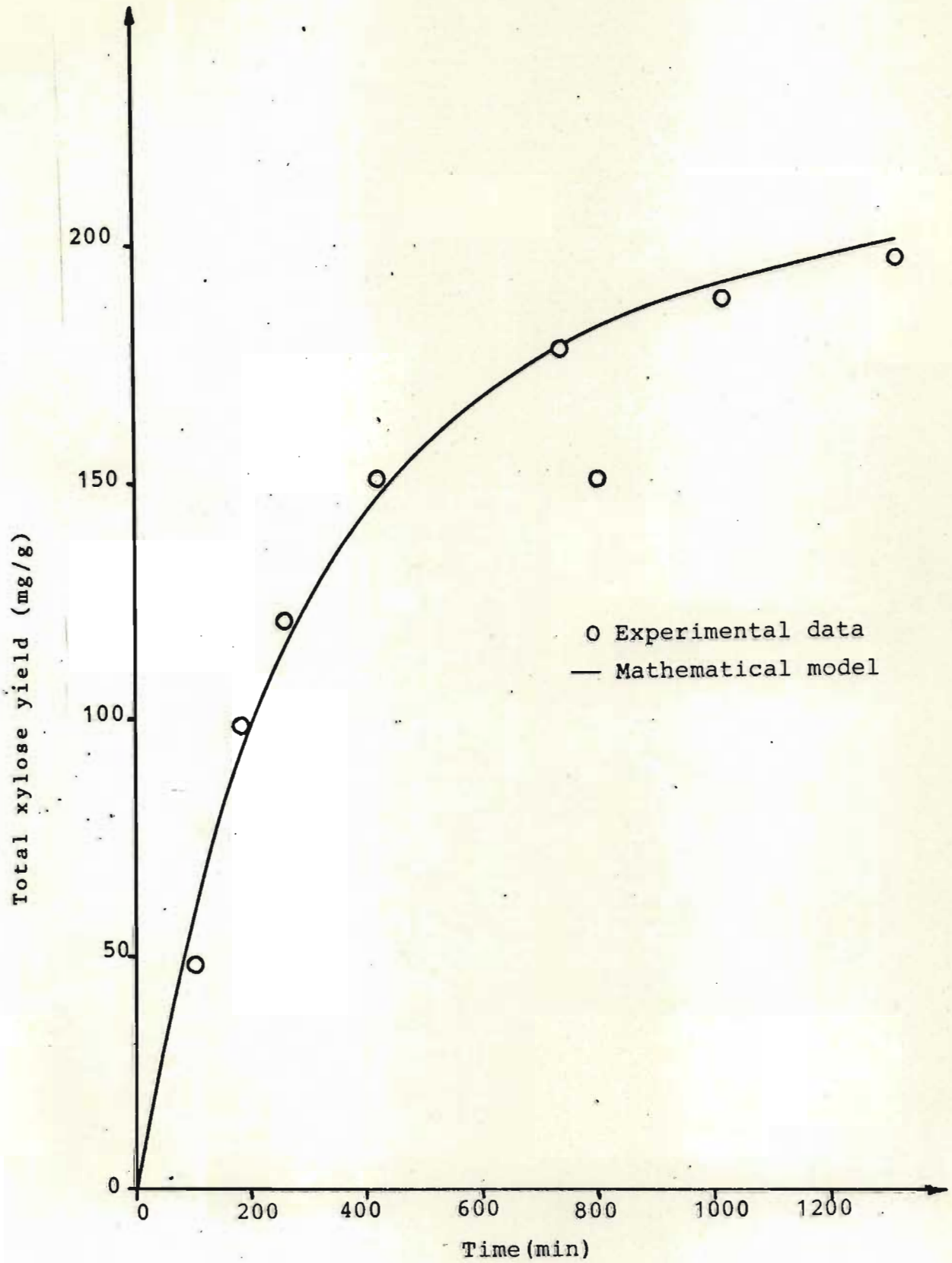


FIGURE 11.9 Comparison of model-predicted and experimental xylose yields at 80°C and 20g/ℓ H_2SO_4

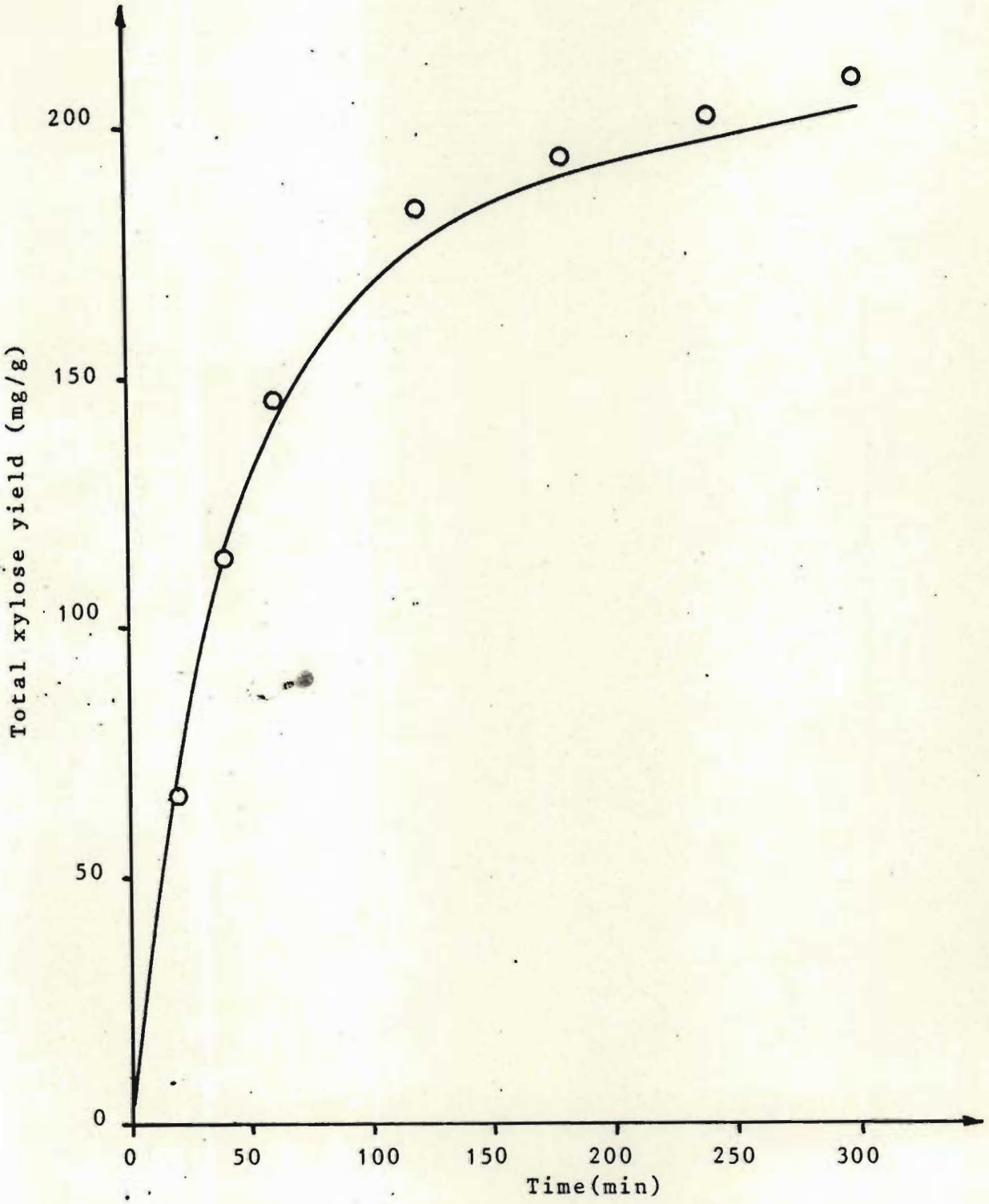


FIGURE 11.10 Comparison of model predicted and experimental xylose yields at 100°C and 20 g/ℓ H_2SO_4

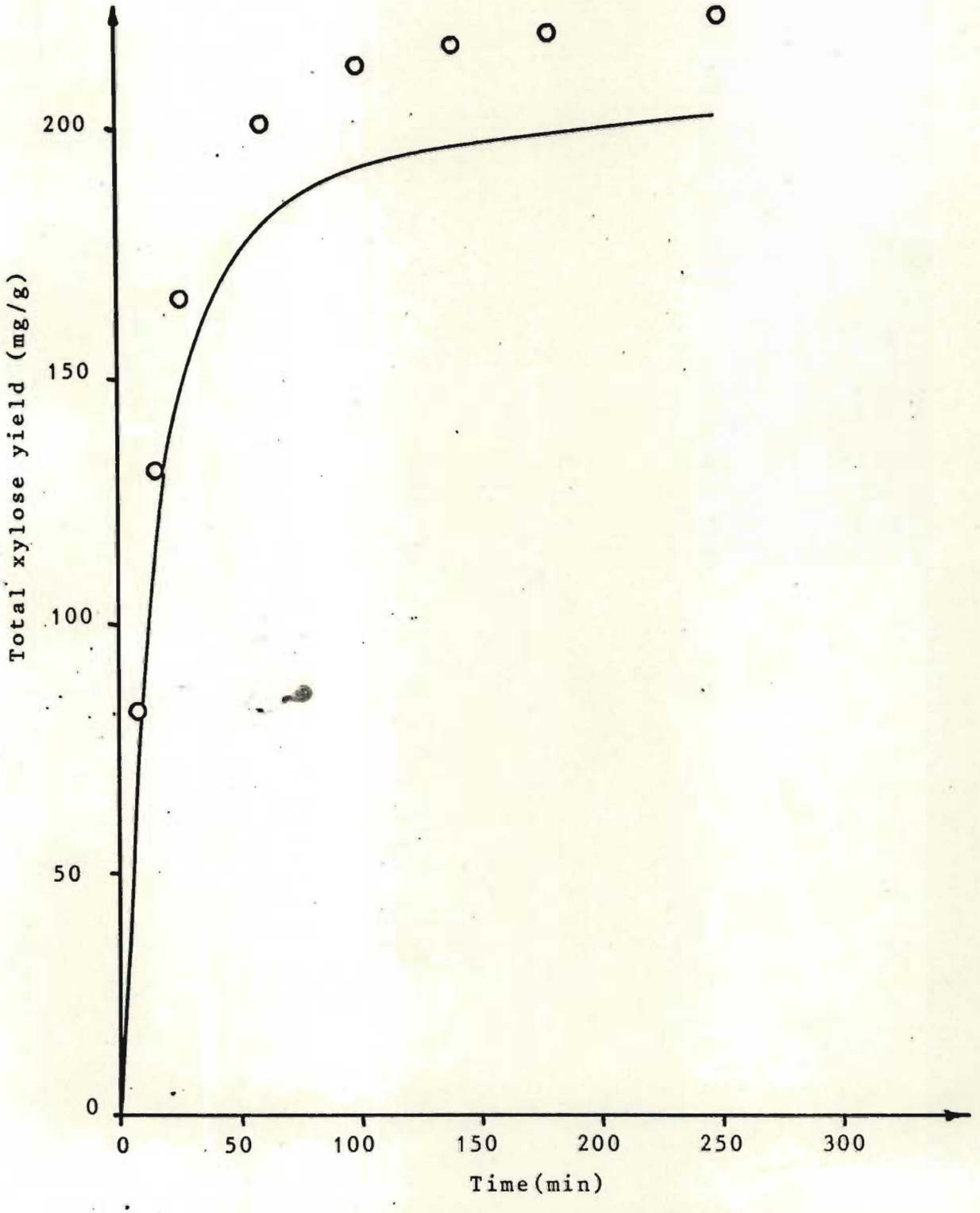


FIGURE 11.11 Comparison of model-predicted and experimental xylose yields at 120°C and 10 g/ℓ H_2SO_4

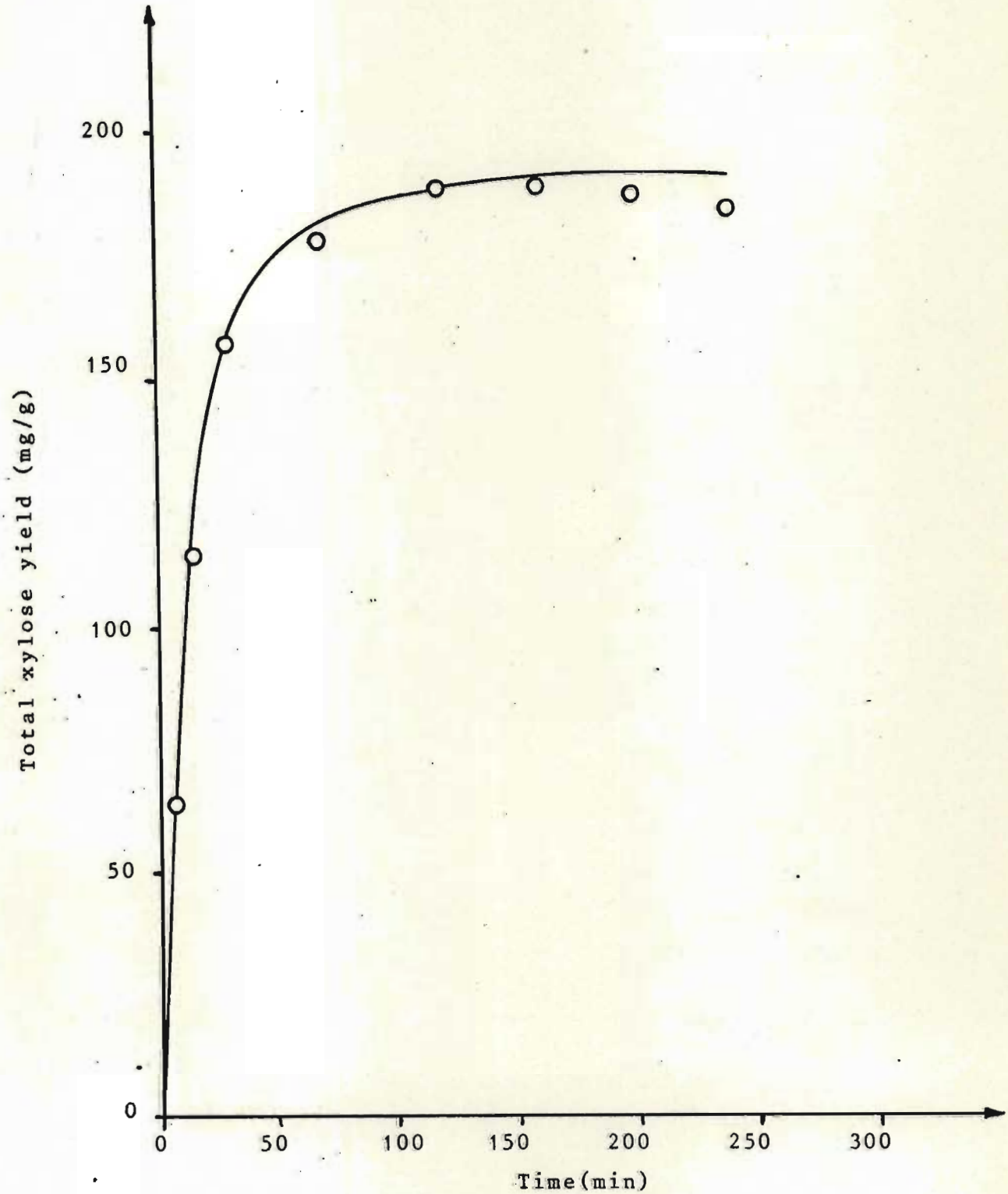


FIGURE 11.12 Comparison of model-predicted and experimental xylose yields at 130°C and 5g/ℓ H_2SO_4

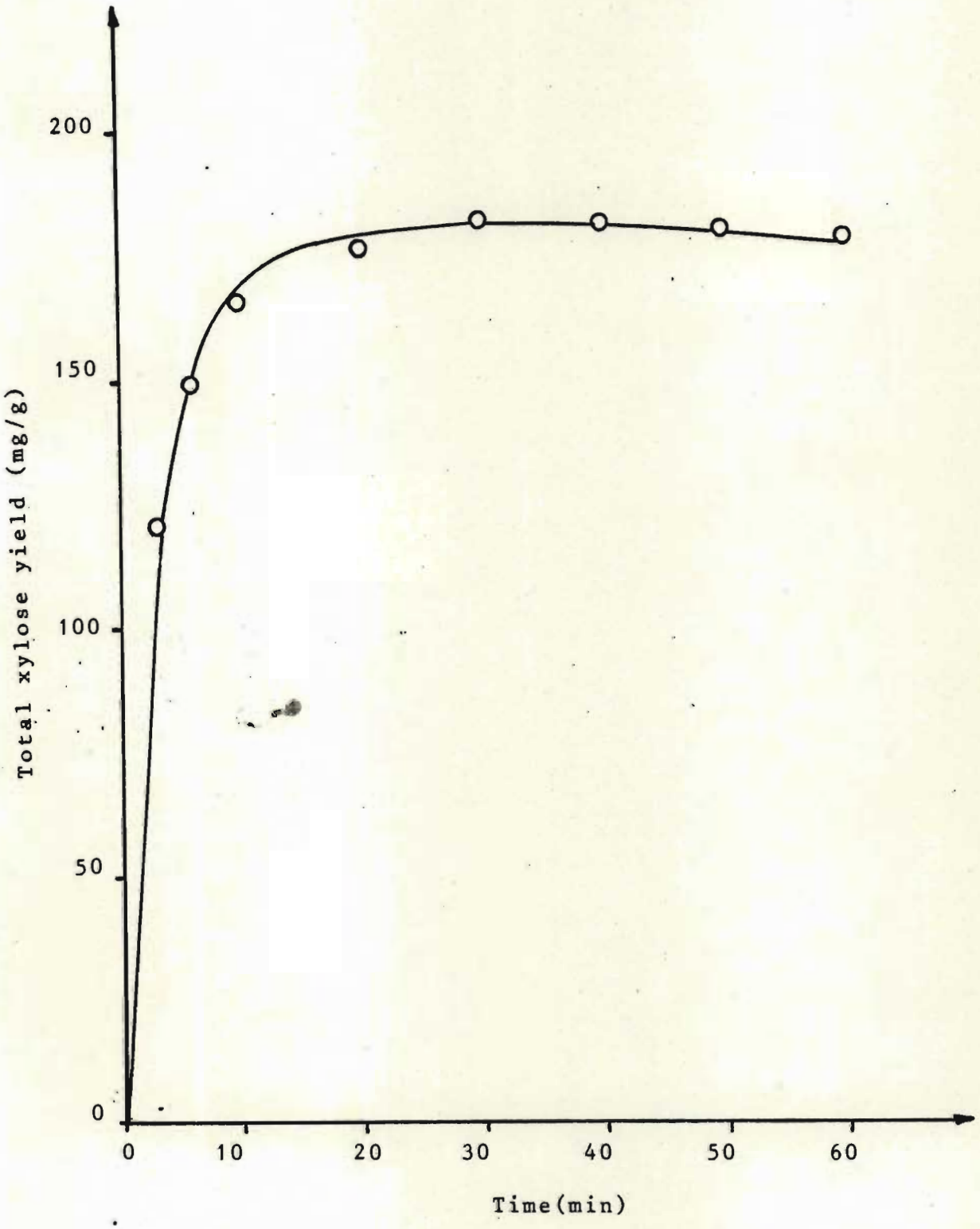


FIGURE 11.13 Comparison of model-predicted and experimental xylose yields at 140°C and 10 g/ℓ H_2SO_4

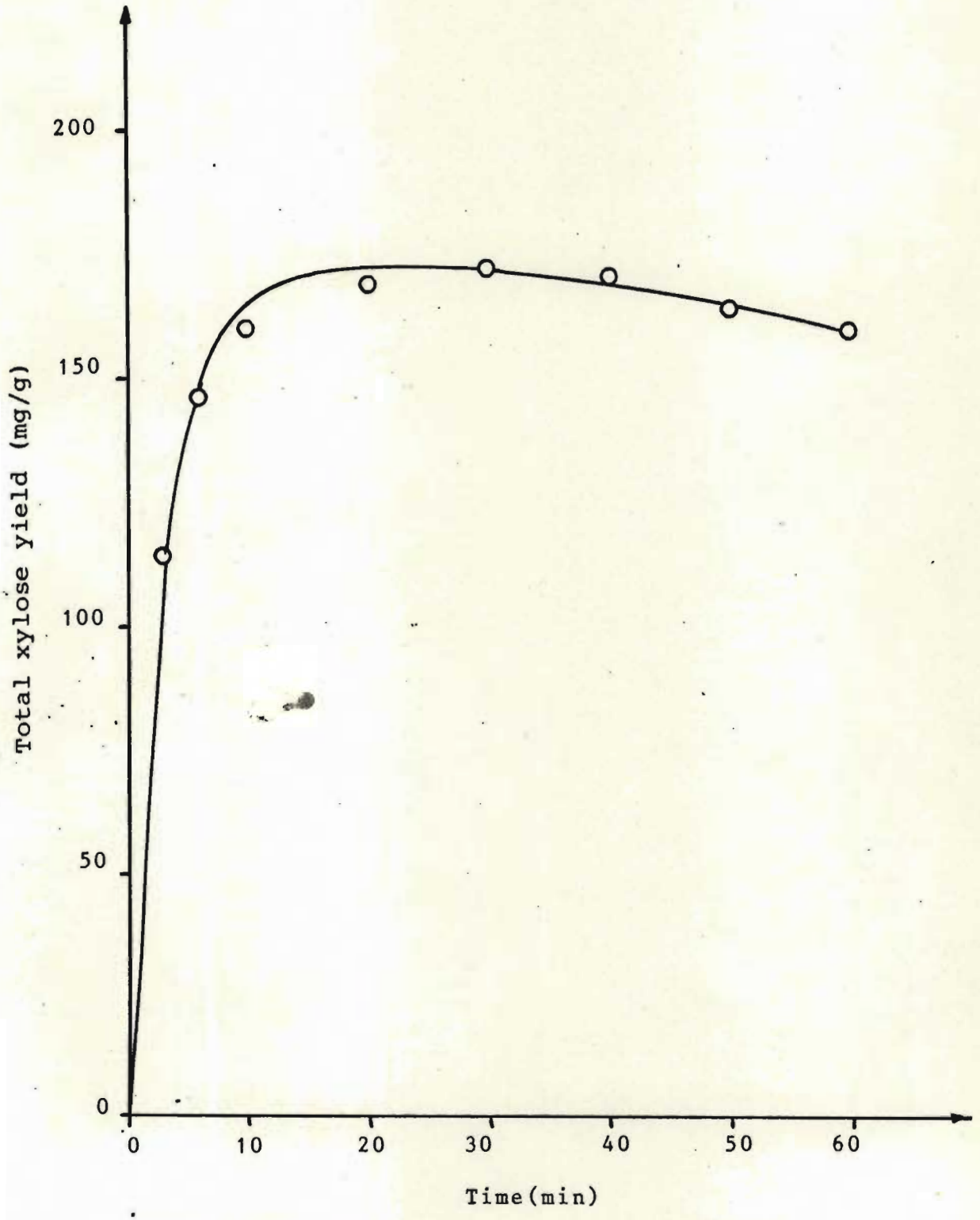


FIGURE 11.14 Comparison of model-predicted and experimental xylose yields at 150°C and 5 g/ℓ H_2SO_4

12. CONTINUOUS DILUTE ACID HYDROLYSIS OF BAGASSE

12.1 Introduction

Batch dilute acid hydrolysis of the hemicellulose fraction of sugarcane bagasse has been investigated at a liquid to solid ratio of 15 : 1 over the temperature range 80° to 150°C (Sections 8.4 and 9.4) with sulphuric acid concentrations at and below 40 g/ℓ.

The xylose yields obtained during these hydrolyses were extremely encouraging and it was decided to construct a continuous hydrolysis reactor to process bagasse at a low liquid to solid ratio (below 5 : 1) and to determine whether xylose yields similar to the batch hydrolysis yields could be obtained at the same hydrolysis conditions.

12.2 Commissioning of Reactor

12.2.1. Bagasse Transport by Reactor Screw

Two bagasse substrates were initially used to check the transport of bagasse by the reactor screw. Dried hammer-milled bagasse (-9 mm) was obtained from Tongaat Milling and a coarse bagasse obtained after sugar extraction in a diffuser was obtained from the Tongaat sugar mill. The bagasse was hand fed into the reactor at a liquid to solid ratio of 5 : 1 with the screw speed set at 1 rpm.

A number of problems were encountered.

The bagasse did not discharge from the reactor and it was discovered that it was packing up against one of the sets of cutter blades on the screw (cutter set A in the digester screw detail in Appendix C). This was avoided by removing the blade set completely.

Even with these blades removed there was still a problem with discharge of the coarse bagasse. It would discharge for a while and then suddenly bridge over the discharge

port which resulted in a compressed bagasse plug preventing further discharge. This blocking tendency was reduced by screening the coarse bagasse to remove the large fragments, especially the sugarcane rind, before charging to the reactor.

There was also a tendency for the bagasse to become compacted just after the feed throat where there was a change in pitch from 75 to 45 mm. Since there was no change in screw flight depth at this point there was a volume decrease which accounted for this compaction. This was avoided by building up the shaft at the feed point so that there was no change in volume over the pitch change.

With this modification to the feed portion of the screw, it was found that the bagasse tended to cling to the screw at the feed point thus preventing any transport. (The fine bagasse was worse than the coarse bagasse in this respect.) It was thought that this tendency could be overcome by ribbing the wall of the reactor so that the bagasse would be held stationary and thus be conveyed by the screw. This theory was tested by replacing the stainless steel reactor tube with a piece of 100 mm perspex tubing with four 2,5 mm ribs 30 cm long at the feed end of the reactor tube. These proved to be satisfactory and it was found that once the bagasse had passed the feed point there was no further problem of bagasse clinging to the screw shaft. The feed portion of the reactor was then ribbed by three 904L weld run 30 cm long and 2 mm high which prevented any build up on the shaft.

These tests on bagasse transport indicated that the bagasse should be hammermilled before charging to the reactor but that excessive size reduction was not necessary. The tests were also hypothetical in that during acid treatment there is a decrease in bagasse volume as the hemicelluloses are solubilised and the discharge would be aided by the reactor pressure. It was, however, reasoned that if the bagasse could be transported 'as is' then it would be transported without any problem during dilute acid hydrolysis.

12.2.2 Autohydrolysis of Bagasse

The operation of all the control systems and the reactor were checked by charging the hammermilled bagasse obtained from Tongaat Milling at 50% moisture to the reactor together with water (in place of acid) and steam injection at 120°C.

12.3 Bagasse Sample

The bagasse obtained from Tongaat sugar mill used for the bagasse transport tests in Section 12.2.1. was used for the continuous dilute acid hydrolyses. It was screened on a 6 mm mesh - the +6 mm fraction was batch hammermilled for 5 minutes and recombined with the -6 mm fraction.

The bagasse composition is shown in Table 12.1.

Table 12.1 : Composition of bagasse used for continuous acid hydrolysis

Analysis	%
Cellulose	37,2
Lignin	22,0
Pentosan	30,0
Ash	6,0

12.4 Results

The continuous hydrolyses performed could be divided into two groups, those performed with acid injection and continuous steam addition and the other group performed using acid impregnated bagasse and controlled steam injection.

12.4.1 Acid Hydrolysates

The actual hydrolysis results appear in Appendix K and the product yield data appear in Tables 12.2 and 12.3.

Table 12.2. : Product yield data for continuous hydrolyses with acid injection

Run Number	1	2	3
Temperature (°C)	130	130	130
Acid Conc. (g/ℓ H_2SO_4)	8,31	7,94	13,14
Based on Acid Concentration			
Liquid to solid ratio	5,36:1	5,71:1	4,56:1
Free xylose (mg/g)	186,2	182,3	205,3
Total xylose (mg/g)	189,4	184,0	205,8
Free glucose (mg/g)	23,6	20,6	27,8
Total glucose (mg/g)	24,7	21,1	28,7
Acetic acid (mg/g)	43,6	37,9	49,1
Furfural (mg/g)	5,1	3,8	6,4
Based on Hydrolysate Mass			
Liquid to solid ratio	5,52:1	6,24:1	4,43:1
Free xylose (mg/g)	191,7	199,2	199,5
Total xylose (mg/g)	195,0	201,1	200,0
Free glucose (mg/g)	24,3	22,5	27,0
Total glucose (mg/g)	25,4	23,1	27,9
Acetic Acid (mg/g)	44,9	41,4	47,8
Furfural (mg/g)	5,2	4,1	6,2

Table 12.3. : Product yield data for continuous hydrolyses with steam injection

Run Number	4	5	6	7
Temperature (°C)	130	120	130	130
Acid Conc. (g/ℓ H_2SO_4)	11,41	19,53	12,52	10,42
Based on Acid Concentration				
Liquid to solid ratio	3,90:1	4,33:1	3,76:1	3,58:1
Free xylose (mg/g)	197,4	210,5	202,9	193,0
Total xylose (mg/g)	204,0	213,5	202,9	193,4
Free glucose (mg/g)	28,5	32,9	30,8	27,2
Total glucose (mg/g)	29,6	34,2	34,2	27,2
Acetic acid (mg/g)	48,3	55,2	50,9	48,3
Furfural (mg/g)	11,5	12,5	16,9	12,5
Based on Hydrolysate Mass				
Liquid to solid ratio	3,81:1	4,23:1	3,66:1	3,61:1
Free xylose (mg/g)	192,5	205,7	197,6	193,3
Total xylose (mg/g)	199,0	208,7	197,6	194,8
Free glucose (mg/g)	27,8	32,2	30,0	27,4
Total glucose (mg/g)	28,9	33,4	33,3	27,4
Acetic acid (mg/g)	47,1	54,0	49,6	48,7
Furfural (mg/g)	11,3	12,2	16,5	12,6

12.4.2 Hydrolysis Residues

Table 12.4 : Continuous hydrolysis residue analyses

Run Number	Bagasse dry mass (g)	Residue dry mass (g)	Residue Analysis (%)				Loss %
			Cellulose	Lignin	Pentosan	Ash	
1	623	399	58,6	32,7	6,9	3,29	35,95
2	682	428	56,3	31,2	6,5	5,88	37,24
3	799	491	58,3	31,4	6,3	6,28	38,55
4	809	527	55,8	32,5	5,0	6,64	34,86
5	787	522	55,0	32,1	6,2	6,10	33,67
6	817	519	58,3	32,8	5,0	4,91	36,47
7	844	541	58,4	31,9	4,8	5,49	35,90

12.5 Discussion

From the results in Tables 12.2 and 12.3 it is evident that the xylose yields at the hydrolysis conditions tested have not been affected by the decrease in liquid to solid ratio and that the yields are comparable with those obtained under similar reaction conditions in the batch hydrolysis tests which were conducted at a liquid to solid ratio of 15 : 1 (see Section 9.4.).

It would thus appear that, even at the high solids level achieved in the continuous reactor, the reactor performance is still controlled by kinetic rather than by other possible limiting factors such as diffusion rate or particle/particle shielding.

This has also been found by Church and Woolridge[136] who have developed a high solids plug flow reactor for processing lignocellulosic materials. They found that glucose yields during dilute acid cellulose hydrolysis were unaffected by varying the feed solids from 8% to 35%.

Although it has been shown that the xylose yields were unaffected at liquid to solid ratios down to 3,61 : 1 at 130°C - and the model given in Section 11.6 is therefore applicable - further tests over a wide range of acid concentrations, temperatures and liquid to solid ratios will still have to be performed to confirm the preliminary findings.

13. NEUTRALISATION OF ACID HYDROLYSATE

13.1 Objective

The hydrolysate produced by the dilute acid hydrolysis of bagasse hemicellulose requires neutralisation before any bio-conversion process to convert the pentose sugars to chemical feedstocks.

In this investigation the amount of calcium ions remaining in the hydrolysate after neutralisation at room temperature to various pH values with $CaCO_3$ and $Ca(OH)_2$ was determined. The effect of pH of neutralisation on the quantities of other metal cations in solution (mainly corrosion products from the continuous hydrolysis reactor) was also noted.

13.2 Hydrolysate Composition

The hydrolysate was a composite sample prepared from a number of dilute acid hemicellulose hydrolyses performed at low liquid to solid ratios (approximately 5 : 1) at various acid concentrations and temperatures.

The hydrolysate had the composition given in Table 13.1

Table 13.1 : Hydrolysate composition

Analysis	g/ℓ
Free Xylose	42,6
Total Xylose	43,1
Free Glucose	7,1
Total Glucose	7,2
Acetic Acid	9,91
Furfural	2,90
Total Acidity (g/ℓ H_2SO_4)	22,23
H_2SO_4	14,14
Cation Analysis	**mg/ℓ**
Calcium	37
Iron	630
Copper	2
Nickel	75
Chromium	65
Molybdenum	<10
Zinc	

13.3 Neutralisation Method

13.3.1 Determination of Precipitation Period

In order to determine how long the hydrolysate should be allowed to stand after neutralisation before the supernatant was sampled for calcium ions, a sample of the hydrolysate composite was neutralised pH 4,6 and then periodically sampled.

200 ml of the hydrolysate at room temperature was continuously agitated by a magnetic stirrer and neutralised by $CaCO_3$ addition. Samples were taken at 5, 15, 30 and 60 minutes after neutralisation and immediately centrifuged at 10 000 rpm for 5 minutes. The supernatant of each was then sampled and diluted 100 times before the calcium ion analysis.

13.3.2. Neutralisation to Various pH Values

Two sets of experiments were performed using either $CaCO_3$ or $Ca(OH)_2$ as the neutralising agent.

In each experiment a number of 100 ml aliquots of the hydrolysate composite were neutralised at room temperature (stirred until pH reached steady value). The pH and the amount of neutralising agent added were recorded. The hydrolysate was then allowed to stand for one hour before filtration through Whatman No. 1 filter paper and sampling for calcium analysis.

The filtrates from the various neutralisation conditions were also analysed for iron, chromium and nickel to determine whether these had been reduced.

13.4 Results

Table 13.2 : Effect of precipitation period on calcium concentratio

Time (min)	Calcium (mg/ℓ)
5	2400
15	2200
30	2000
60	1700

Table 13.3 : Neutralisation with $CaCO_3$

pH	$CaCO_3$ Added (g)	Calcium (mg/ℓ)	Iron (mg/ℓ)	Nickel (mg/ℓ)	Chromium (mg/ℓ)
2,6	0,484	500	650	75	66
3,2	0,520	700	600	75	62
4,0	0,599	800	580	75	57
5,1	0,932	1600	580	71	47
5,6	2,000	1800	540	71	47

Table 13.4 : Neutralisation with $Ca(OH)_2$

pH	$Ca(OH)_2$ Added (g)	Calcium (mg/ℓ)	Iron (mg/ℓ)	Nickel (mg/ℓ)	Chromium (mg/ℓ)
2,6	0,893	300	620	75	45
3,3	0,989	500	570	74	44
3,9	1,107	700	520	70	45
4,5	1,326	850	520	70	45
5,0	1,510	1250	510	70	46
6,0	1,695	1700	430	70	36

13.5 Discussion

The stability of calcium sulphate in water over the range 0° to 200°C has been investigated by Partridge and White[181]. They concluded that gypsum ($CaSO_4.2H_2O$) and anhydrite ($CaSO_4$) are the only stable phases within this range. The transition temperature of gypsum into anhydrite lies near 40°C. Hemihydrate ($CaSO_4.\frac{1}{2}H_2O$) is the only other form of calcium sulphate which has existence in the range 0° - 200°C. It is metastable in the approximate range 90° - 130°C, showing decreasing stability with decrease of temperature below 90°C and with increase of temperature above 130°C. A copy of the equilibrium diagram suggested by the authors is illustrated in Figure 13.1. The neutralisation tests in this work were performed at room temperature and the calcium sulphate precipitate would thus be in the $CaSO_4.2H_2O$ form.

Leonard and Hajny[182] have investigated the fermentation of wood sugars to ethanol. They suggested that the acid hydrolysate be neutralised at an elevated temperature with lime and then held at that temperature to allow calcium sulphate precipitation. It was desirable to suppress the calcium sulphate content to about 750 ppm (405 ppm Ca) for the wood sugar fermentation. Calcium sulphate is at this desired concentration in saturated solutions at approximately 140°C. Less than 700 ppm calcium sulphate remained in a solution neutralised to pH 4,7 after 20 to 30 minutes at 140°C.

The amount of calcium sulphate in solution can also be limited by neutralising to a lower pH value. O'Neil *et al*[120] mention that in the Hokkaido acid hydrolysis process the hydrolysate is neutralised and the resultant calcium sulphate removed by filtration. The pH of the sugar solution is then readjusted to 2,5 to remove the calcium ions.

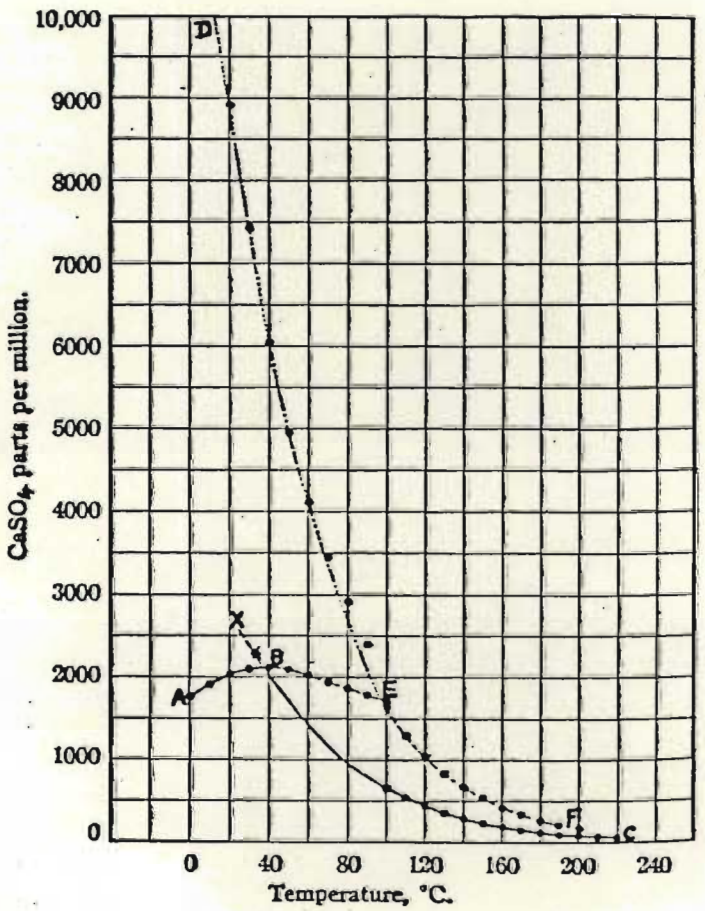
From the test performed on the effect of precipitation period on the calcium ion concentration, it is evident that there is still a gradual decrease in the calcium even after a standing period of one hour (see Figure 13.2). It was decided however that a

holding time of one hour should be used for all subsequent test work as any period longer would tend to be impractical on an industrial scale.

Neutralisation of the hydrolysate composite using $Ca(OH)_2$ was found to be more effective than $CaCO_3$ with regard to the residual calcium ions in solution as shown in Figure 13.3. This difference can be accounted for by the presence of CO_2 in solution in the case of $CaCO_3$ neutralisation which will tend to depress the pH of the solution due to the formation of H_2CO_3. The CO_2 also presented a problem during the neutralisation as it promotes excessive foaming which was avoided only by addition of a small quantity of antifoam.

The effect of pH on neutralisation on the amount of calcium in solution is illustrated in figure 13.3.It is evident that in the case of both $CaCO_3$ and $Ca(OH)_2$ there is an increase in calcium with increasing pH. The significant increase in calcium ion concentration at pH values above 3,5 could, mainly be accounted for by the acetic acid in solution. The $Ca(CH_3COO)_2$ formed is very soluble - 34,2 g $Ca(CH_3COO)_2$ soluble in 100 g H_2O at 25°C [183]. This raises the possibility of neutralising to pH 3, which would minimise the amount of calcium in solution, and then neutralising further with NH_4OH which would serve as a nitrogen source in any subsequent fermentation.

The amounts of nickel, chromium and iron in solution were only slightly decreased by neutralisation. It would appear that these heavy metals cannot be removed from the hydrolysate by $Ca(OH)_2$ or $CaCO_3$ neutralisation up to pH 6.



ABE - Gypsum, $CaSO_4.2H_2O$

DEF - Hemihydrate, $CaSO_4.\frac{1}{2}H_2O$

XBC - Anhydrite, $CaSO_4$

Figure 13.1. Equilibrium diagram of the system calcium sulphate-water at temperatures from 0^0 to 220^0C

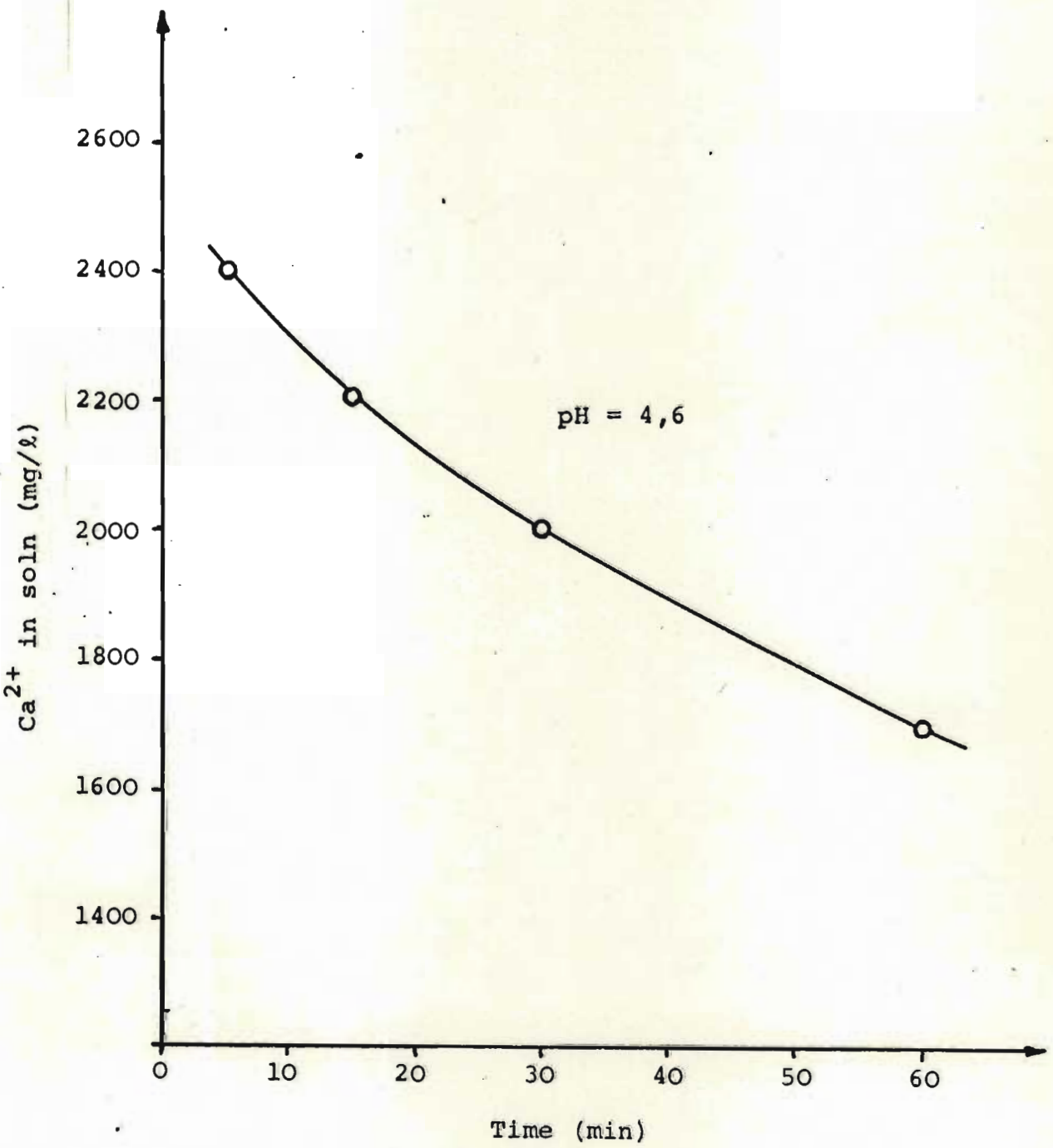


Figure 13.2. Effect of precipitation period on calcium ion concentration

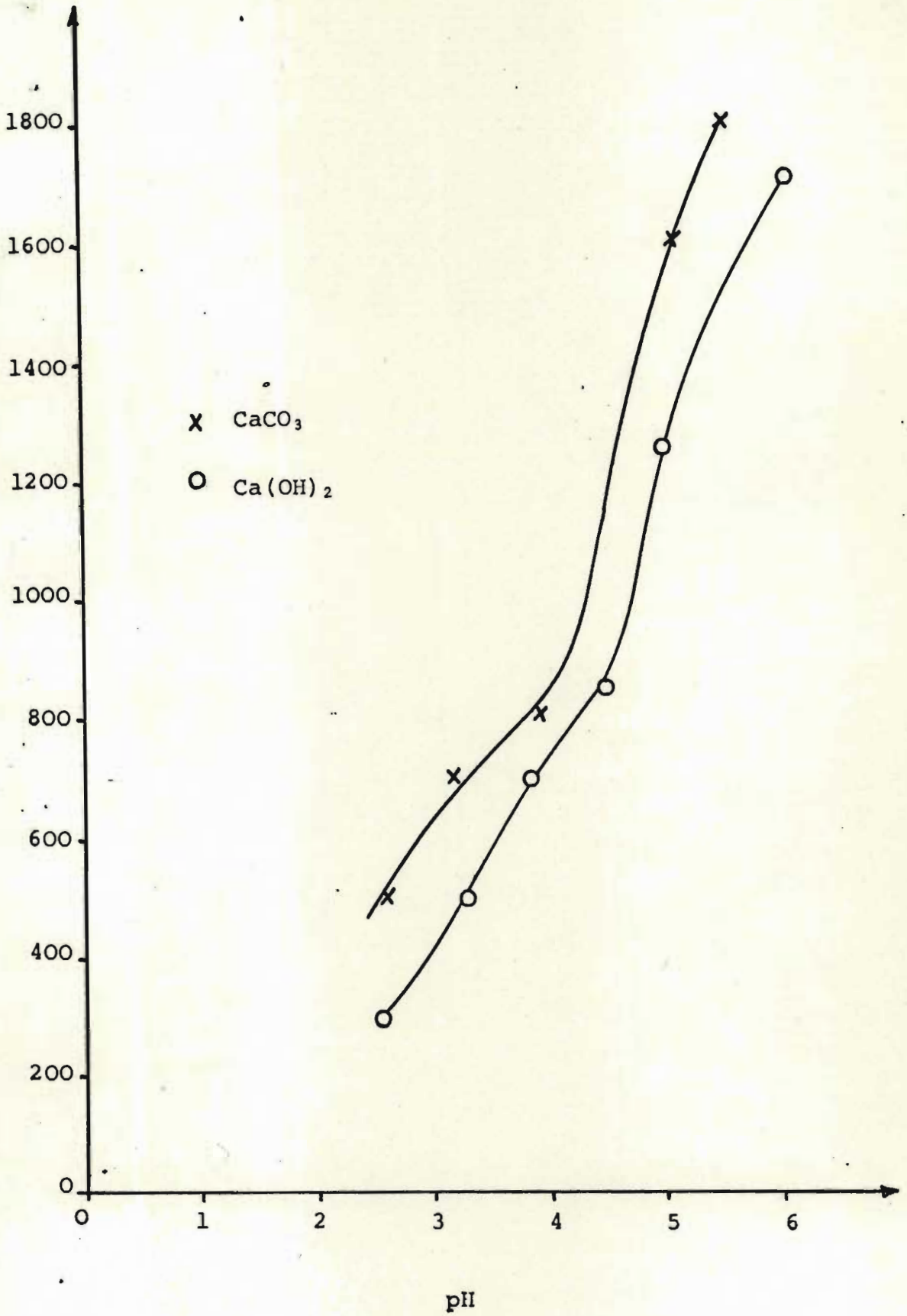


Figure 13.3 Effect of pH of neutralisation on calcium ion concentration Ca^{2+} in soln (mg/ℓ)

14. REACTOR CONFIGURATIONS FOR INDUSTRIAL ACID HYDROLYSIS

14.1 Introduction

Substantial kinetic data has been obtained at a liquid to solid ratio of 15 : 1 (Sections 8.4 and 9.4) and the xylose yield from the hemicellulose fraction of bagasse has been determined. It is also obvious that the hemicellulose fraction can be hydrolysed from the complex lignocellulosic structure without significantly removing any of the cellulose and lignin.

The process envisaged for the utilisation of bagasse for fuel or chemical feedstock is one which involves an initial dilute acid hydrolysis of the hemicellulose fraction. This overall process scheme is depicted in Figure 14.1.

In addition to the kinetic yield and the selectivity of the hydrolysis, there still remains a critical problem if the hemicellulose hydrolysis is to be developed into a chemical process. This problem is related to

(i) recovering sugar in a concentrated form,
(ii) separation of the hydrolysate from the bagasse residue.

As mentioned previously the kinetic experiments were carried out using 15 : 1 liquid to solid ratio which resulted in a low xylose concentration, usually about 14 g/ℓ. This is very undesirable from the process standpoint because the steam requirements increase with the amount of water. The steam is required in reactor heating and evaporation.

It is implied that the liquid to solid ratio should be decreased to increase the sugar concentration and reduce steam consumption. The highest xylose concentration achieved during the continuous hydrolysis tests was 54 g/ℓ. However, as the amount of water is decreased a new problem of product recovery is created. The sugar produced is in the hydrolysate which has to be separated from the liquid - solid mixture.

A number of reactor configurations are proposed in an attempt to overcome these problems. The final selection of hemicellulose extraction system will, of course, depend on an extensive economic and technical investigation.

14.2 Continuous Vapour-Phase Cooking

Vapour phase cooking offers a method of producing a concentrated pentosan solution with a very small requirement for steam and other forms of energy. The key idea is to conduct the hemicellulose hydrolysis at a very low liquid to solid ratio (approximately 1 : 1) and to obtain a relatively concentrated solution by counter-current extraction of the solubilised pentosan from the hydrolysed bagasse.

This reactor scheme is shown in Figure 14.2. It consists of an acid impregnation stage such as spraying the bagasse with an acid solution followed by processing in a plug flow continuous reactor. The xylose solution is then extracted by countercurrent washing using equipment similar to the diffuser used in the sugar industry for sugar extraction.

This process has the advantage that acid requirements and hence neutralisation costs are reduced as it is not the amount of acid but its strength which affects the acid catalysed hemicellulose hydrolysis.

A similar reaction scheme has been proposed by Pfeiffer[141] for the production of xylose from a variety of lignocellulosic materials.

14.3 Continuous Hydrolysis with Wash Recycle

This reactor configuration is shown in Figure 14.3. It is suitable for a hydrolysis process in which bagasse is acid treated at a low liquid to solid ratio (approximately 4 : 1).

The acid impregnated bagasse passes through the plug flow continuous reactor and acid hydrolysate is then removed from the residue by either a twin roll press or a batch basket centrifuge.

Preliminary tests with basket centrifuges indicated that a minimum moisture of content of 67% can be achieved (same moisture content at 1100g and 3500g) whereas moisture contents as low as 50% have been claimed for the twin roll press[184]. This has been attributed to the water in the fine bagasse capillaries which can only be extracted by expelling the water as in the twin roll press.

The sugar containing residue from the hydrolysate extraction process is then washed by countercurrent extraction in a diffuser and the washings containing both acid and sugar are returned to the acid make-up stream.

14.4 Continuous Hydrolysis with Recycle

This hydrolysis reactor configuration illustrated in Figure 14.4 relies on the recycle of a fraction of the bagasse hydrolysate to produce a concentrated xylose solution.

The acid impregnated bagasse passes through the hydrolysis reactor and acid hydrolysate is then removed from the residue and a fraction of this is recycled to the reactor. The hydrolysate recycle need not be injected at the feed end of the reactor but at any point along its length.

The sugar containing residue from the hydrolysate extraction process is then washed by countercurrent extraction in a diffuser and the washings are returned to the acid make-up stream.

A similar reactor system has been patented by Savo and Nyman[142]. They have also suggested a countercurrent process which is illustrated in Figure 14.5.

14.5 Two-Stage Continuous Hydrolysis Reactor

Bagasse hemicellulose contains two fractions which hydrolyse at different rates (see section 11.6).

In a two-stage continuous hydrolysis reactor system the easily hydrolysable hemicellulose fraction is hydrolysed in the first stage under mild conditions. The resistant hemicellulose fraction is then hydrolysed under more severe hydrolysis conditions in the second stage.

This system shown in Figure 14.6 has the advantage that the xylose produced from the easily hydrolysable hemicellulose is removed before the more severe secondary hydrolysis. In a one-stage hydrolysis system this xylose would remain in the reactor and undergo some decomposition while the resistant hemicellulose was hydrolysed.

14.6 Percolation Reactor

A percolation reactor is a packed-bed type reactor which is run in a semi-continuous manner. Feed material is packed in the reactor and preheated acid is continuously fed and withdrawn.

There are a number of advantages in using this reactor. The product stream is continuously collected as the reaction proceeds; an additional sugar recovery step is thus not necessary. The acid flow rate can also be adjusted such that the product stream is withdrawn before xylose undergoes significant decomposition which thus increases the kinetic yield. (This yield increase has been theoretically verified by Veeraraghavan et al[139]).

This reactor scheme which is illustrated in Figure 14.7 does have a major disadvantage in that it is a batch operation and a number of percolators with staggered operating times would be required for a continuous sugar stream.

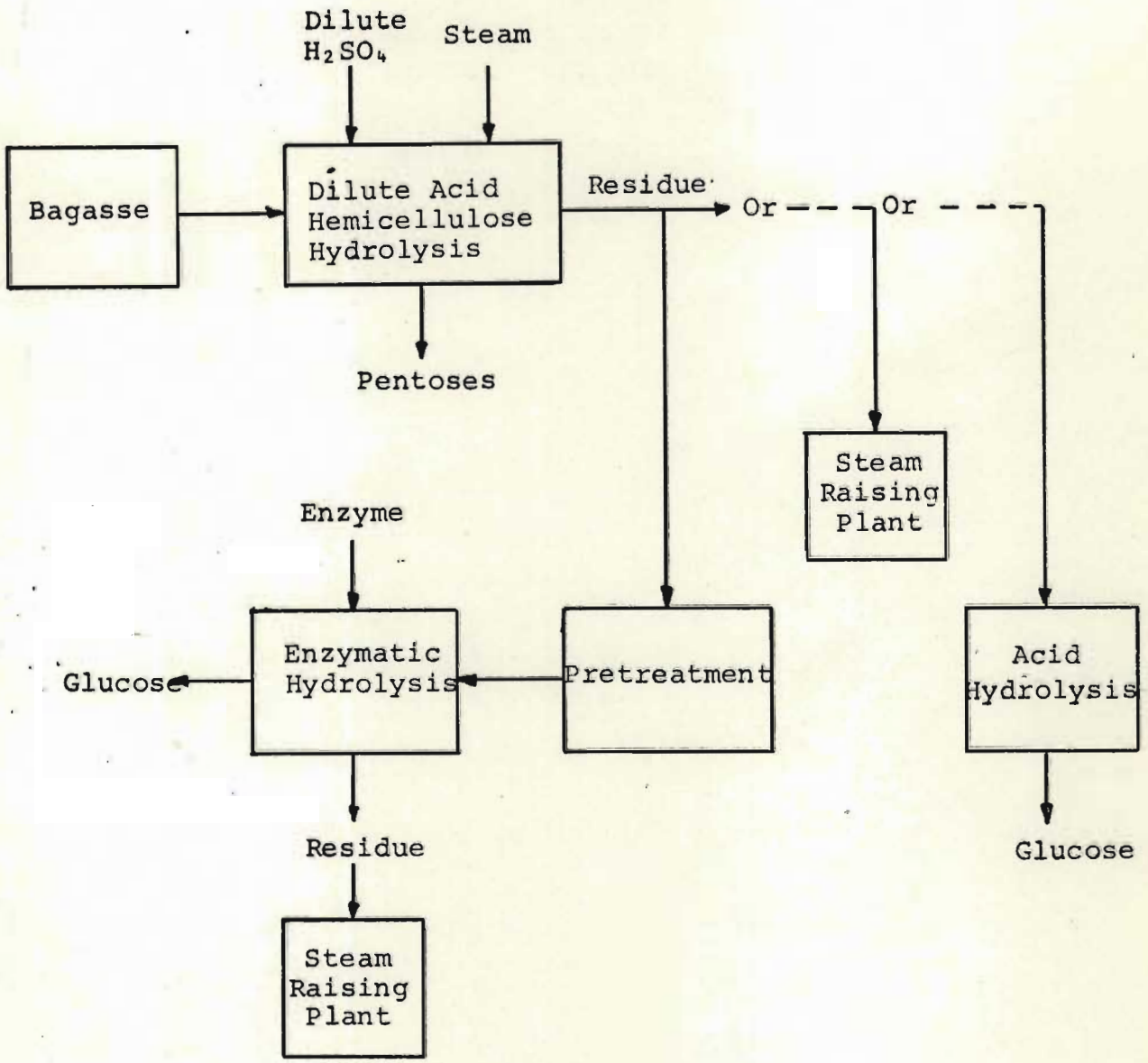


Figure 14.1 Proposed scheme for utilisation of bagasse

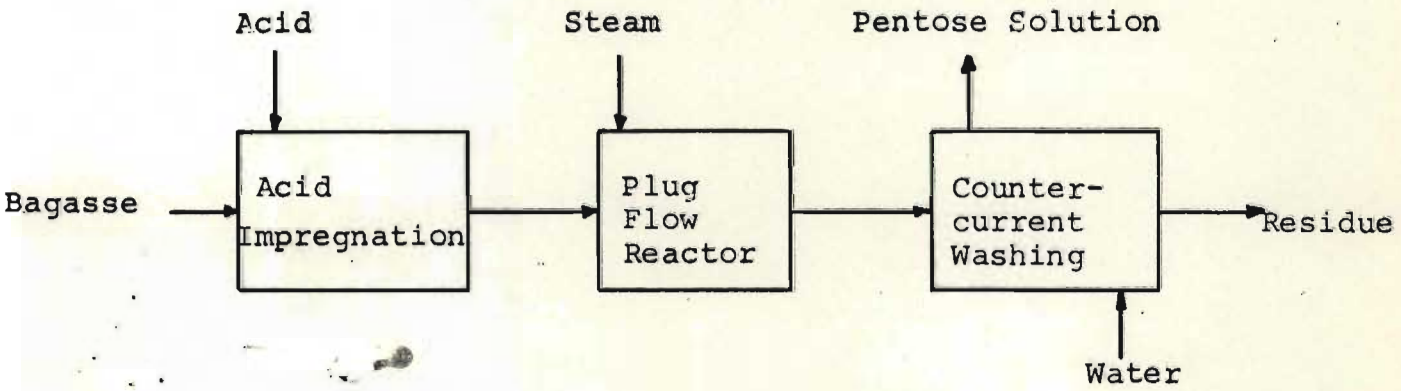


Figure 14.2 Continuous vapour-phase cooking

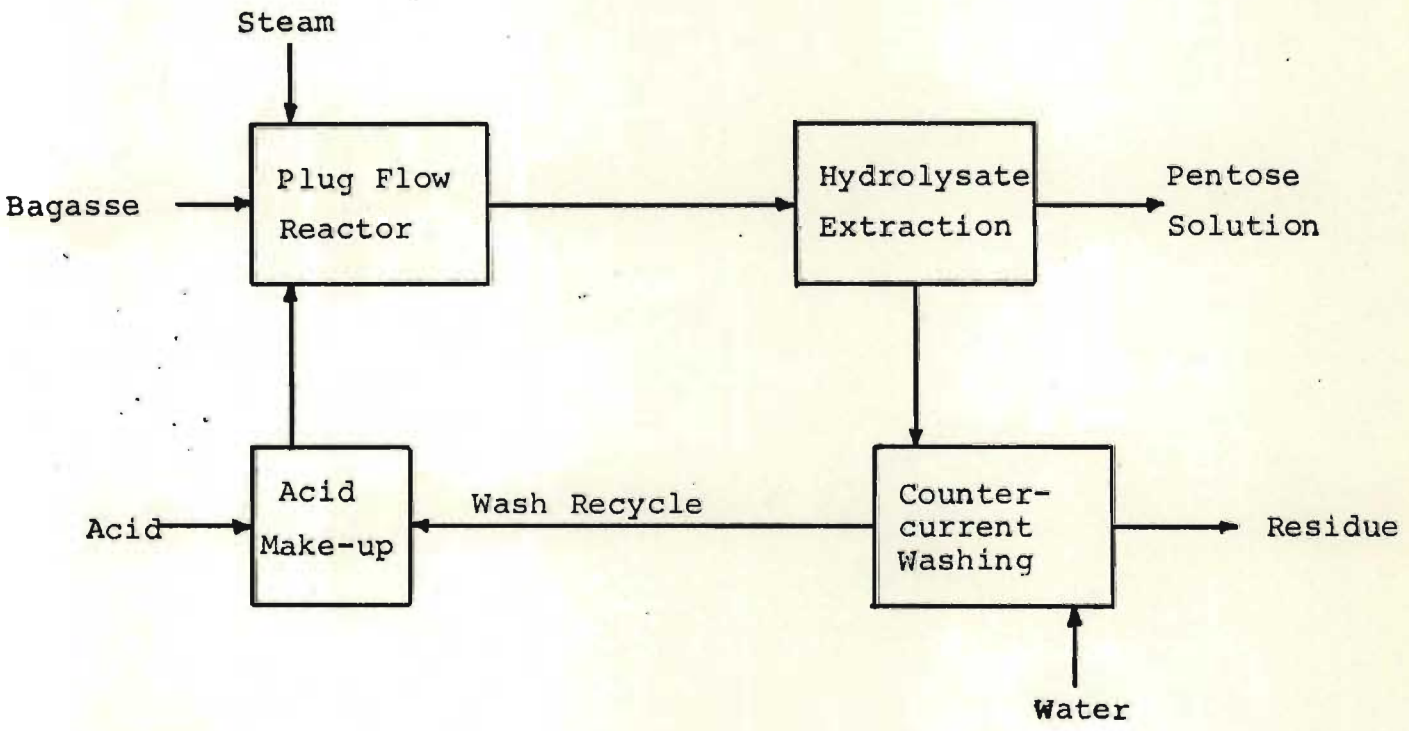


Figure 14.3 Continuous hydrolysis with wash recycle

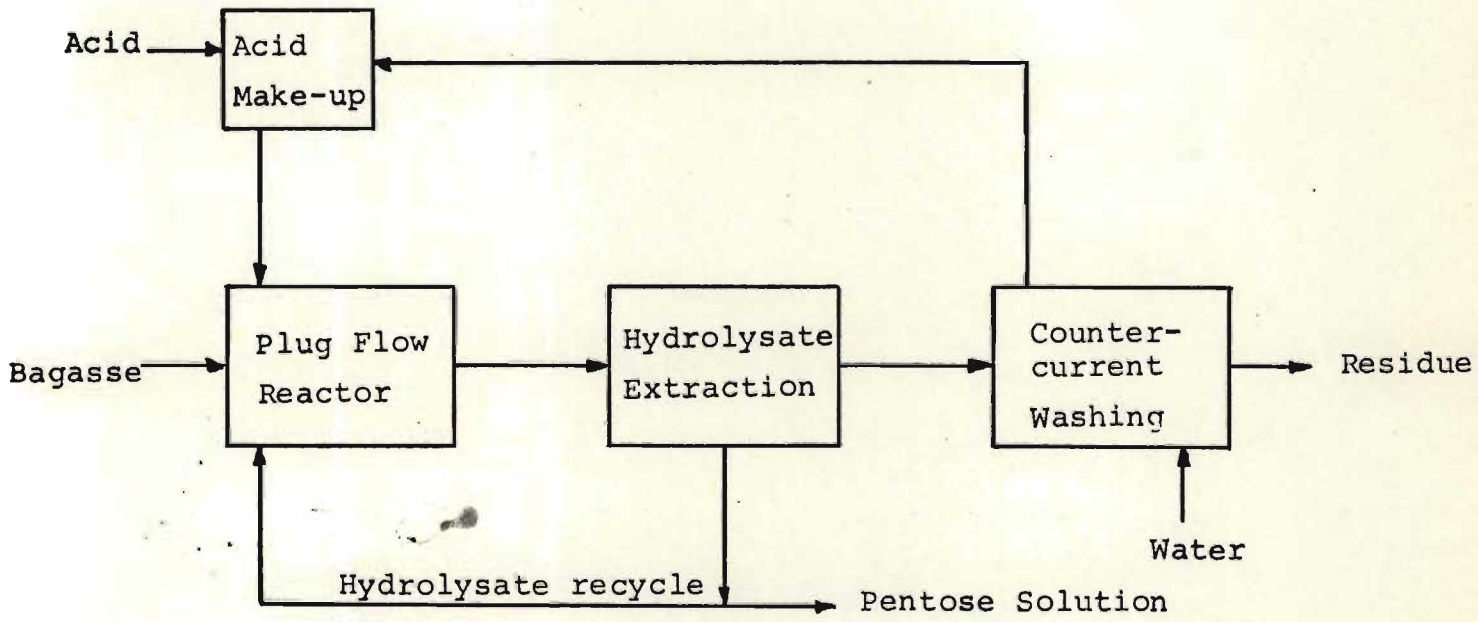


Figure 14.4 Continuous hydrolysis with recycle

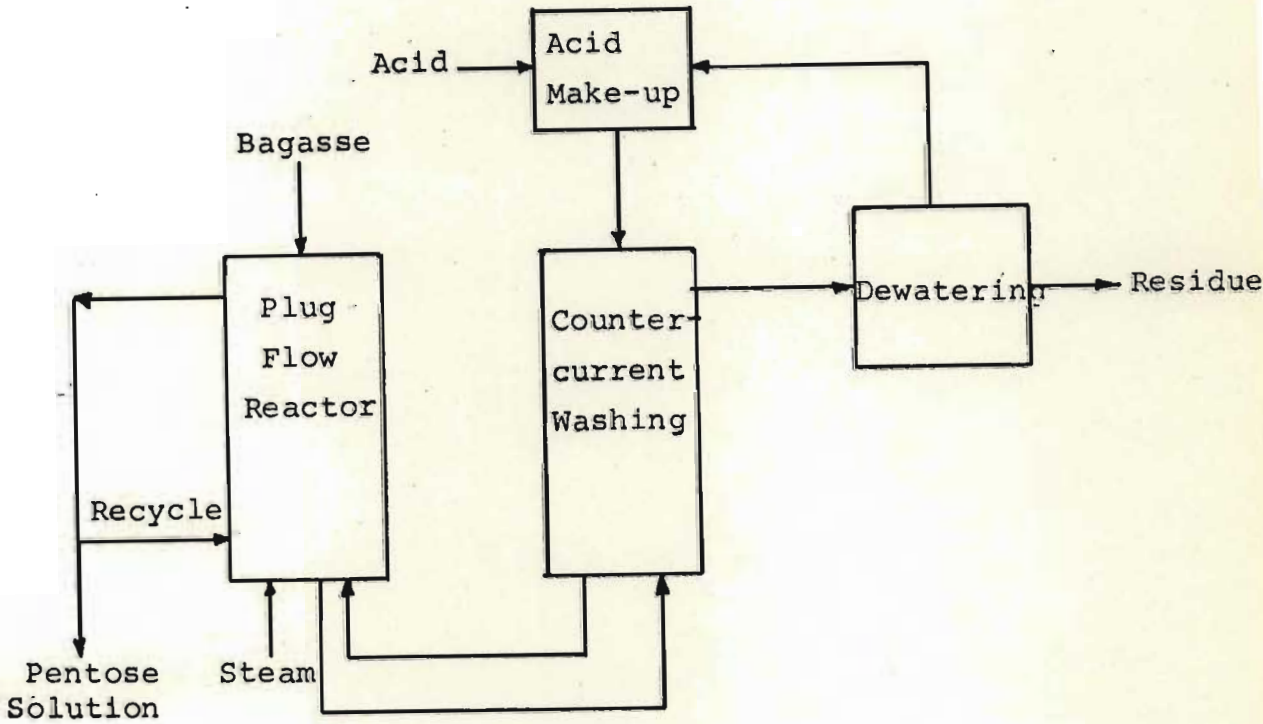


Figure 14.5 Continuous countercurrent hydrolysis with recycle

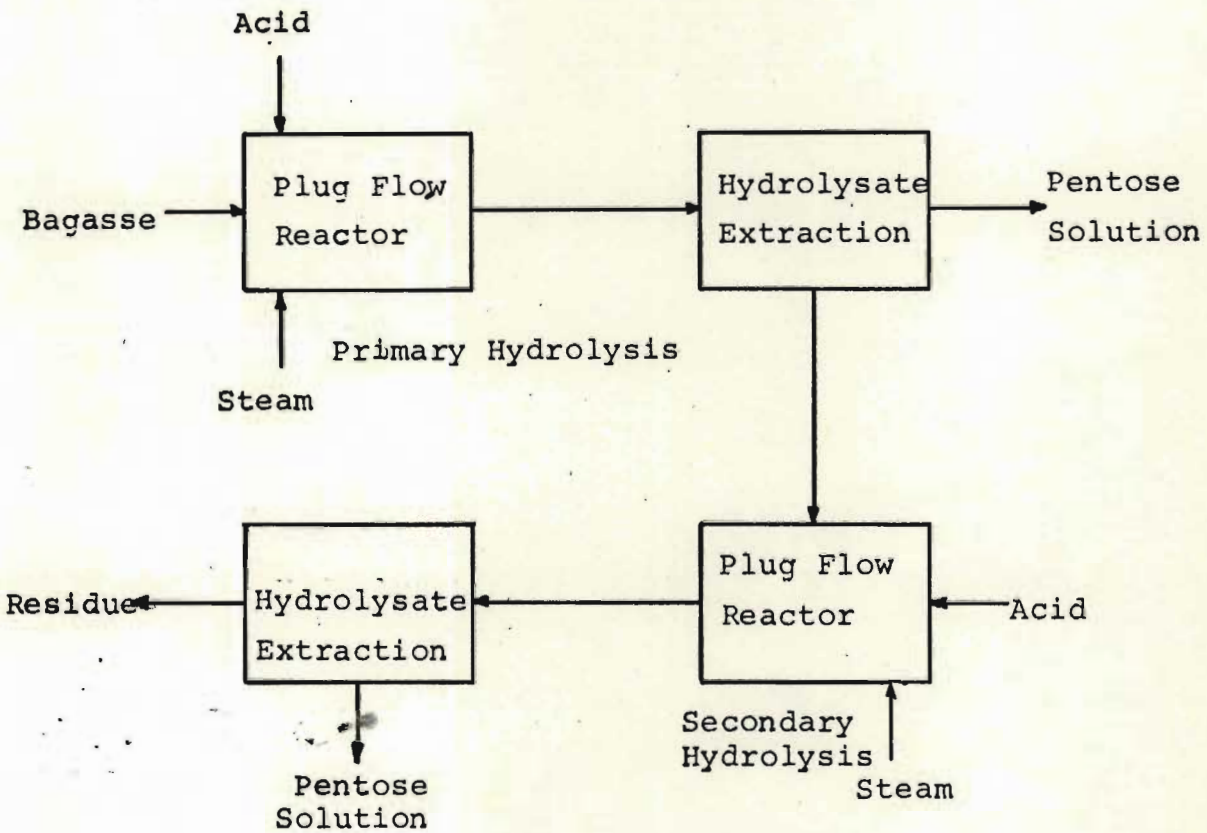


Figure 14.6 Continuous two-stage hydrolysis

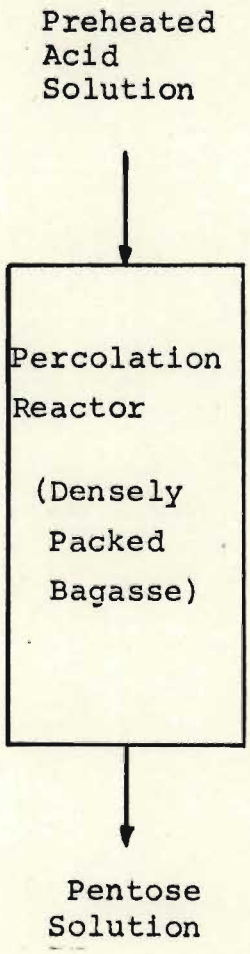


Figure 14.7. Percolation reactor

15. CONCLUSIONS

Autohydrolysis tests on bagasse show that hemicellulose hydrolysis only becomes significant at temperatures above 140°C. However, the autohydrolysis liquor still requires dilute mineral acid hydrolysis to convert the pentose oligomers produced to their monomeric forms.

Batch hemicellulose hydrolysis (75% to 95% extraction based on the pentosan analysis of the bagasse residue) can be readily achieved with dilute sulphuric acid at temperatures from 80° to 150°C and acid concentrations from 3 to 4 g/ℓ at a liquid to solid ratio of 15 : 1 without significant attack of the other lignocellulose components. At the upper end of the temperature range acid concentrations below 20 g/ℓ are sufficient for hemicellulose hydrolysis due to the effect of temperature on reaction rate.

The acid hydrolysate contains three principle sugars : xylose, arabinose, and glucose. Sugar yields at conditions at which there is not excessive pentose degradation are approximately 220, 40 and 35 mg/g dry bagasse for xylose, arabinose and glucose respectively.

The bagasse hemicellulose consists of two fractions which hydrolyse at different rates. The easily hydrolysable portion contains 165 mg of potential xylose/g dry bagasse and the more resistant fraction 105 mg of potential xylose/g dry bagasse. A first order reaction model based on two hemicellulose fractions reacting simultaneously to produce xylose has been developed to predict total xylose yields for a given set of acid hydrolysis conditions.

The bagasse residue from acid hydrolysis is brittle and amenable to milling. Attritor milling results in increased cellulose susceptibility to *Trichoderma viride* cellulase and the enzymatic susceptibility appears to be unaffected by the acid hydrolysis conditions.

Continuous acid hydrolysis of bagasse hemicellulose can be achieved at low liquid to solid ratios to produce higher final xylose concentrations in the hydrolysate. Even at a liquid to solid ratio of 3,6 : 1 (54 g/ℓ xylose) it would appear that, for the hydrolysis conditions tested, the reactor performance is still controlled by reaction kinetics rather than by other factors such as diffusion.

16. RECOMMENDATIONS FOR FUTURE WORK

16.1 Continuous Acid Hydrolysis of Bagasse Hemicellulose

With the continuous hydrolysis reactor fully operational it will now be possible to produce substantial amounts of acid hydrolysate and prehydrolysed bagasse for any further research into the utilisation of these substrates.

It is recommended that further work be done on the continuous acid extraction of the bagasse hemicellulose fraction. This work should include :

i) Accurate residence time determinations for the whole range of operating conditions using either radioactive tracer or an acid and high temperature stable dye.

ii) Hydrolyses performed over a wide range of hydrolysis conditions.

iii) Minimisation of the liquid to solid ratio to determine whether this affects the xylose yield.

iv) Comparison of xylose yields with those obtained during the batch hydrolysis tests and from the hydrolysis model developed in this work.

v) Use of various bagasse substrates in the reactor such as bagasse pith.

16.2 Neutralisation of Acid Hydrolysate

The preliminary investigation in Section 13 has indicated what calcium levels can be achieved by neutralising a hydrolysate composite produced by continuous acid hydrolysis to various pH values at room temperature with $Ca(OH)_2$ and $CaCO_3$.

Future work should concentrate on the neutralisation at elevated temperatures of hydrolysate produced by continuous hydrolysis with both $CaCO_3$ and $Ca(OH)_2$ to determine conditions that will minimise the calcium ion concentration of

the neutralised hydrolysate. Advantages of high temperature neutralisation include better precipitation at elevated temperatures and the effect of CO_2 in solution during $CaCO_3$ neutralisation will decrease due to decreasing solubility with increasing temperature. Neutralisation under pressure (above 100°C) will also result in a lower calcium ion concentration in the hydrolysate due to the lower solubility of $CaSO_4.\frac{1}{2}H_2O$ as shown in Figure 13.1.

Another method of hydrolysate neutralisation is by sulphuric acid recovery. Recovery systems such as dialysis also could be investigated to determine their economics.

16.3 Utilisation of Hydrolysate

The acid extract produced by dilute acid hemicellulose hydrolysis contains three main sugar components ; xylose, arabinose and glucose with xylose the principle sugar.

It is recommended that the various chemical processes (as distinct from biological conversion processes) for the conversion of the hydrolysate sugars into useful components should be investigated to determine whether any show economic promise for the utilisation of the acid hydrolysate.

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APPENDIX A

PRECISION OF ANALYTICAL RESULTS

1. Precision of Lignin, Cellulose and Pentosan Results

A bagasse sample obtained from the Tongaat sugar mill was hammer-milled and analysed a number of times to obtain the precision of the bagasse component analyses. The results are shown in Table A.1.

Table A.1. : Reproducibility of bagasse analyses

Analysis	1	2	3	4	5	6	7	8
Lignin	22,59	22,64	22,56	22,68	22,68	22,86	21,72	22,00
Cellulose	39,36	39,32	39,46	38,98	37,35	37,01	37,78	37,50
Pentosan	30,69	31,84	31,46	31,92	32,88	31,42	31,91	31,88
Ash	3,00	2,30	3,20	3,00	2,41	4,43	3,00	3,50
Total	95,64	96,10	96,68	96,58	95,32	95,72	94,41	94,88

The totals in Table A.1. do not add up to 100% as the water and solvent solubles have not been included.

The percent relative deviation is defined :

$$\% \text{ relative deviation} = \frac{\text{average/deviation from mean}/x}{\text{mean value}} \times 100$$

The percent relative deviations for the lignin, cellulose and pentosan analyses were calculated to be 1,35%, 2,45% and 1,32% respectively.

2. Precision of Xylose and Glucose Analysis

One percent standard solutions of xylose and glucose were prepared and analysed by HPLC. Peak height measurements were used to determine the precision of the analyses. The results are shown in Table A.2.

Table A.2. : Reproducibility of xylose and glucose analyses

Sample Number	Xylose Peak Height (mm)	Glucose Peak Height (mm)
1	141,5	107,5
2	140,2	108,1
3	139,8	109,3
4	139,0	108,9
5	136,0	108,3
6	138,9	110,1

The percent relative deviations for the xylose and glucose analyses were calculated to be 0,84% and 0,69% respectively.

APPENDIX B

BATCH PRESSURE HYDROLYSIS REACTOR DESIGN

1. Determination of Pipe Schedule Number

$$\text{Schedule No.} = 1000 \frac{P_s}{S_s} \quad (173)$$

where P_s = safe working pressure (psi)

S_s = safe working stress (psi)

For operation at 150 psi (10 bar) and a safe working stress of 18000 psi[174].

Schedule No. = 8,33

Specify Schedule 40 316L stainless steel pipe for the hydrolysis and preheat vessels and schedule 10 pipe for the steam jackets.

2. Calculation of End Flange Thickness

The thickness of flat covers and end plates can be calculated from[175]:

$$t = D\sqrt{\frac{P}{kf}}$$

where t = thickness (in)
P = design pressure (psi)
D = plate diameter (in)
k = constant (2,6 for flanges)
f = design stress (psi)

$$t = 8{,}07\sqrt{\frac{150}{2{,}6 \times 18000}}$$

$$= 0{,}46 \text{ inches}$$

$$\equiv 11{,}7 \text{ mm}$$

Specified 16 mm blank flanges for vessel ends.

3. Determination of Flange Thickness

The required flange thickness was calculated according to the method presented in Section 3M of B.S. 1500 Part 1 : 1958[175].

A flange thickness of 16 mm was found to be suitable for both reactor vessels.

4. Bolt Specifications

The number of bolts required for the flanges was obtained from a table in the Chemical Engineer's Handbook[174] which gives the bolt requirements for 150 lb flanges.

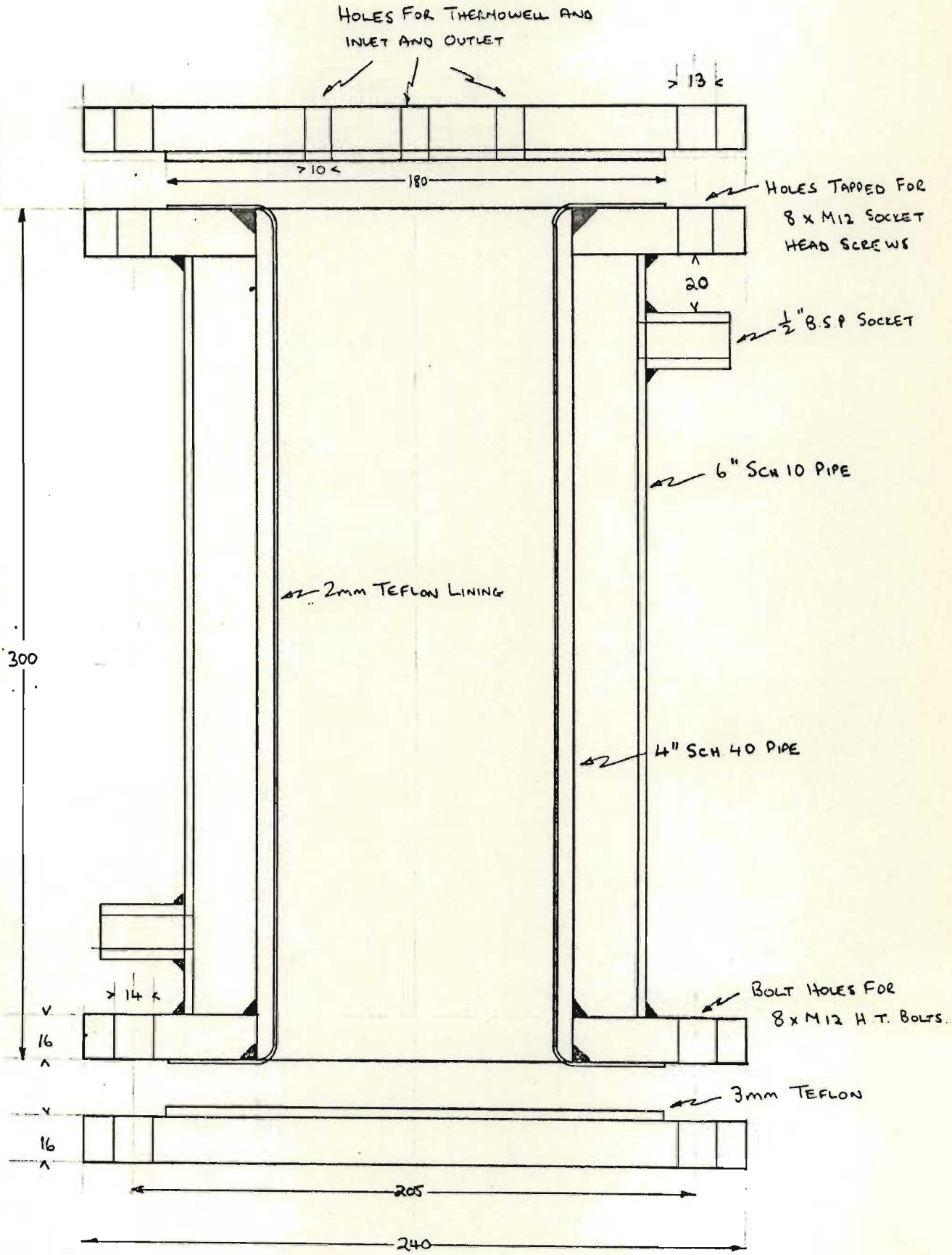
All the bolts used were M12 high tensile bolts or socket head screws.

5. Reactor System Drawings

The reactor system drawings are shown in Figures B.1., B.2 and B.3.

Figure B.1.

Acid Preheat Vessel Detail



NOTE 1. ALL DIMENSIONS IN MILLIMETERS

2. ALL MATERIALS 316L S. STEEL UNLESS OTHERWISE STATED

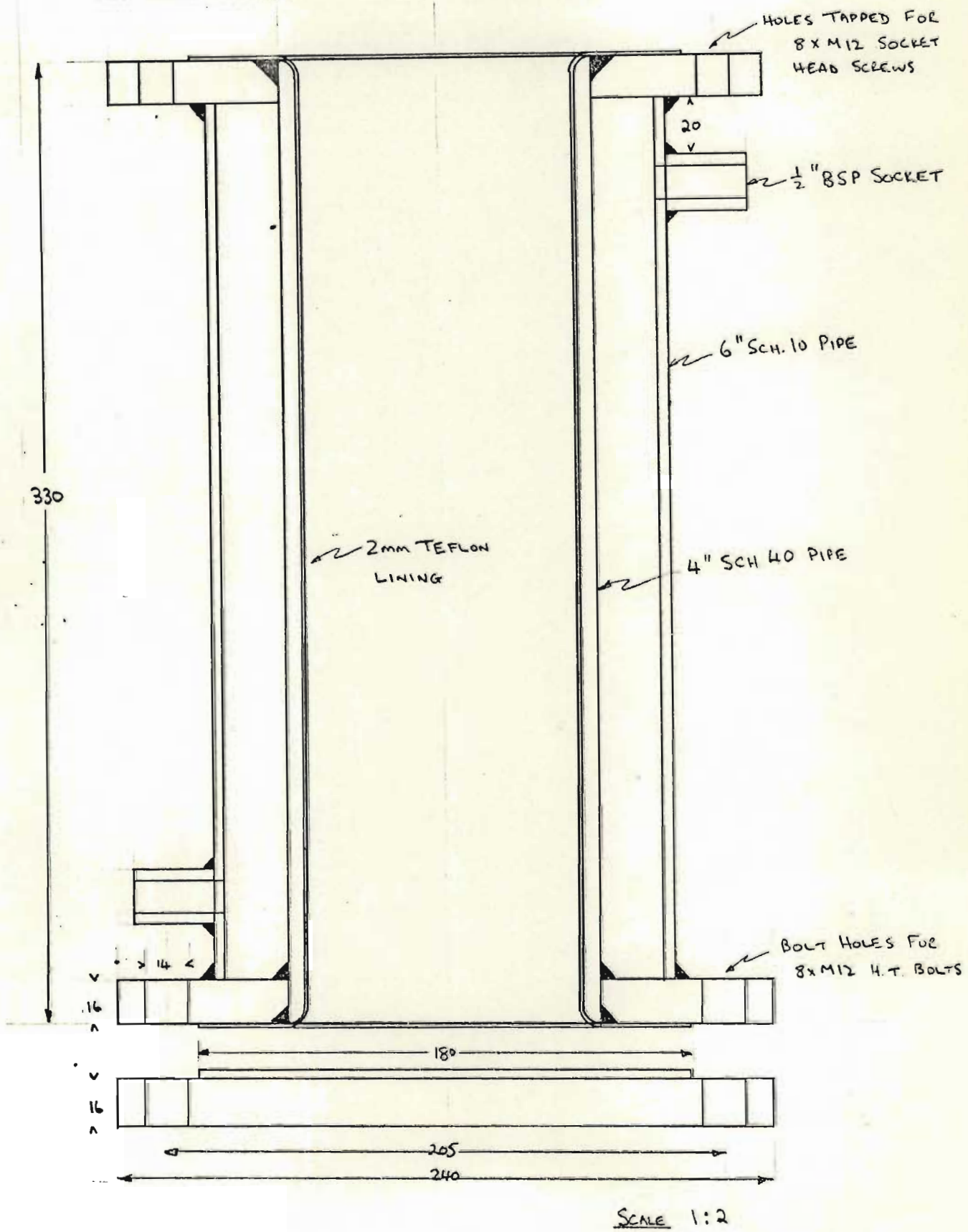
3. ALL FLANGES SOLID TYPE, FLAT

SCALE 1:2

Figure B.2.

Hydrolysis Reactor Detail

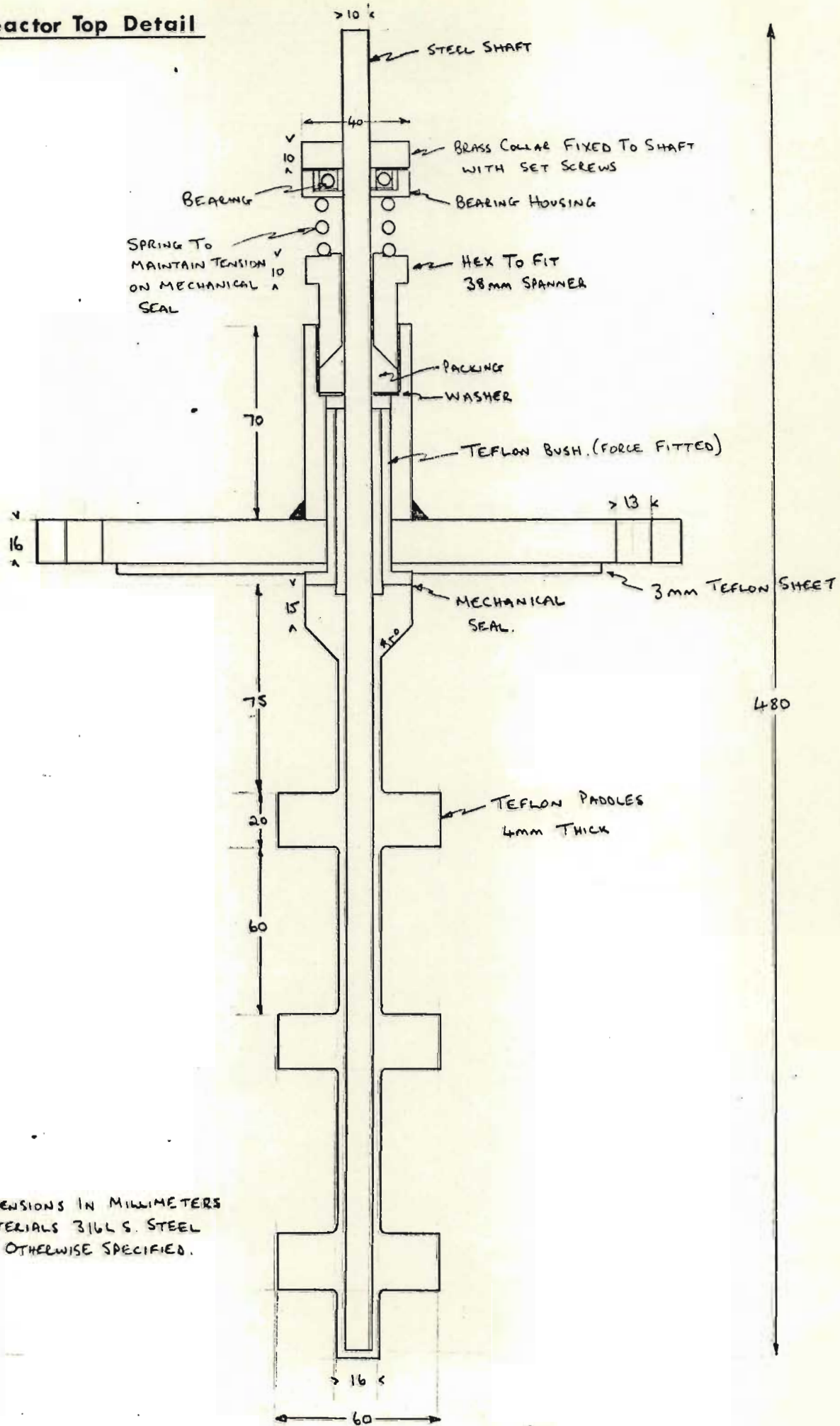
NOTE 1. ALL DIMENSIONS IN MILLIMETERS.
2. ALL MATERIALS 316L S. STEEL UNLESS OTHERWISE SPECIFIED.
3. ALL FLANGE FACES TURNED FLAT AFTER WELDING.



SCALE 1:2

Figure B.3.

Hydrolysis Reactor Top Detail



NOTE 1. ALL DIMENSIONS IN MILLIMETERS
2. ALL MATERIALS 316L S. STEEL UNLESS OTHERWISE SPECIFIED.

SCALE 1:2

APPENDIX C

CONTINUOUS PRESSURE HYDROLYSIS REACTOR DESIGN

1. Determination of Pipe Schedule Number

$$\text{Schedule No.} = 1000 \frac{P_s}{S_s} \quad (173)$$

where P_s = safe working pressure (psi)
S_s = safe working stress (psi)

For operation at 150 psi (10 bar) and a safe working stress of 18000 psi[174].

Schedule No. = 8,33

Thus specify schedule 40 pipe for all pressurised portions of the hydrolysis reactor (includes allowance for corrosion).

2. Calculation of End Flange Thickness

The thickness of flat covers and end plates can be calculated from[175]:

$$t = D\sqrt{\frac{P}{kf}} + C$$

where t = thickness (in)
P = design pressure (psi)
D = plate diameter (in)
k = constant (2,6 for flanges)
C = corrosion allowance (in)
f = design stress (psi)

$$t = 7,1\sqrt{\frac{150}{2,6 \times 18000}} + C$$

$$= 0,40 + C \text{ inches}$$

Specified 16 mm blank flanges for reactor ends.

3. Determination of Flange Thickness

The required flange thickness was calculated according to the method presented in section 3M of B.S.1500 : Part I : 1958[175].

A flange thickness of 16 mm was found to be suitable for both the 3" and 4" schedule 40 pipes.

4. Bolt Specifications

The number of bolts required for the flanges was obtained from a table in the Chemical Engineers' Handbook[174] which gives the bolt requirements for 150 lb flanges.

All the bolts used were M16 high tensile bolts (or their imperial equivalents, ⅝", as the slide valves were fitted with Whitworth tappings).

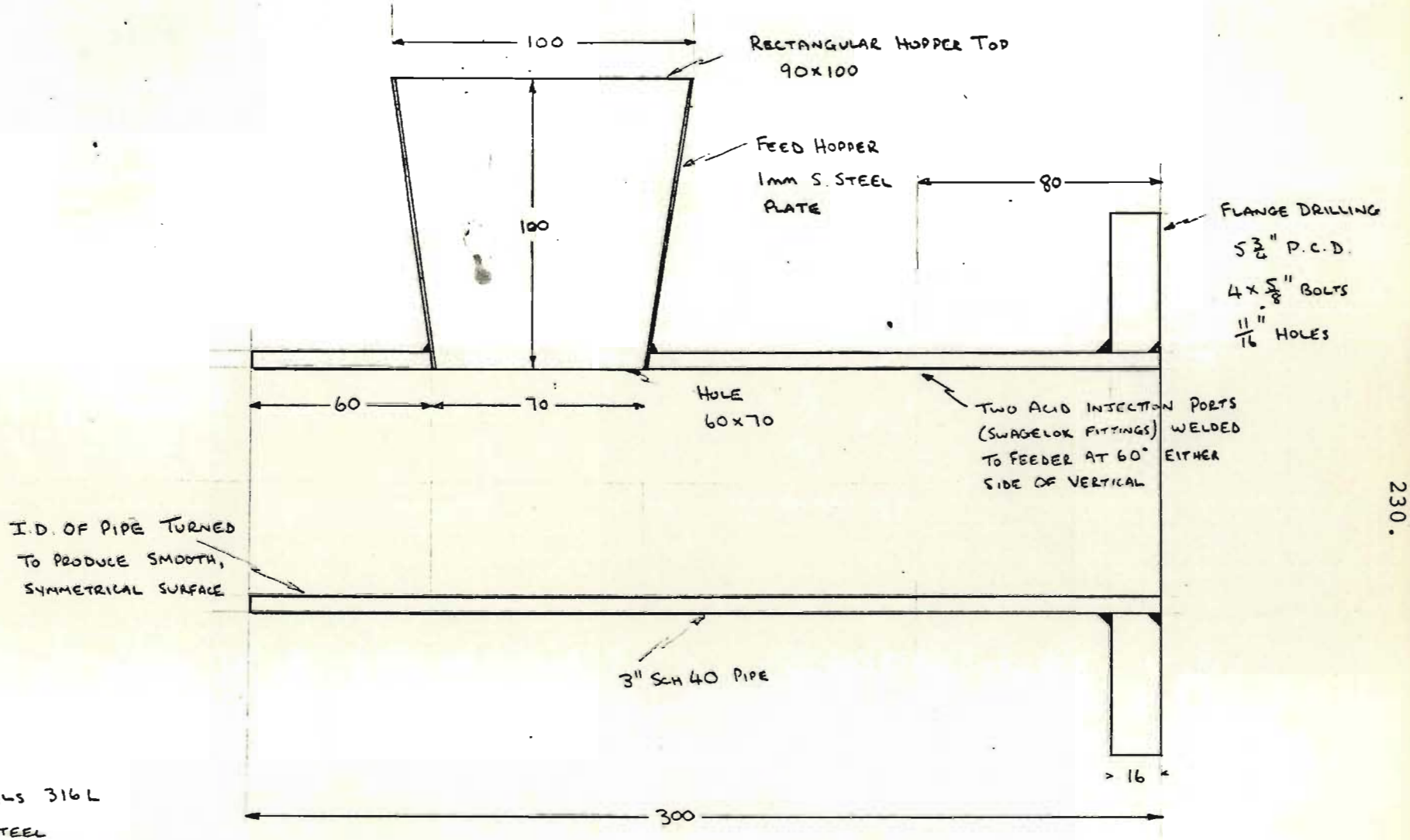
5. Reactor System Drawing

The reactor drawings are shown in Figures C.1, C.2, C.3, C.4, C.5, C.6, C.7, C.8 and C.9.

The reactor pneumatic control systems are shown in Figures C.10 and C.11.

Feeder Detail

Figure C.1.



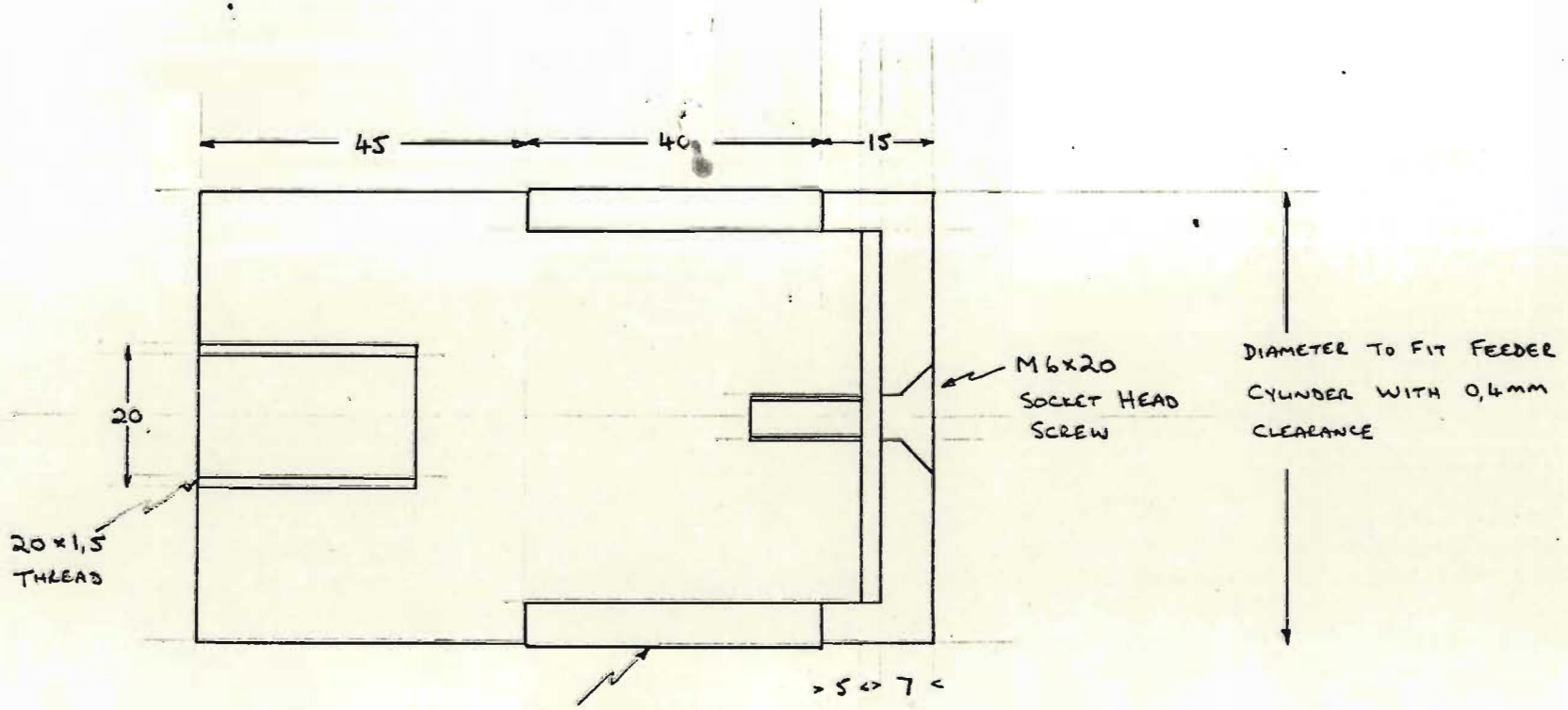
NOTE ALL MATERIALS 316L STAINLESS STEEL

ALL DIMENSIONS IN MILLIMETERS UNLESS SPECIFIED.

SCALE 1:2

Plunger Detail

Figure C.2.



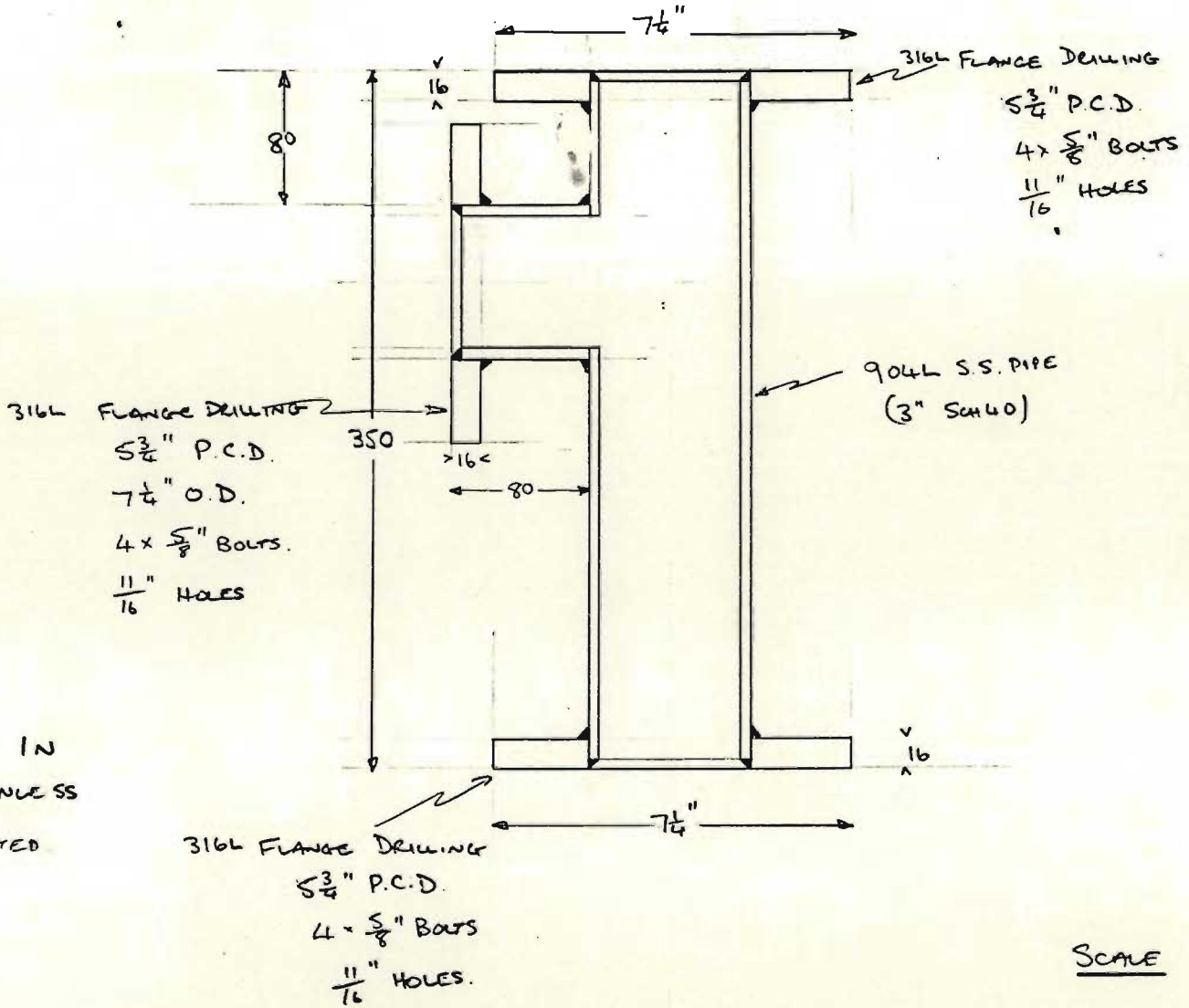
OTE ALL MATERIALS 316L STAINLESS STEEL UNLESS OTHERWISE SPECIFIED.

DIMENSIONS IN MILLIMETERS

SCALE 1:1

T-Pipe Detail

Figure C.3.

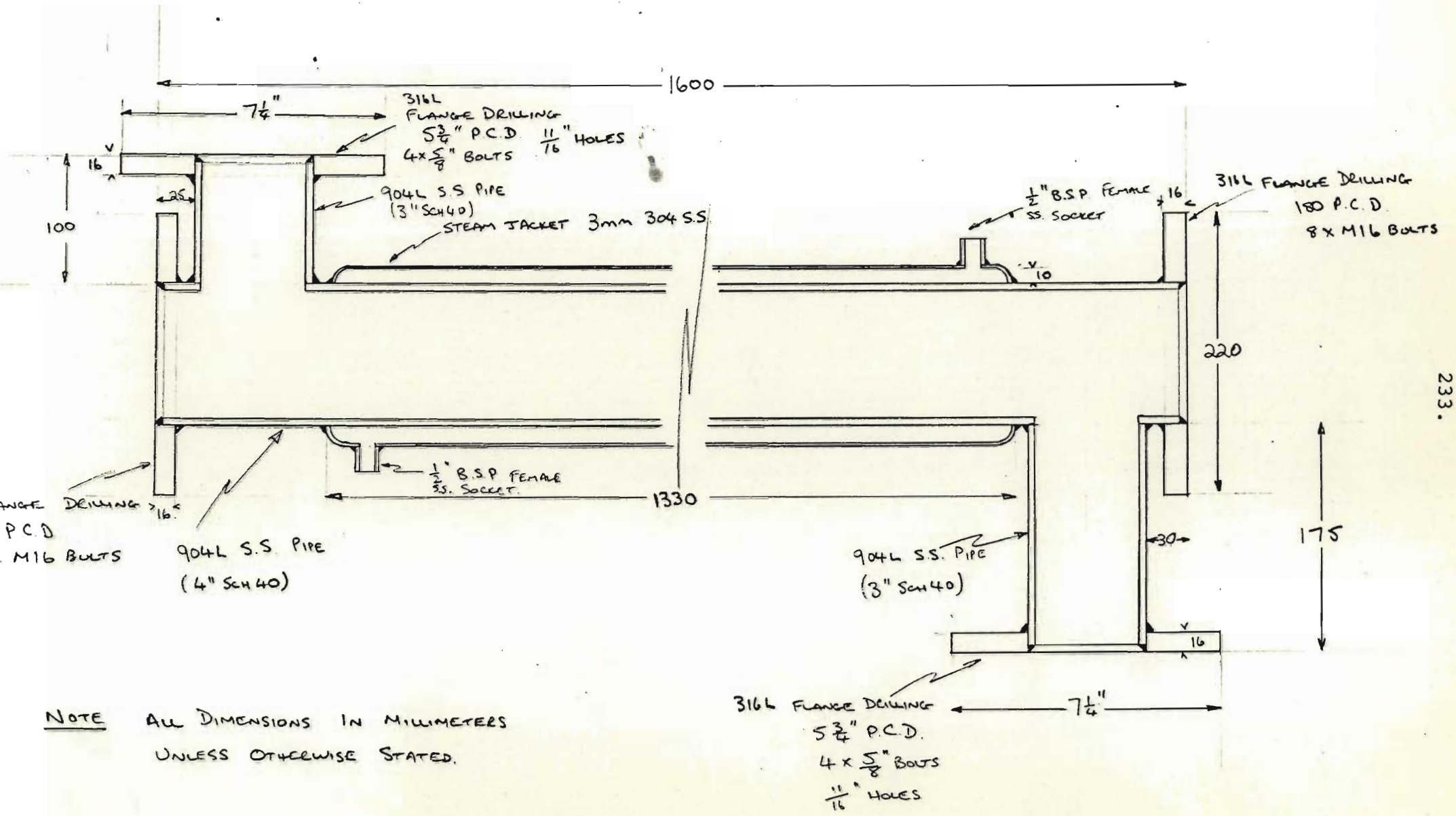


E ALL DIMENSIONS IN MILLIMETRES UNLESS OTHERWISE STATED

SCALE 1:4

Digester Detail

Figure C.4.



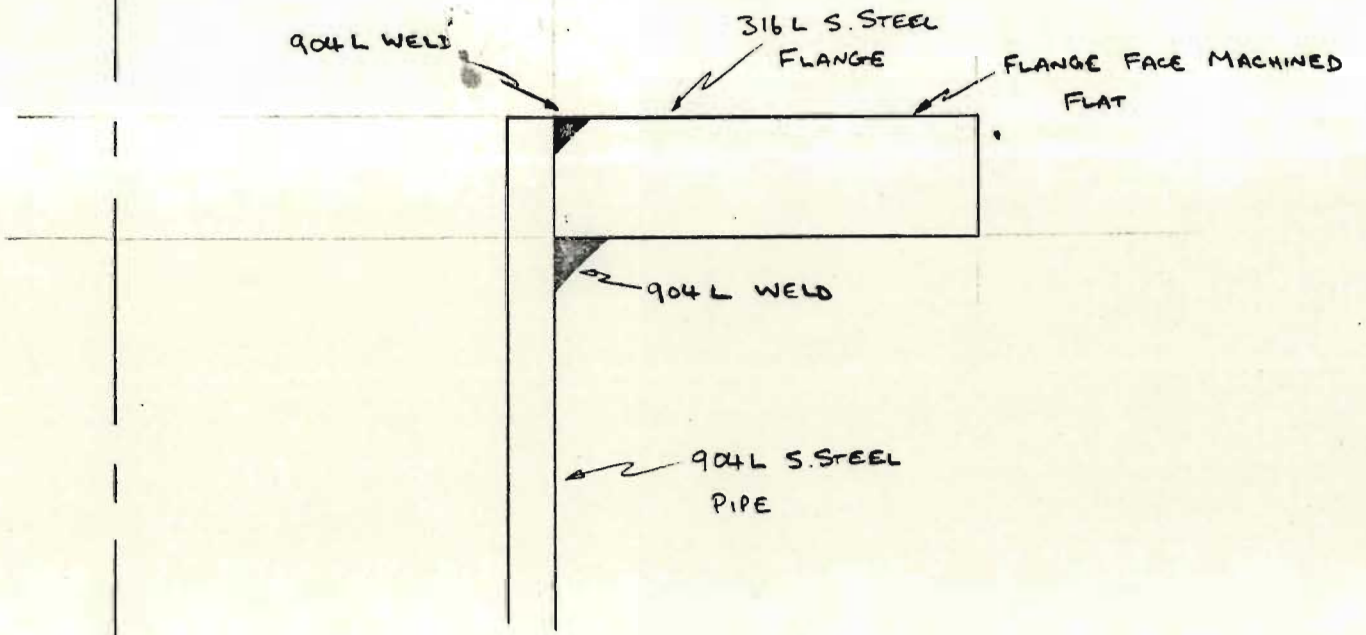
NOTE ALL DIMENSIONS IN MILLIMETERS UNLESS OTHERWISE STATED.

SCALE 1:4

Flange Weld Detail

Figure C.5.

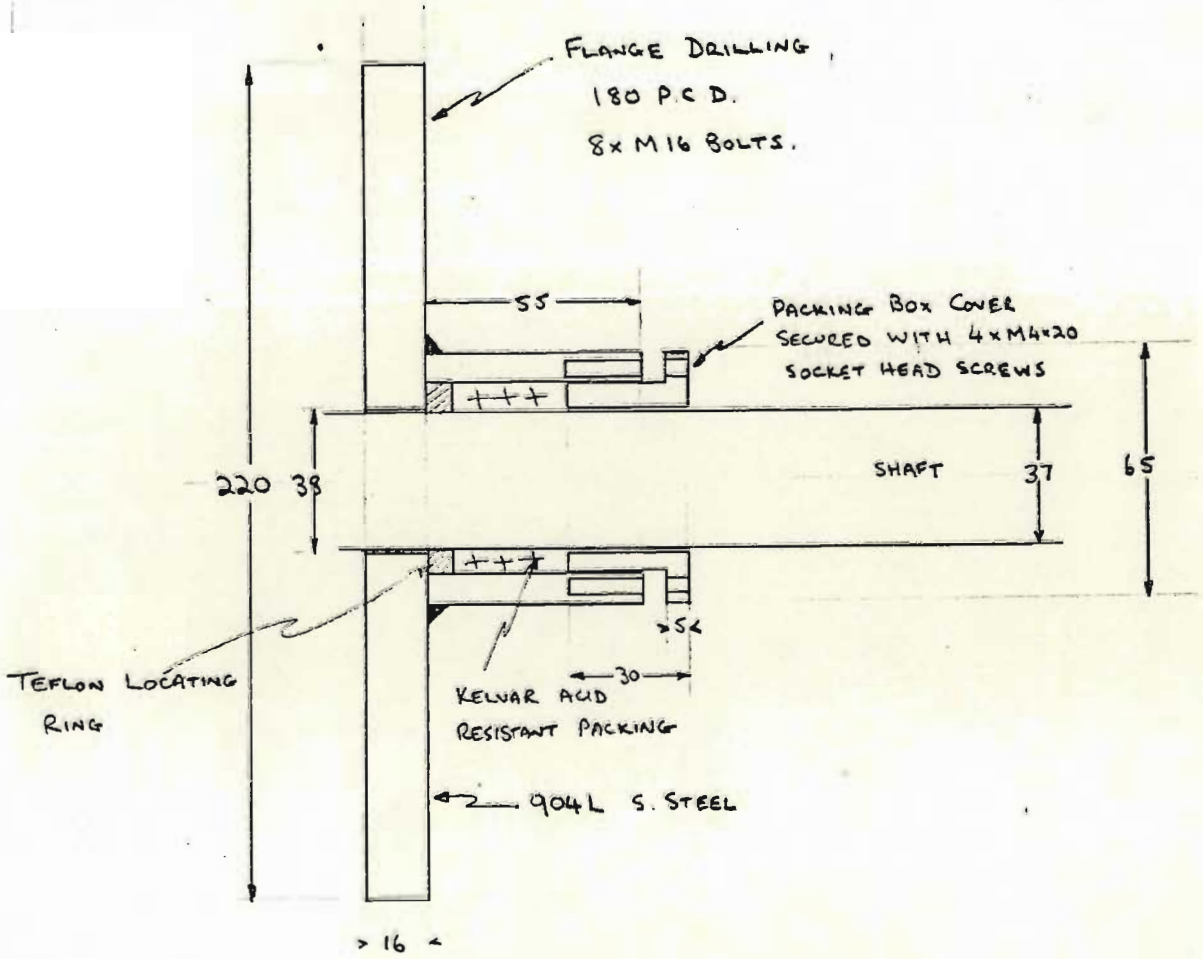
NOTE 904L S.STEEL PIPE FLUSH WITH FLANGE FACE



SCALE 1:1

Packing Box Detail

Figure C.6.



NOTE ALL DIMENSIONS IN MILLIMETERS UNLESS SPECIFIED

ALL MATERIALS 316L STAINLESS STEEL UNLESS SPECIFIED

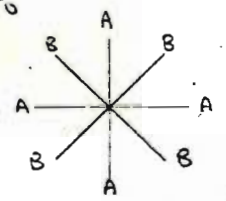
SCALE 1:2

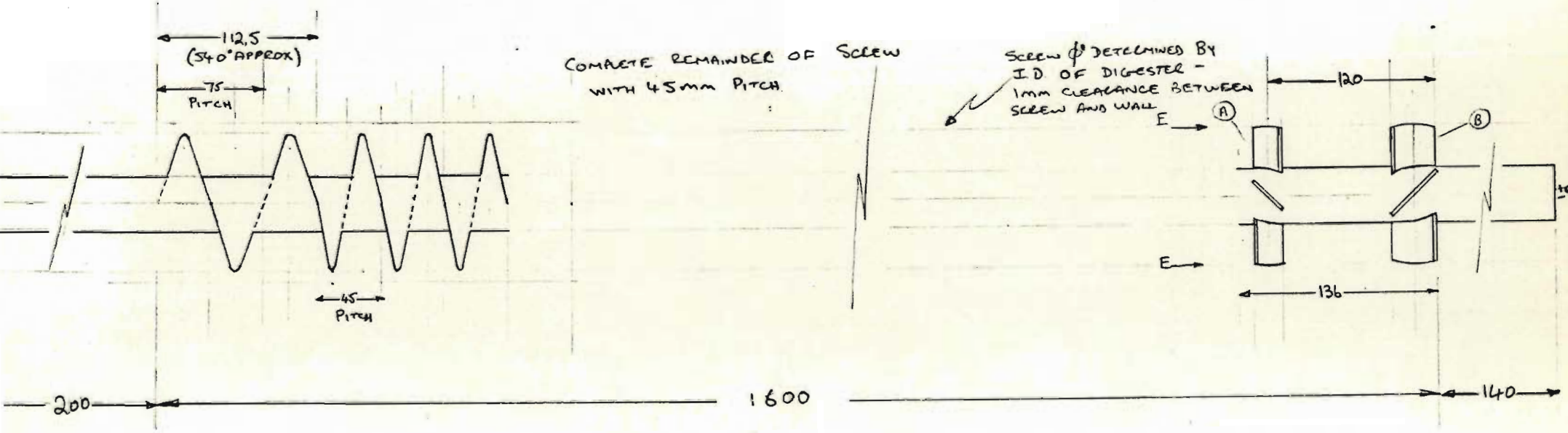
igester Screw Detail

Figure C.7.

SCREW FLIGHTS 4mm S STEEL PLATE WELDED TO SHAFT ON BOTH SIDES BY CONTINUOUS WELD.

STAGGER (A) and (B) AT 45° VIEW EE →





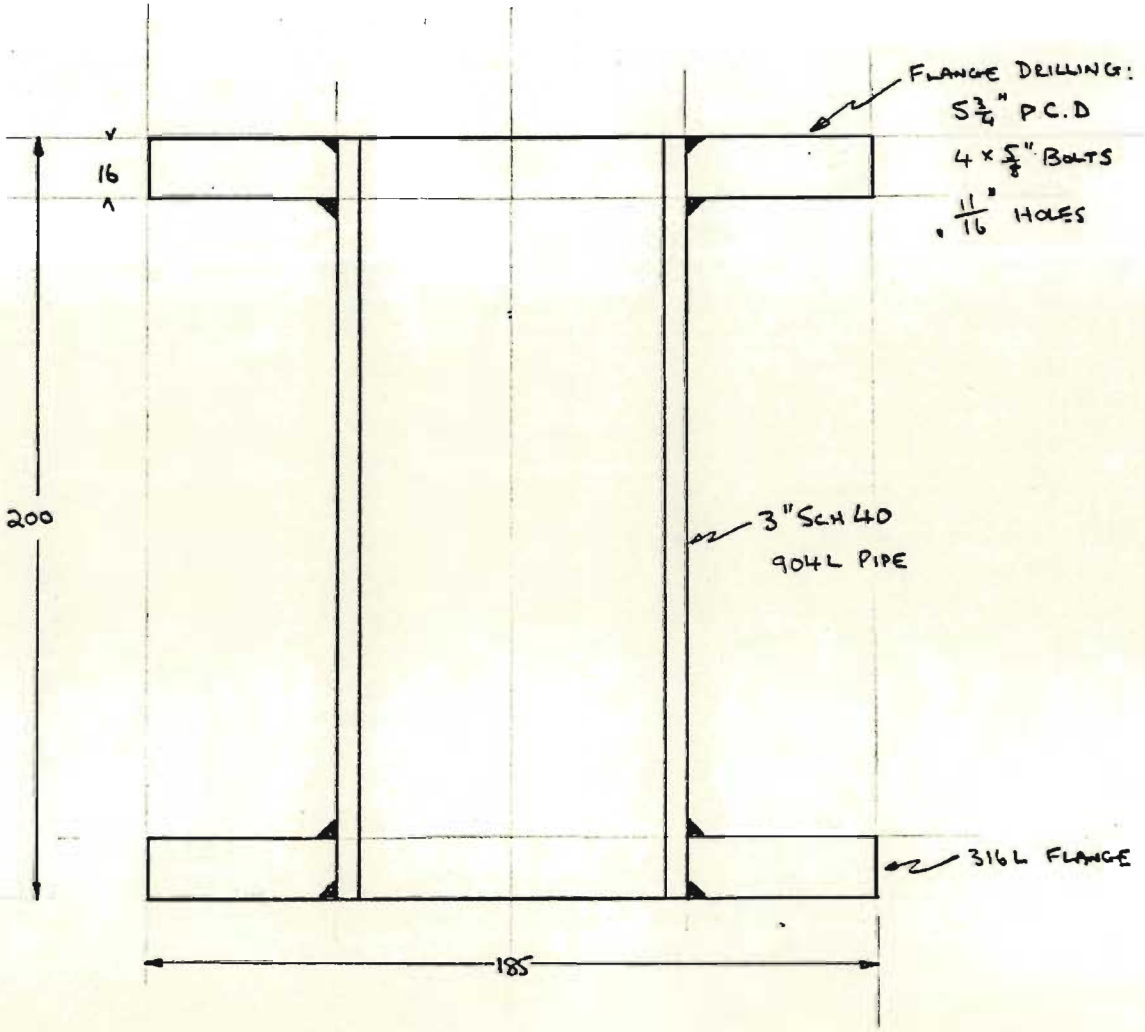
NOTE ALL DIMENSIONS IN MILLIMETERS

ALL MATERIAL 904L S. STEEL.

SCALE 1:4.

Transfer Leg Detail

Figure C.8.

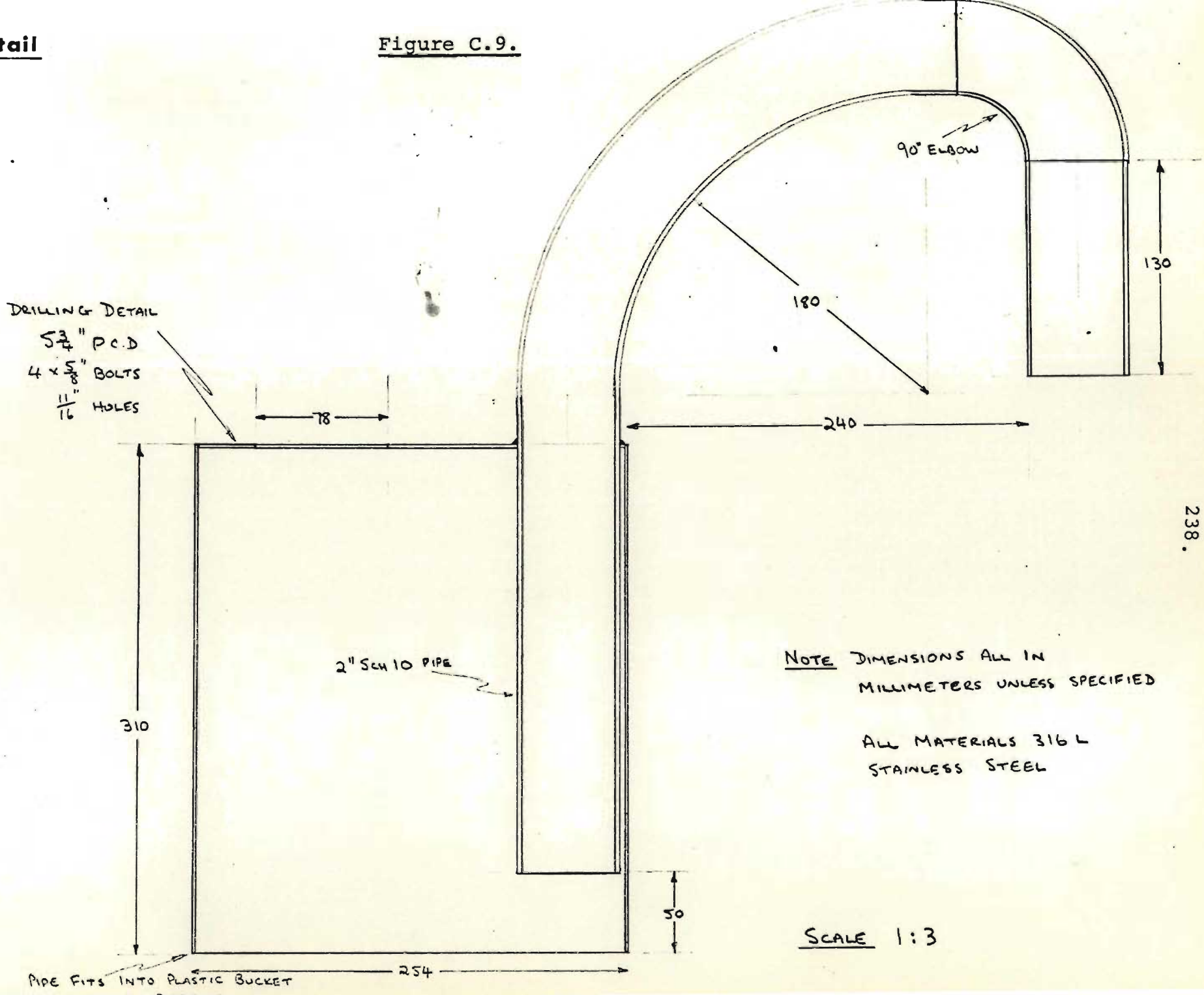


NOTE ALL DIMENSIONS IN MILLIMETERS UNLESS SPECIFIED

SCALE 1:2

Residue Bin Detail

Figure C.9.



NOTE DIMENSIONS ALL IN MILLIMETERS UNLESS SPECIFIED

ALL MATERIALS 316 L STAINLESS STEEL

SCALE 1:3

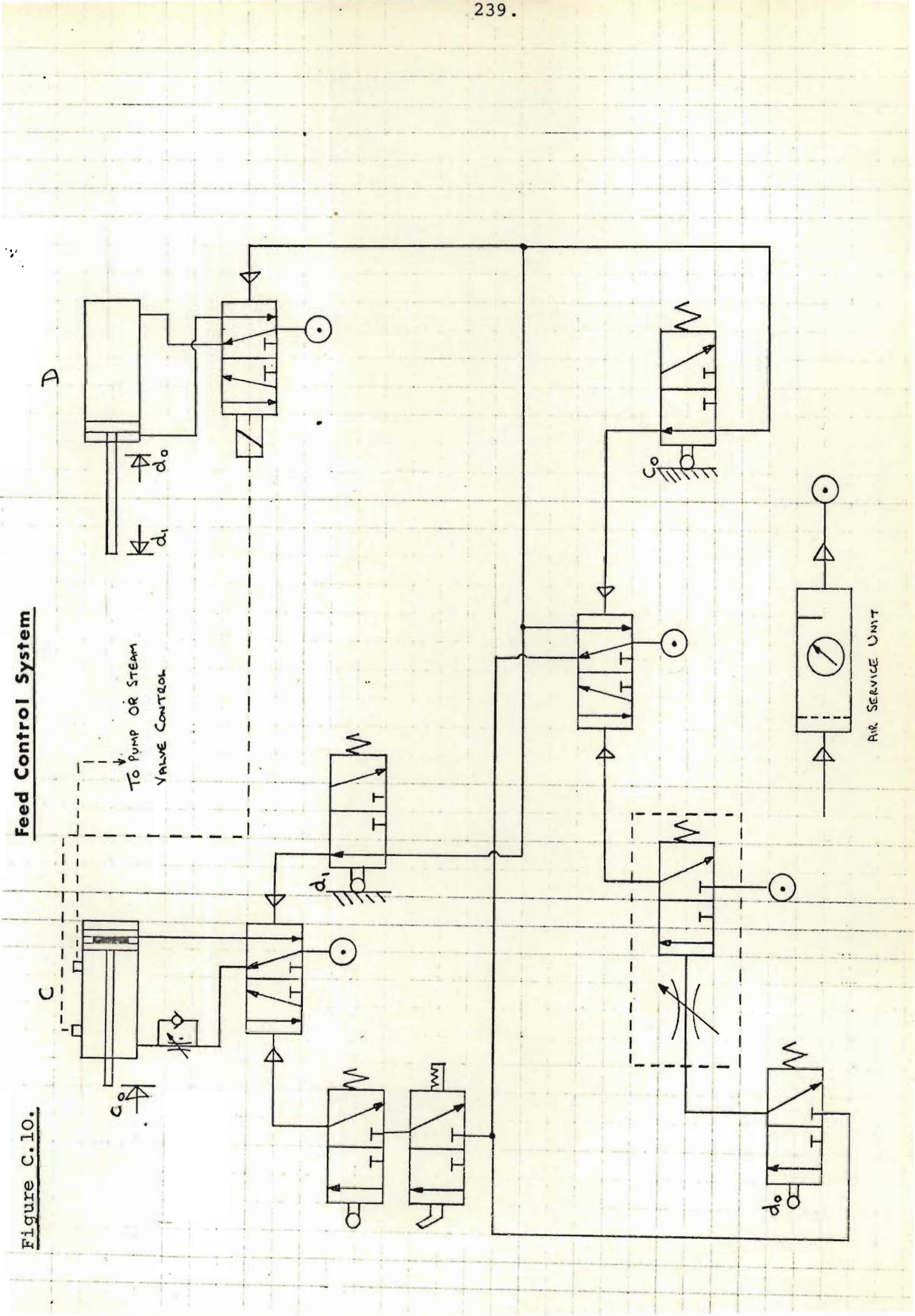

Feed Control System
A
C
d_o
d_i
c_o
TO PUMP OR STEAM VALVE CONTROL
AIR SERVICE UNIT

Figure C.10.

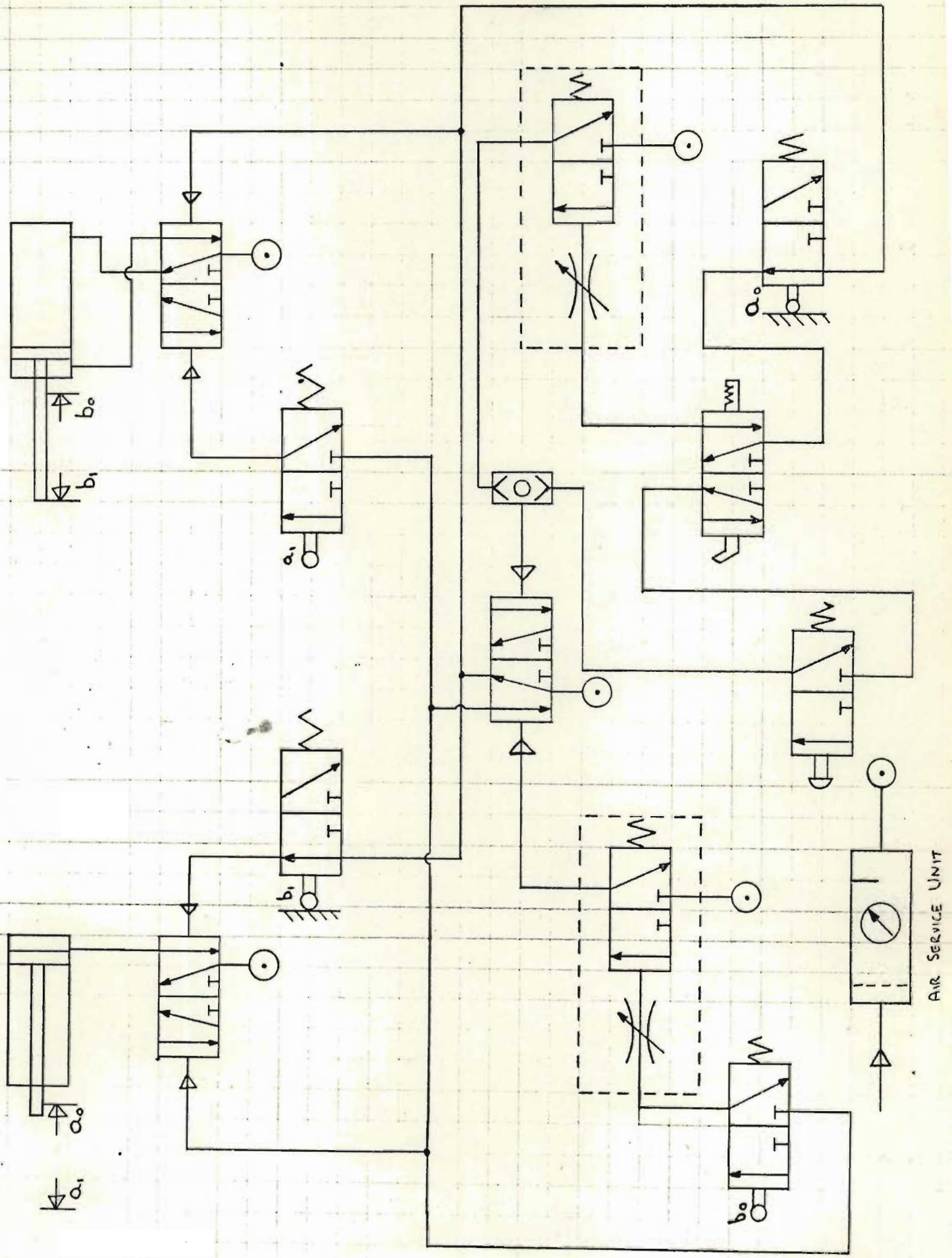


Figure C.11.

APPENDIX D.

OPERATING PROCEDURE FOR CONTINUOUS ACID HYDROLYSIS REACTOR

The hydrolysis reactor can be operated in two modes :

i) controlled acid injection with continuous steam injection into the reactor
ii) controlled steam injection using a solenoid valve with acid impregnated bagasse feed.

Start Up

1) Ensure that steam injection to the reactor is shut-off.
2) Allow steam at the required pressure to flow to the vessel jacket to preheat the reactor.
3) Check the air and electricity supplies to the feed and discharge control systems.
4) Check that the discharge control system is in the manual mode.
5) Check that the feed pneumatic control system is off.
6) Check that all the slide valve sealing glands are fully packed with high temperature packing.
7) If the reactor is operated in the acid injection mode, check that the correct volume of acid is injected during each cycle by activating the acid pump using reset button P. (Can change acid volume by varying pump timer and/or stroke but must check that the pump stops completely before the feed piston passes the acid injection ports).
8) If the reactor is operated in the controlled steam injection mode, check that the required amount of steam is injected during each cycle by using reset button P. (Can vary the steam injected by changing the timer P and the setting on the flow control valve but must check that the solenoid valve closes before the feed piston is retracted).
9) Switch on the motor drive and set the screw speed (this also determines the feed rate).
10) Set the discharge cycle timer to the required value.

11) If the reactor is operated in the acid injection mode, set the steam injection control valve, open the gate vale to supply injection steam and ensure that the solenoid valve is energised i.e. open.

12) If the reactor is operated in the controlled steam injection mode, open the gate vale to supply injection steam.

13) Open the drain valve on the steam injection leg (one full turn) to allow any condensate to escape.

14) Check the reactor temperature : Channel 1 - inlet temp °C
Channel 2 - outlet temp °C

Operation

1. Switch to automatic mode on discharge control system.
2. Feed hammer milled bagasse into hopper (± 150 mℓ of moist bagasse - 50% H_2O or acid impregnated).
3. Switch on feed control system.
4. Feed bagasse to hopper each time piston has completed cycle.
5. Check that all red lights on the electric relays are out at the end of each cycle. If not, switch off the feed control systems, rectify the problem using the correct reset button (P or V) and then reactivate the feed control system.
6. Switch off the feed control system when bagasse feed is completed.

Shut Down

1. Close steam supply to the reactor
2. Allow reactor to cool below 100°C.
3. Remove flange on top of T-pipe and fill reactor with water.
4. Discharge and collect washings at a rapid discharge cycle time (screw speed must be increased for efficient cleaning).
5. Repeat washing until no bagasse present in the discharge.
6. Switch off motor drive.
7. Set feed discharge control system to manual mode.

APPENDIX E

BAGASSE COMPOSITION BY COMPLETE HYDROLYSIS

1. Calculation of pentosan and cellulose content of bagasse

The amount of sugars (g) in the concentrate (including the sugars in 5 mℓ withdrawn for acetic acid and furfural analyses) were calculated from the results in Table 5.3.

Table E.1. : Amounts of sugar obtained by complete hydrolysis

Sample	Xylulose (g)	Xylose (g)	Arabinose (g)	Glucose (g)	Cellobiose (g)
SMRI bagasse A	0,004	0,231	0,041	0,355	0,008
SMRI bagasse B	0,005	0,260	0,049	0,418	0,010
Control	*0,016	0,265	0,065	0,382	-

The control sample had the following initial sugar content :

xylose = 0,3000 g
arabinose = 0,1000 g
glucose = 0,4000 g

Thus the following correction factors were applied to account for sugar degradation :

xylose X 1,132
arabinose X 1,538
glucose X 1,047

*No xylulose added to the control sample.

The corrected amounts of sugar obtained by complete hydrolysis appear in the following table :

Table E.2 : Corrected amount of sugar obtained by complete hydrolysis

Sample	Xylulose (g)	Xylose (g)	Arabinose (g)	Glucose (g)	Cellobiose (g)
SMRI bagasse A	0,004	0,261	0,063	0,372	0,008
SMRI bagasse B	0,005	0,294	0,075	0,438	0,010
Control	0,016	0,300	0,100	0,400	-

The amounts of pentosan and cellulose equivalent to the above sugars were then calculated according to the following equations:

$$(C_5H_8O_4)_n + n\,H_2O \rightarrow nC_5H_{10}O_5$$

pentosan → pentose

i.e. 132,1 kg of pentosan produce 150,1 kg pentose

$$(C_6H_{10}O_5)_n + n\,H_2O \rightarrow nC_6H_{12}O_6$$

cellulose → glucose

i.e. 162,1 kg of cellulose produce 180,2 kg of glucose.

Table E.3 : Carbohydrate constituents of bagasse obtained by complete hydrolysis

Sample	Pentosan (mg/g)	Cellulose (mg/g)	Xylose (mg/g)	Arabinose (mg/g)	Glucose (mg/g)
SMRI bagasse A	304	360	275	66	400
SMRI bagasse B	300	368	268	68	409

APPENDIX F

BAGASSE AUTOHYDROLYSIS HYDROLYSATE ANALYSES

1. Hydrolysate Analyses

The actual autohydrolysis hydrolysate analyses are shown in Table F.1.

Table F.1 : Bagasse autohydrolysis hydrolysate analyses

Temp	Initial Volume (mℓ)	Time (min)	Free Xylose (g/ℓ)	Total Xylose (g/ℓ)	Total Glucose (g/ℓ)	Acetic Acid (g/ℓ)	Furfural (g/ℓ)
100°C	1500	120	no trace	no trace	1,0	not analysed	not analysed
		255			1,1		
		485			1,1		
		730			1,1		
		1290		0,2	1,0		
		1565		0,5	1,1	0,44	
120°C	1500	30	no trace	no	0,6	not analysed	not analysed
		60		trace	0,7		
		120		0,2	0,9		
		165		0,3	0,9		
		243		0,4	0,9		
		300		0,5	0,9		
		360		0,7	0,9	0,35	
140°C	1495	60	no trace	0,5	0,5	0,11	0,130
		120		1,8	0,7	0,25	0,151
		180		2,8	1,0	0,36	0,187
		240	<0,2	3,9	1,0	0,44	0,213
		300	<0,2	4,9	0,9	0,66	0,226
		360	<0,2	5,6	1,0	0,77	0,252
160°C	1515	30	no	1,9	0,9	0,27	0,100
		60	trace	4,6	0,9	0,36	0,136
		90	0,3	6,3	1,2	0,67	0,182
		125	0,6	7,7	1,2	0,80	0,436
		165	1,3	8,6	1,2	1,17	1,364

2. Calculation of Product Yields

Sugar, acetic acid and furfural yields during hydrolysis, expressed as mg/g of dry bagasse, were calculated from the preliminary results by the following H.P. 25 calculator programme :

1.	STO 1	
2.	RCL 3	
3.	X	
4.	RCL 2	Calculation of product yield in mg/g dry bagasse
5.	+	
6.	RCL 0	
7.	÷	
8.	R/S	
9.	RCL 4	
10.	RCL 1	Calculation of product mass removed in sample and
11.	X	total product mass removed in all samples
12.	STO +2	
13.	RCL 3	
14.	RCL 4	Volume correction for sample withdrawal
15.	-	
16.	STO 3	
17.	GTO 00	Return to beginning of programme

MEMORY :

0	Bagasse mass (g)
1	Analytical result (g/ℓ)
2	Product mass removed in sample (g)
3	Original acid vol (ℓ)
4	Sample volume (ℓ)

APPENDIX G

BATCH ATMOSPHERIC ACID HYDROLYSIS HYDROLYSATE ANALYSES

1. Hydrolysate Analyses

Table G.1. : Batch atmospheric acid hydrolysis at 80°C

Acid Conc (g/ℓ)	Time (min)	Free Xylose (g/ℓ)	Total Xylose (g/ℓ)	Free Glucose (g/ℓ)	Total Glucose (g/ℓ)	Acetic Acid (g/ℓ)	Furfural (mg/ℓ)	Acidic Organics (as Acetic Acid) (g/ℓ)	Acid Consumption (Moles/ℓ)
	100	0,7	3,3	1,0	1,3	1,48			
	180	1,7	6,7	1,4	1,6	2,40	30		
	260	3,4	8,2	1,6	1,4	2,90	41		
20	420	7,0	10,3	1,8	1,5	3,11	76		
	745	11,8	12,2	1,9	1,9	3,21	112		
	1025	12,8	12,9	2,0	2,1	3,26	158		
	1320	13,5	13,5	2,3	2,4	3,26	188	4,08	0,0045
	50	1,1	4,3	0,8	1,3	1,45			
	90	2,3	7,2	1,1	1,4	2,34	31		
	130	3,7	8,9	1,4	1,5	2,50	45		
40	180	6,5	10,6	1,5	1,5	2,71	61		
	300	10,9	12,7	1,8	1,9	2,88	92		
	420	12,8	13,0	1,9	2,1	3,06	111		
	540	13,4	13,4	2,0	2,1	3,10	142		
	660	13,6	13,8	2,5	2,4	3,12	163	4,46	0,0055

Table G.2. : Batch atmospheric acid hydrolysis at 90°C

Acid Conc (g/ℓ)	Time (min)	Free Xylose (g/ℓ)	Total Xylose (g/ℓ)	Free Glucose (g/ℓ)	Total Glucose (g/ℓ)	Acetic Acid (g/ℓ)	Furfural (mg/ℓ)	Acidic Organics (as Acetic Acid) (g/ℓ)	Acid Consumption (Moles/ℓ)
	90	0,7	3,8	1,0	1,2	1,58			
	180	3,5	7,1	1,3	1,3	1,82	52		
	260	5,2	8,9	1,4	1,3	2,08	72		
10	480	9,6	11,0	1,7	1,7	2,33	118		
	720	11,8	12,4	1,8	2,0	2,60	189		
	955	12,8	13,1	2,0	2,2	2,78	245		
	1200	13,6	13,7	2,4	2,4	2,85	313	4,03	0,0047
	50	1,0	4,6	1,2	1,3	2,02			
	90	2,5	7,1	1,4	1,5	2,59	60		
	130	4,6	9,1	1,5	1,5	2,77	95		
20	245	10,5	11,6	1,6	1,7	3,02	138		
	360	12,5	12,7	1,8	2,0	3,01	186		
	480	13,4	13,5	2,2	2,4	3,03	220		
	600	13,7	13,8	2,6	2,9	3,14	270	4,23	0,0062
	25	1,0	4,2	1,1	1,3	1,15			
	45	2,6	7,6	1,2	1,3	1,96	53		
	68	5,3	9,5	1,6	1,7	2,17	100		
40	122	10,8	12,4	1,9	2,0	2,46	182		
	180	12,6	13,0	2,0	2,4	2,75	200		
	240	13,1	13,4	2,3	2,7	2,84	240		
	300	13,5	13,7	2,5	2,6	2,92	260	4,69	0,0146

Table G.3. Batch atmospheric acid hydrolysis at 100°C

Acid Conc (g/ℓ)	Time (min)	Free Xylose (g/ℓ)	Total Xylose (g/ℓ)	Free Glucose (g/ℓ)	Total Glucose (g/ℓ)	Acetic Acid (g/ℓ)	Furfural (mg/ℓ)	Acidic Organics (as acetic acid) (g/ℓ)	Acid Consumption (Moles/ℓ)
	38	1,0	4,5	1,5	1,8	1,68	40		
	80	3,2	7,8	1,8	1,9	2,04	76		
	120	6,0	9,9	2,0	2,1	2,54	95		
10	240	11,4	12,3	2,5	2,3	2,86	149		
	375	13,3	13,4	2,6	2,7	3,16	217		
	480	13,9	13,9	2,7	2,6	3,20	236		
	600	14,2	14,4	3,1	3,1	3,23	279	4,17	0,0057
	20	0,9	4,5	1,2	1,5	1,50	45		
	40	3,2	7,7	1,8	1,7	2,00	110		
	60	5,9	9,9	1,8	1,7	2,66	132		
20	120	12,1	12,6	2,0	2,2	2,80	207		
	180	13,0	13,3	2,2	2,7	2,92	252		
	240	13,6	13,8	2,5	2,9	3,09	310		
	300	14,3	14,4	2,9	2,8	3,14	334	3,98	0,0061
	10	1,1	4,2	1,1	1,2	1,44	44		
	20	4,2	7,7	1,3	1,3	2,14	80		
	30	7,2	9,8	1,5	1,6	2,72	127		
40	60	12,0	12,5	1,9	2,3	3,00	161		
	90	13,3	13,4	2,5	2,7	3,07	222		
	120	13,6	13,7	2,5	2,5	3,13	263		
	150	13,9	14,1	2,9	2,8	3,20	288	4,56	0,0093

APPENDIX H

BATCH PRESSURE ACID HYDROLYSIS HYDROLYSATE ANALYSES

1. Hydrolysate Analyses

Table H.1. : Batch pressure acid hydrolysis at 110°C

Acid Conc. (g/ℓ)	Acid Vol. (mℓ)	Time (min)	Free Xylose (g/ℓ)	Total Xylose (g/ℓ)	Free Glucose (g/ℓ)	Total Glucose (g/ℓ)	Acetic Acid (g/ℓ)	Furfural (mg/ℓ)	Acidic Organics (as acetic acid) (g/ℓ)	H_2SO_4 Consumption Moles
		15	0,9	4,1	0,7	0,8	1,16	95		
		30	3,1	7,5	1,0	1,3	1,72	146		
		50	6,3	9,5	1,1	1,5	1,93	196		
10	1494	120	10,8	11,1	1,6	1,8	2,48	257		
		200	12,0	12,3	2,3	2,3	2,60	390		
		300	12,8	12,8	2,6	2,7	2,76	446		
		400	13,2	13,3	2,7	2,8	2,88	500		
		510	13,4	13,5	2,8	2,8	2,88	600	4,08	0,0084
		8	0,7	2,2	0,6	0,8	0,54	43		
		16	3,5	6,5	1,1	1,2	1,66	105		
		25	6,6	8,7	1,3	1,3	2,02	168		
20	1490	60	10,9	11,2	2,0	1,9	2,58	250		
		110	12,5	12,6	2,5	2,6	2,91	335		
		160	12,8	12,8	2,8	2,8	2,96	475		
		210	13,2	13,3	2,9	3,0	2,96	590		
		260	13,4	13,5	3,0	3,0	2,96	690	3,93	0,0078
		4	1,3	3,8	0,8	0,9	1,28	110		
		8	3,9	7,0	1,1	1,2	1,98	200		
		12	7,1	9,2	1,2	1,5	2,32	240		
40	1427	30	11,9	12,2	1,9	2,1	3,07	350		
		50	13,2	13,3	2,6	2,5	3,20	405		
		70	13,7	13,8	2,9	2,9	3,20	480		
		95	13,9	14,0	3,0	3,0	3,20	545		
		120	14,1	14,2	3,0	3,1	3,20	650	4,46	0,0092

Table H.2. : Batch pressure acid hydrolysis at 120°C

Acid Conc. (g/ℓ)	Acid Vol (ml)	Time (min)	Free Xylose (g/ℓ)	Total Xylose (g/ℓ)	Free Glucose (g/ℓ)	Total Glucose (g/ℓ)	Acetic Acid (g/ℓ)	Furfural (mg/ℓ)	Acidic Organics (as acetic acid) (g/ℓ)	H_2SO_4 Consumption Moles
		15	1,8	5,4	0,9	1,1	1,25	36		
		30	3,8	6,9	1,0	1,1	1,74	88		
		46	6,4	9,3	1,2	1,4	1,98	158		
5	1495	121	11,1	11,6	1,9	2,1	2,60	235		
		200	12,4	12,6	2,4	2,5	2,95	290		
		280	12,9	12,9	2,7	2,7	3,00	420		
		370	13,2	13,3	2,9	2,9	3,00	575		
		460	13,6	13,6	3,0	3,1	3,00	782	3,71	0,0070
		7,75	2,4	5,8	1,1	1,3	1,63	75		
		15,5	5,8	9,2	1,3	1,6	1,91	170		
		25	9,4	11,7	1,7	1,8	2,28	205		
10	1442	60	13,9	14,4	2,4	2,8	2,72	295		
		100	15,1	15,1	2,9	2,9	2,80	483		
		143	15,3	15,4	3,2	3,2	3,04	608		
		180	15,5	15,6	3,3	3,4	3,04	810		
		240	15,7	15,9	3,5	3,5	3,10	1080	3,79	0,0076
		4	2,9	6,7	0,9	1,0	1,18	75		
		8	7,0	10,2	1,1	1,3	1,77	105		
		12	10,9	12,8	1,5	1,6	2,37	205		
20	1360	30	15,4	15,9	2,3	2,5	3,94	332		
		50	16,6	16,6	2,9	2,9	3,13	520		
		70	16,9	16,9	3,0	3,1	3,20	610		
		90	17,0	17,0	3,2	3,2	3,20	860		
		110	17,1	17,1	3,3	3,4	3,20	1090	4,50	0,0085

Table H.3. : Batch pressure acid hydrolysis at 130°C

Acid Conc. (g/ℓ)	Acid Vol (mℓ)	Time (min)	Free Xylose (g/ℓ)	Total Xylose (g/ℓ)	Free Glucose (g/ℓ)	Total Glucose (g/ℓ)	Acetic Acid (g/ℓ)	Furfural (mg/ℓ)	Acidic Organics (as acetic acid) (g/ℓ)	H_2SO_4 Consumption Moles
		6	1,6	4,3	0,8	1,0	1,03	96		
		15	5,1	7,7	1,0	1,3	1,61	149		
		30	9,1	10,6	1,5	1,6	1,90	278		
5	1510	70	12,0	12,0	2,2	2,3	2,45	404		
		120	12,6	12,7	2,5	2,7	2,55	606		
		160	12,6	12,7	2,7	2,8	2,64	798		
		200	12,5	12,6	2,8	2,9	2,67	1011		
		240	12,4	12,4	2,8	2,9	2,69	1170	3,57	0,0066
		4	2,5	5,5	1,0	1,1	1,24	170		
		8	6,3	8,5	1,2	1,4	1,76	200		
		12	10,0	10,9	1,7	1,9	2,06	225		
10	1525	30	12,4	12,9	2,4	2,5	2,58	315		
		55	13,7	13,6	2,7	2,7	2,68	605		
		80	13,8	13,7	2,8	3,0	2,86	832		
		110	13,5	13,5	3,0	3,1	2,86	1024		
		135	13,2	13,4	3,0	3,2	2,86	1183	3,79	0,0039
		2,75	4,6	7,4	1,3	1,3	1,64	200		
		5,75	8,6	10,1	1,5	1,5	2,08	238		
		10	11,2	11,7	1,6	2,0	2,24	282		
20	1459	20	13,0	13,1	2,2	2,5	2,54	458		
		30	13,7	13,8	2,6	2,7	2,60	632		
		40	13,6	13,7	2,8	2,8	2,70	844		
		52	13,5	13,5	2,8	2,9	2,80	1146		
		65	13,4	13,3	3,0	3,0	2,80	1300	4,26	0,0081

Table H.4. : Batch pressure and acid hydrolysis at 140°C

Acid Conc. (g/ℓ)	Acid Vol (mℓ)	Time (min)	Free Xylose (g/ℓ)	Total Xylose (g/ℓ)	Free Glucose (g/ℓ)	Total Glucose (g/ℓ)	Acetic Acid (g/ℓ)	Furfural (mg/ℓ)	Acidic Organics (as acetic acid) (g/ℓ)	H_2SO_4 Consumpt Moles
		4	1,1	4,4	0,6	0,8	1,04	0,080		
		8	3,3	6,8	0,8	1,0	1,53	0,210		
		16	6,5	9,2	1,0	1,4	1,85	0,450		
3		35	10,2	10,9	1,5	1,9	2,15	0,980		
		60	11,3	11,4	2,0	2,0	2,26	1,450		
		80	11,6	11,6	2,3	2,4	2,45	1,800		
		110	11,7	11,8	2,4	2,5	2,61	2,150		
		140	11,8	12,0	2,5	2,6	2,65	2,550	3,45	0,0065
		4,5	2,9	6,8	0,8	1,1	1,04	151		
		9	5,9	9,0	1,2	1,6	1,41	292		
		16	8,5	10,4	1,5	1,9	1,67	321		
5	1510	30	11,1	11,8	2,2	2,4	1,94	453		
		55	12,4	12,4	2,6	2,6	2,36	811		
		80	12,1	12,0	2,8	2,6	2,55	1245		
		105	11,8	11,7	2,8	2,7	2,55	2019		
		130	10,7	10,7	2,8	2,8	2,58	2396	3,57	0,0071
		3	5,3	8,0	0,9	1,1	1,62	179		
		6	8,5	9,9	1,2	1,5	1,87	251		
		10	10,3	11,0	1,7	1,9	2,13	335		
10	1515	20	11,8	11,7	2,1	2,3	2,27	502		
		30	12,1	12,1	2,4	2,4	2,46	800		
		40	12,0	12,0	2,5	2,7	2,50	1163		
		50	12,0	11,9	2,7	2,8	2,58	1526		
		60	11,9	11,8	2,8	2,9	2,60	1991	3,59	0,0064
		3	8,7	9,7	1,4	1,6	2,29	212		
		6	10,7	11,0	2,0	2,1	2,32	330		
		10	11,8	11,7	2,3	2,4	2,50	495		
20	1515	15	12,0	11,9	2,5	2,4	2,58	697		
		20	12,2	12,1	2,7	2,6	2,58	991		
		25	12,0	12,0	2,7	2,8	2,60	1743		
		30	12,0	12,0	3,0	2,9	2,60	1963		
		35	11,9	11,9	3,2	3,2	2,60	2220	4,08	0,009

Table H.5. : Batch pressure acid hydrolysis at 150°C

Acid Conc. (g/ℓ)	Acid Vol. (mℓ)	Time (min)	Free Xylose (g/ℓ)	Total Xylose (g/ℓ)	Free Glucose (g/ℓ)	Total Glucose (g/ℓ)	Acetic Acid (g/ℓ)	Furfural (mg/ℓ)	Acidic Organics (as acetic acid) (g/ℓ)	H_2SO_4 Consumption Moles
3		3	2,2	5,3	0,7	0,9	0,97	0,090		
		8	5,9	8,6	1,1	1,4	1,39	0,200		
		15	9,0	10,2	1,4	1,9	1,92	0,450		
		31	10,7	10,7	2,0	2,4	2,20	1,000		
		47	10,8	11,0	2,3	2,5	2,29	1,700		
		60	10,9	10,8	2,4	2,6	2,49	2,250		
		80	10,5	10,5	2,4	2,5	2,49	2,700		
		100	9,9	9,9	2,5	2,6	2,49	2,950	3,40	0,0062
5	1505	3	5,5	7,6	1,1	1,2	1,09	250		
		6	8,2	9,7	1,4	1,8	1,62	288		
		10	10,2	10,7	1,9	2,1	1,94	654		
		20	11,3	11,3	2,3	2,5	2,15	856		
		30	11,6	11,5	2,7	2,6	2,23	1038		
		40	11,5	11,4	2,8	2,8	2,34	1808		
		50	11,1	10,9	3,1	2,9	2,34	2058		
		60	10,8	10,6	3,1	3,1	2,34	2481	3,46	0,0065
10	1520	3	8,4	9,4	1,4	1,5	1,62	327		
		6	10,6	10,8	1,9	2,0	1,97	400		
		10	11,2	11,2	2,3	2,3	2,02	600		
		15	11,3	11,3	2,4	2,4	2,12	964		
		20	11,2	11,1	2,6	2,8	2,26	1582		
		25	10,8	10,8	2,7	2,9	2,31	2364		
		29	10,5	10,6	2,8	3,0	2,31	2800		
		34	10,4	10,4	3,1	3,2	2,34	2982	3,75	0,0068

APPENDIX I.

XYLOSE YIELD DATA FROM BATCH DILUTE ACID HYDROLYSES

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R BAGHYD.DATA
-ONLY MODE
 UPPER ASSUMED
6R1-THU-12/10/81-14:41:46-(62,)

  1:5.          5.
  2:   HEMICELLULOSE ACID HYDROLYSIS ##########################################
  3:TEMP     H2SO4      TIME       FREE       TOTAL
  4:(C)      (%)        (MIN)      XYLOSE     XYLOSE
  5:80.         2.         100.       10.36      40.48
  6:80.         2.         180.       25.01      98.65
  7:80.         2.         260.       49.66     120.40
  8:80.         2.         420.      101.34     150.55
  9:80.         2.         740.      169.53     172.54
 10:80.         2.        1025.      183.59     187.38
 11:80.         2.        1320.      193.33     195.73
 12:80.         4.          50.       16.17      63.23
 13:80.         4.          90.       33.64     105.45
 14:80.         4.         130.       53.82     129.94
 15:80.         4.         180.       93.76     154.19
 16:80.         4.         300.      155.87     183.03
 17:80.         4.         420.      182.41     188.03
 18:80.         4.         540.      190.70     193.55
 19:80.         4.         660.      193.44     199.02
 20:90.         1.          90.       10.44      56.66
 21:90.         1.         180.       51.77     105.37
 22:90.         1.         260.       76.61     131.67
 23:90.         1.         480.      140.25     162.05
 24:90.         1.         720.      171.74     182.09
 25:90.         1.         955.      185.90     192.00
 26:90.         1.        1200.      197.12     200.41
 27:90.         2.          50.       14.84      68.27
 28:90.         2.          90.       36.88     105.00
 29:90.         2.         130.       67.42     134.09
 30:90.         2.         245.      152.36     170.88
 31:90.         2.         360.      180.85     185.75
 32:90.         2.         480.      193.54     197.03
 33:90.         2.         600.      197.73     201.22
 34:90.         4.          25.       14.70      61.76
 35:90.         4.          45.       38.00     111.25
 36:90.         4.          68.       76.90     138.63
 37:90.         4.         122.      155.35     180.00
 38:90.         4.         180.      180.76     188.47
 39:90.         4.         240.      187.75     194.05
 40:90.         4.         300.      193.28     198.20
 41:100.        1.          30.       14.87      66.91
 42:100.        1.          60.       47.25     115.48
 43:100.        1.         120.       88.05     146.07
 44:100.        1.         240.      165.92     180.69
 45:100.        1.         375.      193.04     196.39
 46:100.        1.         480.      201.52     203.45
 47:100.        1.         600.      205.71     210.44
 48:100.        2.          20.       13.32      66.59
 49:100.        2.          40.       47.02     113.48
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50:	100.		60.	86.17	145.38
51:	100.		120.	175.17	184.14
52:	100.		180.	187.96	194.09
53:	100.		240.	196.39	201.11
54:	100.		300.	206.13	209.46
55:	100.		10.	16.13	61.60
56:	100.	4.	20.	61.14	112.41
57:	100.	4.	30.	104.26	142.59
58:	100.	4.	60.	172.54	181.00
59:	100.	4.	90.	190.84	193.67
60:	100.	4.	120.	195.02	197.05
61:	100.	4.	150.	199.16	203.37
62:	110.	1.	15.	13.53	60.72
63:	110.	1.	30.	45.56	110.53
64:	110.	1.	50.	91.94	135.17
65:	110.	1.	120.	156.44	163.40
66:	110.	1.	200.	173.45	179.42
67:	110.	1.	300.	184.66	186.42
68:	110.	1.	400.	190.21	193.35
69:	110.	1.	510.	192.95	196.09
70:	110.		6.	10.29	32.35
71:	110.		16.	51.02	94.89
72:	110.		25.	95.62	126.54
73:	110.		60.	156.80	162.12
74:	110.		110.	179.32	181.02
75:	110.		160.	183.49	184.60
76:	110.		200.	189.00	191.48
77:	110.		260.	191.72	192.84
78:	110.	4.	4.	10.21	53.22
79:	110.	4.	8.	54.21	97.54
80:	110.	4.	12.	98.02	127.66
81:	110.	4.	30.	162.99	168.26
82:	110.	4.	50.	180.38	182.97
83:	110.	4.	71.	186.99	189.58
84:	110.	4.	95.	189.60	192.20
85:	110.	4.	120.	192.18	194.78
86:	120.	0.	15.	26.70	80.09
87:	120.	0.	30.	56.04	102.10
88:	120.	0.	46.	93.78	136.93
89:	120.	0.	121.	161.25	169.95
90:	120.	0.	200.	172.70	174.14
91:	120.	0.	280.	176.72	180.36
92:	120.	0.	370.	178.88	182.91
93:	120.	0.	460.	186.37	188.02
94:	120.	1.	7.75	34.27	80.03
95:	120.	1.	15.50	82.28	130.82
96:	120.	1.	25.	132.54	165.73
97:	120.	1.	60.	194.65	203.00
98:	120.	1.	100.	211.03	213.55
99:	120.	1.	143.	213.73	216.60
100:	120.	1.	180.	216.39	219.26
101:	120.	1.	240.	219.03	223.21
102:	120.		4.	38.91	89.90
103:	120.		8.	93.28	136.31
104:	120.		12.	144.37	170.37
105:	120.		30.	202.62	210.50
106:	120.		50.	217.96	219.45
107:	120.		70.	221.75	223.45
108:	120.		90.	223.00	224.48
109:	120.		110.	224.23	225.71

112:	130.	0.	30.	134.35	157.16
113:	130.	0.	70.	176.36	172.44
114:	130.	0.	120.	184.96	187.47
115:	130.	0.	160.	184.96	187.47
116:	130.	0.	200.	183.56	186.07
117:	130.	0.	240.	182.17	183.30
118:	130.	1.	4.	37.47	82.44
119:	130.	1.	8.	93.83	126.94
120:	130.	1.	16.	148.13	162.16
121:	130.	1.	30.	182.97	191.19
122:	130.	1.	55.	201.64	201.24
123:	130.	1.	80.	203.06	202.66
124:	130.	1.	105.	198.85	199.85
125:	130.	1.	130.	194.68	190.46
126:	130.	2.	2.75	65.79	105.83
127:	130.	2.	5.75	122.36	144.02
128:	130.	2.	10.	158.73	166.40
129:	130.	2.	20.	183.62	185.76
130:	130.	2.	30.	193.20	195.33
131:	130.	2.	40.	191.84	193.98
132:	130.	2.	50.	190.51	191.31
133:	130.	2.	60.	189.19	180.67
134:	140.	0.	4.	16.59	66.36
135:	140.	0.	8.	49.41	102.17
136:	140.	0.	16.	96.65	137.59
137:	140.	0.	35.	150.66	162.41
138:	140.	0.	60.	166.54	169.63
139:	140.	0.	86.	170.83	172.48
140:	140.	0.	110.	172.24	175.31
141:	140.	0.	140.	173.63	170.10
142:	140.	0.	4.5	43.70	102.48
143:	140.	0.	9.	80.43	135.28
144:	140.	0.	16.	126.78	155.93
145:	140.	0.	30.	164.72	176.36
146:	140.	0.	55.	183.48	185.01
147:	140.	0.	80.	179.20	179.31
148:	140.	0.	105.	174.97	175.07
149:	140.	0.	130.	159.62	161.12
150:	140.	1.	3.	80.13	120.26
151:	140.	1.	6.	128.01	149.38
152:	140.	1.	10.	154.65	165.66
153:	140.	1.	20.	176.61	175.91
154:	140.	1.	30.	180.95	181.70
155:	140.	1.	40.	179.52	180.27
156:	140.	1.	50.	179.52	178.86
157:	140.	1.	60.	178.12	177.46
158:	140.	2.	3.	130.24	145.22
159:	140.	2.	6.	160.72	165.35
160:	140.	2.	10.	176.93	175.66
161:	140.	2.	15.	179.88	178.58
162:	140.	2.	20.	178.73	181.46
163:	140.	2.	30.	179.88	180.04
164:	140.	2.	40.	179.88	180.04
165:	140.	2.	55.	178.48	178.64
166:	150.	0.	3.	33.40	80.46
167:	150.	0.	8.	88.98	130.03
168:	150.	0.	15.	135.04	153.81
169:	150.	0.	31.	160.03	161.16

```
170:150.          0.3          47.          161.49       165.52
171:150.          0.33         60.          162.93       163.65
172:150.          0.33         80.          159.24       158.38
173:150.          0.33         100.         148.81       149.95
174:150.          0.           3.           82.61        114.15
175:150.          0.           6.           122.73       145.36
176:150.          0.           10.          152.13       160.06
177:150.          0.           20.          168.13       168.78
178:150.          0.           30.          172.44       171.66
179:150.          0.           40.          171.02       170.24
180:150.          0.           50.          165.40       163.21
181:150.          0.5          60.          161.23       159.04
182:150.          1.           3.           127.43       142.59
183:150.          1.           6.           160.45       163.61
184:150.          1.           10.          169.36       169.55
185:150.          1.           15.          170.83       171.02
186:150.          1.           20.          169.37       168.11
187:150.          1.           25.          163.62       163.80
188:150.          1.           29.          159.36       160.96
189:150.          1.           34.          157.96       158.15
190:-1.
191:1.            150.         0.0100       75.
192:                           0.0001000    0.0005000
193:                           25.00        0.05
194:                           0.0001000    0.0000050
195:                           21.00        0.05
196:                           0.0000010    0.00000050
197:                           33.56        0.0
198:                           165.         0.0
199:                           105.         0.0
200:
:200
CORRECTIONS APPLIED.

EAK,P S03PR1/DATA
```

APPENDIX J.

COMPUTER PROGRAMME AND RESULTS OF BEST HYDROLYSIS CURVE FIT

```
RUNID * FIT          USER ID * TRICKE          PART NUMBER * 00      INPUT DEVICE *                OUTPUT DEV
 FILE NAME * PR@000FIT                CREATED AT:   08:44:04 DEC 08,1981          PRINTED AT:   08:45:19 D

FIT,CHEN-DPGRAD/TRICKE,CHEN-DTRICKE,1,9

PRINT$,,S03PR1

,A BAGHYD.

,SFX BAGHYD.SUBPRG,BAGHYD.SUBPRG
3R1   *12/08/81-08:44(26,)
      1.          SUBROUTINE SIZ(NDIMA,NTDEPA)
      2.          DIMENSION NDIMA(1),NTDEPA(1)
      3.          NDIMA(1)=8
      4.          NTDEPA(1)=3
      5.          RETURN
      6.          END

      7.          SUBROUTINE CAPT
      8.          WRITE(6,50)
      9.    50    FORMAT(20X,'FIRST ORDER CONSECUTIVE REACTION SYSTEM')
     10.          RETURN
     11.          END

     12.          SUBROUTINE GANDSD(Y,X)
     13.          COMMON /SIMPY/ NEXP,NGSET,REFT
     14.          DIMENSION X(16),Y(16)
     15.          Y(1)=X(5)
     16.          Y(2)=REFT-503.52/(X(1)+273.15)
     17.          Y(3)=X(3)
     18.          Y(4)=X(4)
     19.          Y(5)=X(2)
     20.          RETURN
     21.          END
```

```
      22.           FUNCTION RCALC(E,S,Y)
      23.           COMMON /SIMPY/ NEXP,NGSET,REFT
      24.           DIMENSION S(8),Y(5),E(3)
      25.           REAL K1,K2,K3
      26.           K1=E(1)*Y(5)
      27.           K2=E(2)*Y(5)
      28.           K3=E(3)*Y(5)
      29.           RCALC=K1*S(7)*(EXP(-K1*Y(3))-EXP(-K3*Y(3)))/(K3-K1)
      30.          1       +K2*S(8)*(EXP(-K2*Y(3))-EXP(-K3*Y(3)))/(K3-K2)
      31.           RETURN
      32.           END

FTN 151 IBANK 74 DBANK 3 COMMON


,BAGHYD.FIT
30R1Q1+1 S74T11 12/08/81 08:44:12
T=003673, PROG SIZE(I/D)=6256/17654
*RLIB$, LEVEL 74R1N2
MAP.  ERRORS: 0  TIME: 29.929  STORAGE: 19328/6/037777/077777

 BAGHYD.FIT
```

EXPRESSION FITTED FIRST ORDER CONSECUTIVE REACTION SYSTEM

DATA SET TITLE HEMICELLULOSE ACID HYDROLYSIS ##

CONVERGENCE REACHED ON QUADRATIC FIT TEST AFTER 27 EVALUATIONS

OF SQUARES (SS) .1883+005 NUMBER OF DATA POINTS (ND)= 185 NUMBER OF PARAMETERS FITTED (NP) = 5

STANDARD DEVIATION (SQRT(SS/(ND - NP)) .1023+002

REFERENCE TEMPERATURE 75.00 DEGREES CENTIGRADE

AMETERS

ST	2 ND	3 RD	4 TH	5 TH	6 TH	7 TH	8 TH
22381-002	.246804+002	.107803-003	.223434+002	.279327-005	.335600+002	.165000+003	.105000+003

AMETER STANDARD ERRORS

45715-004	.117785+000	.938972-005	.673122+000	.290623-006	.000000	.000000	.000000

ELATION MATRIX

1.0000	-.3976	.3731	-.2062	.0670
-.3976	1.0000	-.6973	.8793	.4171
.3731	-.6973	1.0000	-.6673	-.0742
-.2062	.8793	-.6673	1.0000	.7441
.0670	.4171	-.0742	.7441	1.0000

EXPRESSION FITTED

FIRST ORDER CONSECUTIVE REACTION SYSTEM

DATA SET TITLE

HEMICELLULOSE ACID HYDROLYSIS ##

OBSERVED	CALCULATED	DIFFERENCE	DIFF**2	DIFFERENCE STD. DEV.	100*DIFFERENCE OBSERVED
.4848+002	.5744+002	-.8956+001	.8021+002	-.88	-18.47
.9865+002	.9022+002	.8430+001	.7106+002	.82	8.55
.1204+003	.1147+003	.5694+001	.3242+002	.56	4.73
.1505+003	.1471+003	.3422+001	.1171+002	.33	2.27
.1775+003	.1785+003	-.9881+000	.9764+000	-.10	-.56
.1874+003	.1910+003	-.3659+001	.1339+002	-.36	-1.95
.1957+003	.1994+003	-.3633+001	.1320+002	-.36	-1.86
.6323+002	.5744+002	.5794+001	.3357+002	.57	9.16
.1054+003	.9022+002	.1523+002	.2320+003	1.49	14.44
.1299+003	.1147+003	.1523+002	.2321+003	1.49	11.72
.1542+003	.1370+003	.1720+002	.2957+003	1.68	11.15
.1838+003	.1681+003	.1577+002	.2487+003	1.54	8.58
.1880+003	.1836+003	.4433+001	.1965+002	.43	2.36
.1935+003	.1928+003	.7136+000	.5092+000	.07	.37
.1990+003	.1994+003	-.3433+000	.1178+000	-.03	-.17
.5666+002	.6557+002	-.8910+001	.7938+002	-.87	-15.73
.1054+003	.1077+003	-.2313+001	.5348+001	-.23	-2.19
.1317+003	.1326+003	-.9121+000	.8319+000	-.09	-.69
.1620+003	.1689+003	-.6817+001	.4647+002	-.67	-4.21
.1821+003	.1856+003	-.3466+001	.1201+002	-.34	-1.90
.1920+003	.1944+003	-.2443+001	.5969+001	-.24	-1.27
.2004+003	.2010+003	-.5699+000	.3248+000	-.06	-.28
.6827+002	.7121+002	-.2941+001	.8648+001	-.29	-4.31
.1050+003	.1077+003	-.2683+001	.7197+001	-.26	-2.55
.1341+003	.1326+003	.1508+001	.2274+001	.15	1.12
1701+003	.1699+003	.2163+000	.4680-001	.02	.13
1857+003	.1856+003	.1942+000	.3770-001	.02	.10
1970+003	.1946+003	.2434+001	.5924+001	.24	1.24
2012+003	.2010+003	.2401+000	.5763-001	.02	.12
6176+002	.7121+002	-.9451+001	.8932+002	-.92	-15.30
1112+003	.1077+003	.3567+001	.1273+002	.35	3.21
1386+003	.1356+003	.3049+001	.9293+001	.30	2.20
1800+003	.1697+003	.1033+002	.1068+003	1.01	5.74
1885+003	.1856+003	.2914+001	.8492+001	.28	1.55
1940+003	.1946+003	-.5460+000	.2981+000	-.05	-.28
1982+003	.2010+003	-.2780+001	.7728+001	-.27	-1.40
6691+002	.6812+002	-.1208+001	.1459+001	-.12	-1.81
1155+003	.1141+003	.1369+001	.1874+001	.13	1.19
1461+003	.1409+003	.5184+001	.2687+002	.51	3.55
1807+003	.1766+003	.4129+001	.1705+002	.40	2.29

EXPRESSION FITTED

FIRST ORDER CONSECUTIVE REACTION SYSTEM

DATA SET TITLE

. HEMICELLULOSE ACID HYDROLYSIS ##

SERVED	CALCULATED	DIFFERENCE	DIFF**2	DIFFERENCE STD. DEV.	100*DIFFERENCE OBSERVED
1964+003	.1906+003	.5805+001	.3370+002	.57	2.96
2034+003	.1969+003	.6526+001	.4259+002	.64	3.21
2104+003	.2025+003	.7890+001	.6226+002	.77	3.75
6659+002	.7089+002	-.4296+001	.1846+0	-.42	-6.45
.135+003	.1141+003	-.6312+000	.3984+00	-.06	-.56
1454+003	.1409+003	.4494+001	.2019+002	.44	3.09
1841+003	.1766+003	.7579+001	.5745+002	.74	4.12
1941+003	.1895+003	.4604+001	.2120+002	.45	2.37
2011+003	.1969+003	.4186+001	.1753+002	.41	2.08
2095+003	.2025+003	.6910+001	.4775+002	.68	3.30
6160+002	.7089+002	-.9286+001	.8623+002	-.91	-15.07
1124+003	.1141+003	-.1701+001	.2894+001	-.17	-1.51
.426+003	.1409+003	.1704+001	.2902+001	.17	1.19
1810+003	.1766+003	.4439+001	.1971+002	.43	2.45
.937+003	.1895+003	.4184+001	.1751+002	.41	2.16
.978+003	.1969+003	.9263+000	.8581+000	.09	.47
2034+003	.2025+003	.8204+000	.6730+000	.08	.40
6072+002	.6466+002	-.3941+001	.1553+002	-.39	-6.49
.105+003	.1060+003	.4511+001	.2035+002	.44	4.08
.352+003	.1394+003	-.4241+001	.1798+002	-.41	-3.14
624+003	.1801+003	-.1769+002	.3128+003	-1.73	-10.89
794+003	.1926+003	-.1314+002	.1726+003	-1.28	-7.32
864+003	.2012+003	-.1478+002	.2183+003	-1.44	-7.93
933+003	.2070+003	-.1368+002	.1871+003	-1.34	-7.07
961+003	.2111+003	-.1501+002	.2253+003	-1.47	-7.66
235+002	.6802+002	-.3567+002	.1273+004	-3.49	-110.28
489+002	.1103+003	-.1539+002	.2370+003	-1.50	-16.22
265+003	.1394+003	-.1287+002	.1657+003	-1.26	-10.17
621+003	.1801+003	-.1797+002	.3229+003	-1.76	-11.08
818+003	.1946+003	-.1278+002	.1634+003	-1.25	-7.03
846+003	.2025+003	-.1795+002	.3222+003	-1.75	-9.72
915+003	.2070+003	-.1555+002	.2417+003	-1.52	-8.12
928+003	.2114+003	-.1853+002	.3433+003	-1.81	-9.61
322+002	.6802+002	-.1480+002	.2192+003	-1.45	-27.82
754+002	.1103+003	-.1274+002	.1624+003	-1.25	-13.06
277+003	.1369+003	-.9196+001	.8457+002	-.90	-7.20
583+003	.1801+003	-.1183+002	.1398+003	-1.16	-7.03
330+003	.1926+003	-.9589+001	.9195+002	-.94	-5.24
396+003	.2000+003	-.1046+002	.1093+003	-1.02	-5.52
922+003	.2060+003	-.1384+002	.1915+003	-1.35	-7.20

EXPRESSION FITTED

FIRST ORDER CONSECUTIVE REACTION SYSTEM

DATA SET TITLE

HEMICELLULOSE ACID HYDROLYSIS ##

SERVED	CALCULATED	DIFFERENCE	DIFF**2	DIFFERENCE STD. DEV.	100*DIFFERENCE OBSERVED
1948+003	.2102+003	-.1542+002	.2378+003	-1.51	-7.92
8009+002	.7134+002	.8751+001	.7658+002	.86	10.93
1021+003	.1141+003	-.1196+002	.1431+003	-1.17	-11.72
1369+003	.1413+003	-.4346+001	.1889+002	-.42	-3.17
1699+003	.1808+003	-.1089+002	.1186+003	-1.06	-6.41
1841+003	.1904+003	-.6221+001	.3870+002	-.61	-3.38
1884+003	.1958+003	-.7463+001	.5570+002	-.73	-3.96
1939+003	.1997+003	-.5774+001	.3334+002	-.56	-2.98
1980+003	.2017+003	-.3663+001	.1342+002	-.36	-1.85
8282+002	.7314+002	.9679+001	.9368+002	.95	11.69
1308+003	.1162+003	.1460+002	.2133+003	1.43	11.16
1657+003	.1461+003	.1959+002	.3836+003	1.91	11.82
2030+003	.1807+003	.2235+002	.4995+003	2.18	11.01
2125+003	.1904+003	.2219+002	.4923+003	2.17	10.44
2166+003	.1961+003	.2045+002	.4183+003	2.00	9.44
2193+003	.1994+003	.1991+002	.3962+003	1.95	9.08
2232+003	.2019+003	.2130+002	.4535+003	2.08	9.54
8990+002	.7491+002	.1499+002	.2246+003	1.47	16.67
1363+003	.1183+003	.1801+002	.3244+003	1.76	13.21
1704+003	.1438+003	.2658+002	.7067+003	2.60	15.60
2105+003	.1807+003	.2985+002	.8910+003	2.92	14.18
2194+003	.1904+003	.2909+002	.8462+003	2.84	13.26
2234+003	.1958+003	.2763+002	.7633+003	2.70	12.36
2245+003	.1994+003	.2513+002	.6313+003	2.46	11.19
2257+003	.2014+003	.2433+002	.5920+003	2.38	10.78
6434+002	.6411+002	.2294+000	.5262-001	.02	.36
1147+003	.1193+003	-.4591+001	.2107+002	-.45	-4.00
1572+003	.1586+003	-.1473+001	.2171+001	-.14	-.94
1774+003	.1815+003	-.4031+001	.1625+002	-.39	-2.27
1875+003	.1879+003	-.3868+000	.1496+000	-.04	-.21
1875+003	.1901+003	-.2643+001	.6984+001	-.26	-1.41
1861+003	.1907+003	-.4646+001	.2158+002	-.45	-2.50
1833+003	.1900+003	-.6690+001	.4476+002	-.65	-3.65
3244+002	.7977+002	.2673+001	.7147+001	.26	3.24
1269+003	.1234+003	.3524+001	.1242+002	.34	2.78
1622+003	.1614+003	.7572+000	.5734+000	.07	.47
1912+003	.1790+003	.1215+002	.1477+003	1.19	6.36
2012+003	.1870+003	.1426+002	.2033+003	1.39	7.09
2027+003	.1901+003	.1255+002	.1574+003	1.23	6.19
1998+003	.1906+003	.9202+001	.8468+002	.90	4.60

EXPRESSION FITTED

FIRST ORDER CONSECUTIVE REACTION SYSTEM

DATA SET TITLE

. HEMICELLULOSE ACID HYDROLYSIS ##

SERVED	CALCULATED	DIFFERENCE	DIFF**2	DIFFERENCE STD. DEV.	100*DIFFERENCE OBSERVED
1985+003	.1892+003	.9247+001	.8550+002	.90	4.66
1058+003	.9924+002	.6586+001	.4337+002	.64	6.22
1440+003	.1453+003	-.1302+001	.1694+001	-.13	-.90
1664+003	.1695+003	-.3094+001	.9570+001	-.30	-1.86
1858+003	.1833+003	.2473+001	.6115+001	.24	1.33
1953+003	.1879+003	.7473+001	.5585+002	.73	3.83
1940+003	.1901+003	.3867+001	.1496+002	.38	1.99
1913+003	.1907+003	.5943+000	.3532+000	.06	.31
1887+003	.1900+003	-.1320+001	.1743+001	-.13	-.70
6636+002	.5570+002	.1066+002	.1136+003	1.04	16.06
1022+003	.9352+002	.8646+001	.7476+002	.85	8.46
1376+003	.1369+003	.7071+000	.5000+000	.07	.51
1624+003	.1696+003	-.7224+001	.5219+002	-.71	-4.45
1696+003	.1776+003	-.7979+001	.6366+002	-.78	-4.70
1725+003	.1798+003	-.7328+001	.5369+002	-.72	-4.25
1753+003	.1804+003	-.5062+001	.2562+002	-.49	-2.89
1781+003	.1798+003	-.1684+001	.2835+001	-.16	-.95
1025+003	.8955+002	.1293+002	.1671+003	1.26	12.61
1353+003	.1331+003	.2196+001	.4823+001	.21	1.62
1559+003	.1615+003	-.5542+001	.3072+002	-.54	-3.55
1764+003	.1758+003	.5770+000	.3329+000	.06	.33
1850+003	.1800+003	.4972+001	.2472+002	.49	2.69
1793+003	.1800+003	-.7131+000	.5085+000	-.07	-.40
1751+003	.1777+003	-.2587+001	.6693+001	-.25	-1.48
.611+003	.1736+003	-.1244+002	.1549+003	-1.22	-7.72
.210+003	.1076+003	.1333+002	.1777+003	1.30	11.02
.494+003	.1490+003	.3996+000	.1597+000	.04	.27
.657+003	.1684+003	-.2762+001	.7630+001	-.27	-1.67
.759+003	.1784+003	-.2504+001	.6272+001	-.24	-1.42
.817+003	.1803+003	.1437+001	.2065+001	.14	.79
803+003	.1800+003	.2469+000	.6097-001	.02	.14
789+003	.1783+003	.5742+000	.3297+000	.06	.32
775+003	.1754+003	.2084+001	.4345+001	.20	1.17
460+003	.1490+003	-.2990+001	.8942+001	-.29	-2.05
653+003	.1724+003	-.7097+001	.5037+002	-.69	-4.29
757+003	.1784+003	-.2754+001	.7587+001	-.27	-1.57
786+003	.1803+003	-.1683+001	.2832+001	-.16	-.94
815+003	.1800+003	.1437+001	.2065+001	.14	.79
800+003	.1783+003	.1754+001	.3077+001	.17	.97
800+003	.1754+003	.4664+001	.2176+002	.46	2.59

EXPRESSION FITTED

FIRST ORDER CONSECUTIVE REACTION SYSTEM

DATA SET TITLE

HEMICELLULOSE ACID HYDROLYSIS ##

SERVED	CALCULATED	DIFFERENCE	DIFF**2	DIFFERENCE STD. DEV.	100*DIFFERENCE OBSERVED
1786+003	.1716+003	.7084+001	.5019+002	.69	3.97
8046+002	.7710+002	.3361+001	.1129+002	.33	4.18
1300+003	.1367+003	-.6678+001	.4459+002	-.65	-5.14
1538+003	.1634+003	-.9552+001	.9124+002	-.93	-6.21
1612+003	.1722+003	-.1106+002	.1224+003	-1.08	-6.86
1655+003	.1715+003	-.6029+001	.3635+002	-.59	-3.64
1626+003	.1696+003	-.6991+001	.4887+002	-.68	-4.30
1584+003	.1652+003	-.6790+001	.4610+002	-.66	-4.29
1499+003	.1593+003	-.9376+001	.8791+002	-.92	-6.25
1141+003	.1081+003	.6037+001	.3645+002	.59	5.29
1454+003	.1483+003	-.2908+001	.8454+001	-.28	-2.00
1601+003	.1659+003	-.5858+001	.3432+002	-.57	-3.66
1688+003	.1723+003	-.3530+001	.1246+002	-.35	-2.09
1717+003	.1712+003	.4675+000	.2186+000	.05	.27
1702+003	.1683+003	.1906+001	.3633+001	.19	1.12
1632+003	.1643+003	-.1069+001	.1142+001	-.10	-.65
1590+003	.1593+003	-.2862+000	.8194-001	-.03	-.18
1426+003	.1483+003	-.5678+001	.3223+002	-.56	-3.98
1636+003	.1691+003	-.5522+001	.3049+002	-.54	-3.37
1695+003	.1723+003	-.2760+001	.7620+001	-.27	-1.63
1710+003	.1712+003	-.1725+000	.2974-001	-.02	-.10
1681+003	.1683+003	-.2240+000	.5017-001	-.02	-.13
1638+003	.1643+003	-.4786+000	.2290+000	-.05	-.29
1610+003	.1604+003	.5839+000	.3408+000	.06	.36
1581+003	.1549+003	.3268+001	.1068+002	.32	2.07
ALS					
2935+005	.2927+005	.7955+002	.1883+005		

APPENDIX K

CONTINUOUS ACID HYDROLYSIS RESULTS AND CALCULATIONS

1. Continuous Hydrolysis Results

Table K.1. : Continuous acid hydrolyses with acid injection

Run Number	1	2	3
Temperature (°C)	130	130	130
Residence time (min)	55	55	55
Concentration of injected acid (g/ℓ H_2SO_4)	25	25	40
Mass acid injected (kg)	1,373	1,514	1,423
Mass moist bagasse feed (kg)	1,270	1,39	1,63
% moisture of bagasse	50,97	50,97	50,97
Mass dry bagasse feed (kg)	0,623	0,682	0,799
Mass discharged residue and hydrolysate (kg)	3,39	4,28	3,72
Mass dry residue washed from reactor (kg)	0,060	0,051	0,060
Mass air dried discharged residue (kg)	0,358	0,409	0,511
% moisture of air dried residue	5,37	7,70	15,68
Mass dry discharged residue (kg)	0,339	0,377	0,431
Free xylose (g/ℓ)	34,7	31,9	45,0
Total xylose (g/ℓ)	35,3	32,2	45,1
Free glucose (g/ℓ)	4,4	3,6	6,1
Total glucose (g/ℓ)	4,6	3,7	6,3
Acetic acid (g/ℓ)	8,12	6,63	10,77
Furfural (g/ℓ)	0,95	0,66	1,40
Total acidity (g/ℓ H_2SO_4)	14,94	13,35	21,93
H_2SO_4 conc. (g/ℓ)	8,31	7,94	13,14

Table K.2. : Continuous acid hydrolyses with steam injection

Run Number	4	5	6	7
Temperature °C)	130	120	130	130
Residence time (min)	55	85	55	55
Mass acid impregnated feed (kg)	1,86	1,87	1,87	1,92
% liquid in bagasse	56,49	57,91	56,31	56,03
Mass dry bagasse feed (kg)	0,809	0,787	0,817	0,844
Acid content (g/kg acid impreg.bagasse)	23,71	39,99	24,91	20,79
Mass discharged residue and hydrolysate (kg)	3,18	3,38	3,13	3,16
Mass dry residue washed from reactor (kg)	0,087	0,102	0,082	0,090
Mass air dried discharged residue	0,516	0,458	0,541	0,534
% moisture of air dried residue	14,69	8,31	19,26	15,57
Mass dry discharged residue (kg)	0,440	0,420	0,437	0,451
Free xylose (g/ℓ)	50,6	48,6	54,0	53,6
Total xylose (g/ℓ)	52,3	49,3	54,0	54,0
Free glucose (g/ℓ)	7,3	7,6	8,2	7,6
Total glucos (g/ℓ)	7,6	7,9	9,1	7,6
Acetic acid (g/ℓ)	12,38	12,75	13,56	13,50
Furfural (g/ℓ)	2,96	2,88	4,50	3,48
Total acidity (g/ℓ H_2SO_4)	22,78	30,04	23,59	21,44
H_2SO_4 conc (g/ℓ)	11,41	19,63	12,52	10,42

2. Calculation of Product Yields

The product yields and the liquid to solid ratios for the continuous hydrolyses were calculated according to the following methods :

2.2 Calculation Based on Discharged Hydrolysate Mass

This calculation is based on a mass balance on the discharged hydrolysate (all volumes in ℓ and product conc. in g/ℓ).

Mass discharged residue and hydrolysate - Mass discharged residue

Mass discharged residue = mass H_2O + mass xylose + mass glucose + mass acetic acid + mass furfural + mass H_2SO_4

$$\text{Hydrolysate mass (g)} = 1\,000 \left[\text{vol} - \frac{\text{acetic acid conc} \times \text{vol}}{1{,}05 \times 1\,000} - \frac{\text{furfural conc} \times \text{vol}}{1{,}16 \times 1\,000} - \frac{H_2SO_4 \text{ conc} \times \text{vol}}{1{,}84 \times 1\,000}\right]$$

+ xylose conc x vol + glucose conc x vol + furfural conc x vol + acetic acid conc x vol + H_2SO_4 conc x vol.

$$\text{Liquid to solid ratio} = \text{vol (ℓ)} : \text{initial mass bagasse (kg)} \times \frac{\text{mass discharged residue}}{\text{mass total residue}}$$

$$\text{Product yield} = \frac{\text{product conc} \times \text{vol}}{\text{initial mass bagasse (g)}} \times \frac{\text{mass of total residue (g)}}{\text{mass of discharged residue (g)}}$$

Consider Run 1 as an example :

$$(3{,}39 - 0{,}339) \times 1\,000 = 1\,000 \left[\text{vol} - \frac{8{,}12\ \text{vol}}{1{,}05 \times 1\,000} - \frac{0{,}95\ \text{vol}}{1{,}16 \times 1\,000} - \frac{8{,}31\ \text{vol}}{1{,}84 \times 1\,000}\right]$$

$$+ 35{,}3\ \text{vol} + 4{,}6\ \text{vol} + 0{,}95\ \text{vol} + 8{,}12\ \text{v}$$

$$+ 8{,}31\ \text{vol}$$

Solving the equation :

$$\text{Vol} = 2{,}92ℓ$$

$$\text{Liquid to solid ratio} = 2{,}92 : 0{,}623 \times \frac{0{,}339}{0{,}399}$$

$$= \underline{5{,}52 : 1}$$

$$\text{Total xylose yield} = \frac{35{,}3 \times 2{,}92}{623} \times \frac{0{,}399}{0{,}339}$$

$$= \underline{195{,}0\ \text{mg/g bagasse}}$$