

# **Modelling the distribution of micro-nutrient metals in the anaerobic digestion for Fischer-Tropsch reaction water**



**Submitted by: Nomalungelo Thembeka Ntuli**

**Supervisor: Mr. C.J. Brouckaert**

In fulfilment of Master of Science in Chemical Engineering at the University of KwaZulu-Natal

**Pollution Research Group**

**School of Chemical Engineering**

**College of Agriculture, Engineering and Science**

**University of Kwa-Zulu Natal**

**June 2017**

## ABSTRACT

Sasol, one of South Africa's largest petrochemical producers; derives water from the Fischer Tropsch process and is referred to as Fischer-Tropsch Reaction Water (FTRW). FTRW has high hydrocarbon content and a very low pH, hence, it must be treated prior reuse in the system. In the purification process, the organic content in the FTRW is mainly broken down to CO<sub>2</sub> and CH<sub>4</sub> by microorganisms which use the organic content as nourishment, bringing about its decomposition. Macro and micronutrients are necessary for survival of microorganisms, and this study is focused on the optimisation of micronutrients (micro-metals) to supplement the treatment of FTRW.

This study was aimed at developing a model to predict the distribution of micronutrients in the various phases present in anaerobic digester sludge: soluble ions, precipitates and an adsorbed phase. The aim was to introduce an adsorbed phase to the precipitates-soluble phase model to reduce the deviation between model and experimental sludge concentrations. The model would thereafter be used for optimization of micronutrients dosing techniques employed by Sasol to reduce operating costs of anaerobic treatment of FTRW.

The model was sufficiently extended to include the adsorbed phase by ionic representation of the biomass, however, the accuracy to which the model represents reality could not be tested by model validation due to these crucial limitations: incomplete set of experimental soluble phase concentrations, imprecise experimental data for metals entrapped in precipitates only and in the adsorbed phase only, to allow for regression of governing equations, as well lack of experimental representation of the relationship between sulphide and sulphate by concentrations. The integrated Ionic speciation model was used to point out the importance of the sulphate-sulphide system, as the phase control varies between the precipitates and the adsorbed phase, depending on the behaviour of the anions, specifically the sulphides.

A series of further experimental work needs to be completed to ensure a robust model outcome, such that the model best represents the speciation of metals in an anaerobic digester. The hypothesis therefore could not be proven to be true based on the data at hand. The next steps will be to carry out detailed experimental work; showing initial conditions, sulphate and sulphide concentration as well as experimental partitioning of metals in the various phases. In the meantime, Sasol can look into incorporation of a *donor* cation with a greater affinity to sulphide ions and adsorption on biomass while not impacting the decomposition reaction, such that the metal cations essential for anaerobic digestion are kept in solution to enhance the microbial activity for the treatment of FTRW.

## DECLARATIONS

### Supervisor:

As the supervisor for the candidate, I hereby declare that this dissertation is fit for submission and has been completed in fulfilment of Master of Science in Chemical Engineering at the University of KwaZulu-Natal.

Signed: \_\_\_\_\_

***Mr Christopher J. Brouckaert  
Pollution Research Group  
School of Chemical Engineering  
College of Agriculture, Engineering and Science  
University of Kwa-Zulu Natal***

### Student:

I, Nomalungelo Thembeka Ntuli, declare that:

- (i) The research reported in this dissertation/thesis, except where otherwise indicated, is my original work.
- (ii) This dissertation/thesis has not been submitted for any degree or examination at any other university.
- (iii) This dissertation/thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
- (iv) This dissertation/thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
  - a. their words have been re-written but the general information attributed to them has been referenced;
  - b. Where their exact words have been used, their writing has been placed inside quotation marks, and referenced.
- (v) Where I have reproduced a publication of which I am an author, co-author or editor, I have indicated in detail which part of the publication was actually written by myself alone and have fully referenced such publications.
- (vi) This dissertation/thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation/thesis and in the References sections.

Signed: \_\_\_\_\_

***Nomalungelo Thembeka Ntuli  
Student Number: 209505109***

## ACKNOWLEDGEMENTS

I would like to acknowledge the following people and organisations that had a vital contribution to this dissertation and research as a whole:

- To my **God Almighty**, my Beginning and my End, for constantly opening doors of success, paving my way and giving me strength and wisdom to complete this research.
- To **Sasol Ltd**: the initiator and funder of the research. Thank you for the great opportunity and for being a company that greatly invests in research.
- To **Chris Buckley**: the leader of the Pollution Research Group of the University of Kwa-Zulu Natal. Thank you for such joint ventures that allow for students to be a part of such an amazing and diverse world of research, particularly in a field we all have contact with in some or other way.
- To **Dr K M Foxon**: thank you for instilling sound engineering principles, practices and engineering-driven thinking in me. You have greatly increased my ability to think outside the box, sharpened my technical communication skills and professionalism.
- To **Chris Brouckaert**: my supervisor: Thank you for constantly being available when I came across hurdles in the construction of my model, I learnt an enormous amount of modelling techniques and skills from you. I was able to see the importance of applying thermodynamics, aquatic chemistry, biochemistry and pure maths first principles in the construction of the model. I'm yet to see or come across a person with greater modelling skill and insight.
- To **Aarefah Mathir**, thank you for forming a great foundation in the area of work, for the insight and experimental data. Your hard work greatly paved the way for my research and for students who are yet to undertake research in the same area of work.
- To my **family**: Dr M.S Ntuli, Mrs B.N Ntuli, Thuthuka Ntuli, Ntobeko Ntuli, Thamsanqa Mbokazi together with my precious daughter: Yamkelwa Mbokazi. Thank you for being a constant pillar of strength in my academic journey, for constantly making me smile and strengthening me.
- To all my **friends and colleagues**, thank you for all the support given aiding the completion of this research.

## CONTENTS

<b>ABSTRACT</b> .....	<b>ii</b>
<b>DECLARATIONS</b> .....	<b>iii</b>
<b>ACKNOWLEDGEMENTS</b> .....	<b>iv</b>
<b>GLOSSARY</b> .....	<b>viii</b>
<b>LIST OF ACRONYMS AND ABBREVIATIONS</b> .....	<b>x</b>
<b>LIST OF FIGURES</b> .....	<b>xii</b>
<b>LIST OF TABLES</b> .....	<b>xv</b>
<b>1. INTRODUCTION</b> .....	<b>1</b>
1.1    CONTEXT OF THE STUDY .....	1
1.2    ENGINEERING PROBLEM.....	3
1.3    PURPOSE OF THE STUDY .....	3
1.4    RESEARCH OUTCOMES.....	3
1.5    SIGNIFICANCE OF THE STUDY .....	4
1.6    DELIMITATIONS OF THE STUDY.....	4
<b>2. LITERATURE REVIEW</b> .....	<b>5</b>
2.1    FUNDAMENTAL CHEMICAL BACKGROUND .....	5
2.1.1    ANAEROBIC DIGESTION.....	5
2.1.2    SIGNIFICANCE OF NUTRIENTS .....	6
2.1.3    MICRONUTRIENT RECIPES .....	7
2.1.4    MICRONUTRIENT DOSING .....	9
2.1.5    METAL UPTAKE BY MICROORGANISMS.....	13
2.1.6    SPECIATION .....	14
2.2    ANALYTICAL APPROACH.....	15
2.2.1    SEQUENTIAL EXTRACTION PROCEDURE .....	15
2.2.2    CHEMICAL SEQUENTIAL EXTRACTION FOR METAL PARTITIONING IN ENVIRONMENTAL SOLID SAMPLES (Filgueiras, et al., 2002).....	16
2.2.3    HEAVY METAL EXTRACTABLE FORMS IN SLUDGE FROM WASTEWATER TREATMENT PLANTS (Alvarez, et al., 2002).....	17
2.2.4    SIMPLE AND SEQUENTIAL EXTRACTION OF HEAVY METALS FROM DIFFERENT SEWAGE SLUDGES (Feuntes, et al., 2004) .....	20
2.2.5    COMPARISON OF THREE SEQUENTIAL EXTRACTION PROCEDURES TO DESCRIBE METAL FRACTIONATION IN ANAEROBIC GRANULAR SLUDGES (van Hullebusch, et al., 2005a) .....	21
2.2.6    COBALT AND NICKEL BIOAVAILABILITY FOR BIOGAS FORMATION (Gustavsson , 2012) .....	23
2.2.7    CONCLUSIONS ON THE ANALYTICAL APPROACH.....	24
2.3    MODELS .....	26

2.3.1	INTERACTION BETWEEN METALS AND ANAEROBIC SLUDGE.....	26
2.3.2	FREE ION ACTIVITY MODEL (FIAM) AND EXTENDED FIAM.....	27
2.3.3	BIOTIC LIGAND MODEL (BLM) .....	33
2.3.4	ADSORPTION ISOTHERMS .....	36
2.3.5	ANALYSIS OF MICRONUTRIENT REQUIREMENTS FOR THE ANAEROBIC DIGESTION OF FISCHER-TROPSCH REACTION WATER (Mathir, 2013).....	39
<b>3.</b>	<b>SYNTHESIS OF LITERATURE.....</b>	<b>43</b>
3.1	LITERATURE REVIEW SUMMARY .....	43
3.2	GAP ANALYSIS .....	44
3.3	RESEARCH QUESTIONS.....	45
3.4	RESEARCH METHODOLOGY .....	45
3.4.1	HYPOTHESIS .....	46
3.4.2	HYPOTHESIS TESTING .....	46
<b>4.</b>	<b>MODEL DEVELOPMENT AND DESIGN.....</b>	<b>47</b>
4.1	MODELLING OF WASHOUT EXPERIMENT BY Mathir (2013).....	48
4.1.1	EXPERIMENTAL DATA.....	48
4.1.2	BIOLOGICAL CONVERSION .....	48
4.1.3	INITIAL AND FEED CONDITIONS.....	49
4.1.4	REACTOR AND REACTION CONDITIONS.....	49
4.2	SPECIATION MODELING .....	50
4.2.1	PHREEQC .....	50
4.2.2	PRECIPITATION MODELLING IN PHREEQC.....	51
4.2.3	ADSORPTION MODELLING IN PHREEQC.....	52
4.3	MODEL ALGORITHM.....	55
<b>5.</b>	<b>RESULTS.....</b>	<b>59</b>
5.1	BIOLOGICAL CONVERSION OF ORGANIC CONTENT IN FTRW .....	59
5.2	EFFECT OF ADDITIONAL PHASES ON SOLUBLE PHASE.....	61
5.2.1	SLUDGE CONCENTRATIONS – Initial Conditions .....	61
5.3	SULPHATE-SULPHIDE SYSTEM .....	63
5.3.1	SENSITIVITY OF SOLUBLE, PRECIPITATES AND ADSORBED PHASE TO SULPHIDE CONCENTRATION .....	63
5.3.2	WASHOUT EXPERIMENT AT DIFFERENT SULPHIDE CONCENTRATIONS 65	
5.3.3	METAL PARTITIONING AT THE TWO SULPHIDE CONCENTRATION EXTREMES .....	69
5.3.4	COMPARISON BETWEEN MODEL AND EXPERIMENTA DATA.....	71

5.3.5	RELATIONSHIP BETWEEN CONVERSION, BIOAVAILABILITY AND METAL CONCENTRATION AT DIFFERENT SULPHIDE CONCENTRATIONS .....	77
<b>6.</b>	<b>DISCUSSION.....</b>	<b>80</b>
6.1	MODEL DEVELOPMENT AND RESULTS .....	80
6.2	RESPONSE to RESEARCH QUESTION.....	83
<b>7.</b>	<b>CONCLUSIONS.....</b>	<b>84</b>
<b>8.</b>	<b>NEXT STEPS.....</b>	<b>85</b>
<b>9.</b>	<b>BIBLIOGRAPHY.....</b>	<b>87</b>
	<b>APPENDICES.....</b>	<b>91</b>
	APPENDIX A: Supporting Figures and Tables.....	91
	APPENDIX B: Chemical Speciation Mass Balances .....	91
	APPENDIX C: Model Development and Design .....	92
	APPENDIX D: Sequential Extraction Supporting Documentation .....	95
	APPENDIX E: Experimental Results and Initial Conditions .....	95
	APPENDIX F: Sulphate-Sulphide System .....	96
	Appendix F-1: Pilot Plant Reaction and Reactor Conditions.....	96
	Appendix F-2: Sulphate Reducing Bacteria .....	96
	APPENDIX G: Sequential Extraction Results – With Sulphide in the System.....	97
	APPENDIX H: Comparison of P-Model and AP-Model Results to Literature Data .....	106

## GLOSSARY

<b>Adsorption</b>	The accumulation of molecules of a gas or liquid to form a thin film on the surface of a solid.
<b>Aerobic Digestion</b>	The process carrying out the mineralisation of the complex organic matter in wastewater to final products of CH <sub>4</sub> , CO <sub>2</sub> , H <sub>2</sub> and H <sub>2</sub> O <i>in the presence of oxygen</i> .
<b>Anaerobic Digestion</b>	The process carrying out the mineralisation of the complex organic matter in wastewater to final products of CH <sub>4</sub> , CO <sub>2</sub> , H <sub>2</sub> and H <sub>2</sub> O <i>in the absence of oxygen</i> .
<b>Bioavailability</b>	The extent to which metals in a sludge are available for biological uptake by the microorganisms to bring about a biological response.
<b>Biological Response</b>	A response to the catalytic effect of enzymes produced by microorganisms bringing about the degradation of organic content to form CH <sub>4</sub> , CO <sub>2</sub> , H <sub>2</sub> and H <sub>2</sub> O.
<b>Biomass</b>	A measure (quantity or weight) of organisms in a given area or volume, sludge in this case.
<b>Complexation</b>	A mechanism by which metal ions join onto the surface of organic content, resulting in the formation of a metal-surface complex.
<b>Metal Toxicity</b>	The boundary condition for metal addition to activated sludge corresponding to an excess of metals.
<b>Methanogens</b>	Methanogenic bacteria responsible for conversion of acetate and hydrogen into methane in anaerobic digestion.
<b>Microorganism</b>	Minute living organisms that feed on organic material in anaerobic digestion, bringing forth decomposition into various products.
<b>Micronutrients</b>	A mixture (recipe) of trace metals supplemented to sludge, including : Mn, Zn, Co, Mo, Ni, Cu, Va, B, Fe, I, Se, Cr and W.
<b>pH</b>	A measure of the molar concentration of hydrogen ions in the solution, giving a measurement of the acidity or basicity of the solution.



<b>Precipitation</b>	The process of the formation of solids from a solution by a reaction between metal ions (once saturation concentrations have been reached) with anions to form minerals.
<b>Sequential Extraction</b>	An experimental technique in which metal ions are extracted from the different phases in which they exist in a system (sludge) by a solvent.
<b>Sludge</b>	A semi-solid slurry, a mixture of solid and liquid. In this study, the liquid is FTRW and the solid is biomass.
<b>Speciation</b>	The distribution of an element between the chemical species which may exist in a system.
<b>UKZiNe</b>	Ionic representation of anaerobic sludge surface consisting of two carboxylic acid groups, a phosphate and an ammonium group (pr. Youka-zeen)

## LIST OF ACRONYMS AND ABBREVIATIONS

<b>Acronym/Abbreviation</b>	<b>Explanation</b>
<b>ADMLSS</b>	Anaerobic Digester Mixed Liquor Suspended Solids
<b>A<sub>ij</sub></b>	Stoichiometric coefficient giving the number of moles of component <i>j</i> in species <i>i</i> .
<b>Al</b>	Aluminium
<b>AP-Model</b>	Adsorbed and Precipitates Phase Controlled Speciation Model
<b>AVS</b>	Acid Volatile Sulfide
<b>AVS-Me</b>	Semi-continuously extracted metals
<b>B</b>	Boron
<b>BCR</b>	The commission of the European communities Bureau of Reference
<b>β<sub>i</sub></b>	Overall equilibrium formation constant for species <i>i</i> .
<b>BFMpH</b>	Best Fit Model pH
<b>BLM</b>	Biotic Ligand Model
<b>BR</b>	Biological response
<b>BRT</b>	Biological receptor theory
<b>C</b>	Compost
<b>Cd</b>	Cadmium
<b>CH<sub>4</sub></b>	Methane gas
<b>C<sub>j</sub></b>	Concentration of component <i>j</i>
<b>C<sub>n</sub>H<sub>2n</sub></b>	Alkene hydrocarbon functional group
<b>CO</b>	Carbon Monoxide
<b>Co</b>	Cobalt
<b>CO<sub>2</sub></b>	Carbon Dioxide
<b>CO<sub>3</sub><sup>-2</sup></b>	Carbonate ion
<b>COD</b>	Chemical Oxygen Demand
<b>Cr</b>	Chromium
<b>Cu</b>	Copper
<b>D+DS</b>	Dewatered and digested sludge
<b>DOM</b>	Dissolved Organic Matter
<b>ECP</b>	The human eosinophil cationic protein (ECP), also known as RNase
<b>EDTA</b>	Ethylene-diamine-tetra-acetic acid
<b>Fe</b>	Iron
<b>FIAM</b>	Free Ion Activity Model
<b>FTRW</b>	Fischer-Tropsch Reaction Water
<b>GSIM</b>	Gill Surface Interaction Model
<b>H<sub>2</sub></b>	Hydrogen gas
<b>H<sub>2</sub>O</b>	Chemical formula of water
<b>HNO<sub>3</sub></b>	Nitric acid
<b>I</b>	Iodine
<b>K</b>	Potassium
<b>KF</b>	Potassium fluoride
<b>KNO<sub>3</sub></b>	Potassium nitrate
<b>LC<sub>50</sub></b>	Concentrations of the biotic ligand bringing about a mortality of 50%
<b>M or M<sup>Z</sup></b>	Free metal
<b>MgCl<sub>2</sub></b>	Magnesium Chloride

<b>ML</b>	Metal-Ligand complex
<b>M-A<sub>cell</sub>/M-R<sub>cell</sub></b>	Adsorbed metal
<b>M<sub>j</sub></b>	Total mass of component
<b>Mn</b>	Manganese
<b>Mo</b>	Molybdenum
<b>mol/litre</b>	Units of concentration
<b>MPa</b>	Pressure units: Mega pascals
<b>MpH</b>	Model pH
<b>N</b>	Nitrogen
<b>Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub></b>	Sodium di-phosphate
<b>N<sub>c</sub></b>	Number of components
<b>Ni</b>	Nickel
<b>NOM</b>	Natural Organic Matter
<b>OLR</b>	Organic Loading Rate
<b>OM</b>	Organic Matter
<b>P</b>	Phosphorus
<b>P-Model</b>	Precipitates Phase Controlled Speciation Model
<b>Pb</b>	Lead
<b>PHREEQC</b>	PHREEQC stands for pH (PH), redox (RE), equilibrium (EQ) and programme written in C (C)
<b>PS</b>	Primary Sludge
<b>PO<sub>4</sub><sup>3-</sup></b>	Phosphate ion
<b>Qe/Q<sub>max</sub></b>	Uptake and maximum uptake of metal ion by biomass
<b>R&amp;D</b>	Research and Development
<b>S</b>	Sulphur
<b>S<sup>2-</sup></b>	Sulphide ion
<b>SBR</b>	Sequencing Batch Reactor
<b>SCFA</b>	Short Chain Fatty Acid
<b>Se</b>	Selenium
<b>SMP</b>	Soluble Microbial Products
<b>SS</b>	Secondary Sludge
<b>Ti</b>	Titanium
<b>TSS</b>	Total Suspended Solids
<b>UASB</b>	Up-flow Anaerobic Sludge Bed
<b>Va</b>	Vanadium
<b>VS</b>	Volatile Solids
<b>W</b>	Tungsten
<b>WEST</b>	An open source waste water treatment modelling software.
<b>X-cell</b>	Free surface site on cell membrane
<b><i>x<sub>i</sub></i></b>	Activity of the aqueous species <i>i</i>
<b>ZED</b>	Zero Effluent Discharge
<b>Zn</b>	Zinc
<b><i>γ<sub>i</sub></i></b>	Activity coefficient of species <i>i</i>

## LIST OF FIGURES

<b>Figure 1:</b> The Three Stages of Methane Fermentation <sup>a</sup> .....	6
<b>Figure 2:</b> Boundary conditions for metal addition to keep UASB reactor efficiency optimal (Fermoso, et al., 2009) .....	10
<b>Figure 3:</b> Conceptual Framework of the Free Ion Activity Model and Biotic Ligand Model. $M^z$ : free metal ion, L: ligand, ML: hydrophilic complex, $ML_{bio}$ : metal-intracellular ligand complex, $K_{int}$ : internalisation rate constant, M- $A_{cell}$ : non-specific metal-surface adsorption complex, M- $R_{cell}$ : specific metal-surface adsorption complex (Fermoso, et al., 2009).....	14
<b>Figure 4:</b> Metal partitioning of Secondary Sludge in BCR Scheme (Alvarez, et al., 2002). Data plotted from Table 2 and Table 3 of Alvarez (2002) .....	18
<b>Figure 5:</b> Metal partitioning of Dewatered and Digested Sludge in BCR (Alvarez, et al., 2002). Data plotted from Table 2 and Table 3 of Alvarez (2002). .....	19
<b>Figure 6:</b> Percent recoveries of total metals in the sequential extraction fractions for anaerobic sludge using the BCR scheme (Feuntes, et al., 2004). Data plotted from Figure 1 and Table 3 of Feuntes (2004). .....	20
<b>Figure 7:</b> Metal partitioning profiles in Nedalco sludge according to the modified Tessier Sequential extraction scheme (van Hullebusch, et al., 2005a) .....	22
<b>Figure 8:</b> Concentration-response curves for different slope (n) values (Brown & Markich, 2000) .....	30
<b>Figure 9:</b> Cd uptake ( $mg\ kg^{-1}$ ) by <i>Brassica juncea</i> and <i>Zea may</i> shoots modelled by the FIAM assuming a common absorption site for $Cd^{2+}$ and $CdCl^+$ ions (Lo'pez-Chuken, et al., 2010) .....	32
<b>Figure 10:</b> Schematic diagram of the biotic ligand model (Di Toro, et al., 2001).....	33
<b>Figure 11:</b> Comparison between measured and BLM predicted $LC_{50s}$ for Cu for <i>fathead minnow</i> , <i>rainbow trout</i> and <i>daphnia magna</i> (Di Toro, et al., 2001).....	35
<b>Figure 12:</b> Comparison between measured and BLM predicted $LC_{50s}$ for Ag for <i>fathead minnow</i> , <i>rainbow trout</i> and <i>daphnia magna</i> (Di Toro, et al., 2001).....	36
<b>Figure 13:</b> Nickel and cobalt sorption onto Nedalco (a) and Eerbeek (b) anaerobic granular sludges (monometal and competitive (van Hullebusch, et al., 2005b)).....	38
<b>Figure 14:</b> Experimental Metals Speciation Results for Sequential Batch Reactor for Experiment A (Mathir, 2013).....	40
<b>Figure 15:</b> Concentration of precipitated minerals as predicted by model for each successive cycle in Experiment B (Mathir, 2013) .....	41
<b>Figure 16:</b> Concentration profile of $Ca^{+2}$ and $Mg^{+2}$ in the dissolved phase (mg/l) including comparisons to experimental data in Experiment B (Mathir, 2013).....	41
<b>Figure 17:</b> Concentration profile of $Fe^{+2}$ in the dissolved phase (mg/l) including comparisons to experimental data in Experiment B (Mathir, 2013).....	42
<b>Figure 18:</b> Overall Speciation Model Algorithm.....	57

<b>Figure 19:</b> Model construction algorithm for AP-Model and Hypothesis Testing.....	58
<b>Figure 20:</b> Experimental and Model pH per nutrient washout experiment cycle.....	59
<b>Figure 21:</b> Concentration profile of Acetate <sup>-</sup> and Propionate <sup>-</sup> during the nutrient washout experiment.....	60
<b>Figure 22:</b> Model predictions of soluble metal concentrations for the Pilot Plant (Ntuli & Brouckaert, 2014) .....	64
<b>Figure 23 :</b> Model predictions of precipitated metal concentrations for the Pilot Plant (Ntuli & Brouckaert, 2014) .....	64
<b>Figure 24:</b> Model predictions of adsorbed metal concentrations for the Pilot Plant (Ntuli & Brouckaert, 2014) .....	65
<b>Figure 25:</b> Concentration of HS <sup>-</sup> and S <sup>-2</sup> when the PHREEQC reduction reaction is permitted.	66
<b>Figure 26:</b> Soluble Cu <sup>+2</sup> concentration in the AP-Model at different conversions of SO <sub>4</sub> <sup>-2</sup> to HS <sup>-</sup> .....	67
<b>Figure 27:</b> Soluble Co <sup>+2</sup> Concentration in the AP-Model at different conversions of SO <sub>4</sub> <sup>-2</sup> to HS <sup>-</sup> .....	68
<b>Figure 28:</b> Model prediction of Partitioning of Co <sup>+2</sup> in the Soluble, Precipitates and Adsorbed Phases with Sulphide present in the system.....	69
<b>Figure 29:</b> Model prediction of Partitioning of Co <sup>+2</sup> in the Soluble, Precipitates and Adsorbed Phases with no Sulphide present in the system.....	70
<b>Figure 30:</b> Model prediction of Partitioning of Fe <sup>+2</sup> in the Soluble, Precipitates and Adsorbed Phases with Sulphide present in the system.....	70
<b>Figure 31:</b> Model prediction of Partitioning of Fe <sup>+2</sup> in the Soluble, Precipitates and Adsorbed Phases with no Sulphide present in the system.....	71
<b>Figure 32:</b> Co <sup>+2</sup> Concentration in sludge for washout experiment .....	72
<b>Figure 33:</b> Zn <sup>+2</sup> Concentration in sludge for washout experiment .....	73
<b>Figure 34:</b> Mg <sup>+2</sup> Concentration in sludge for washout experiment .....	74
<b>Figure 35:</b> Ca <sup>+2</sup> Concentration in sludge for washout experiment .....	74
<b>Figure 36:</b> Mn <sup>+2</sup> Concentration in sludge for washout experiment .....	75
<b>Figure 37:</b> Cu <sup>+2</sup> Concentration in sludge for washout experiment .....	76
<b>Figure 38:</b> Fe <sup>+2</sup> Concentration in sludge for washout experiment.....	76
<b>Figure 39:</b> Relationship between conversion and Ni <sup>+2</sup> , Zn <sup>+2</sup> , Fe <sup>+2</sup> and Co <sup>+2</sup> soluble Concentration with Sulphide present in the system.....	78
<b>Figure 40:</b> Relationship between conversion and Ni <sup>+2</sup> , Zn <sup>+2</sup> , Fe <sup>+2</sup> and Co <sup>+2</sup> soluble Concentration with no Sulphide present in the system.....	79
<b>Figure 41:</b> HPO <sub>4</sub> <sup>-2</sup> , HS <sup>-</sup> and SO <sub>4</sub> <sup>-2</sup> concentrations per cycle.....	97
<b>Figure 42:</b> CoS, MnHPO <sub>4</sub> and ZnS precipitates formation for P-Model and AP-Model .....	98

<b>Figure 43:</b> Concentration Profile of $Zn^{+2}$ in the Soluble, Precipitates and Adsorbed Phase for AP-Model.....	99
<b>Figure 44:</b> $CuS$ , $Ca_5(PO_4)_3OH$ and $NiS$ precipitates formation for P-Model and AP-Model .....	99
<b>Figure 45:</b> Soluble Phase Concentration of $Ca^{+2}$ for the P-Model and AP-Model.....	100
<b>Figure 46:</b> $Ca^{+2}$ Partitioning in Soluble, Precipitates and Adsorbed Phase for AP-Model Comparing to $PO_4^{-3}$ Concentration .....	100
<b>Figure 47:</b> $Fe_3(PO_4)_2 \cdot 8H_2O$ and $FeS_2$ precipitates formation for P-Model and AP-Model .....	101
<b>Figure 48:</b> Concentration Profile of $Fe^{+2}$ in the Soluble, Precipitates and Adsorbed Phase for AP-Model.....	102
<b>Figure 49:</b> $MoS_2$ precipitates formation for P-Model and AP-Model .....	102
<b>Figure 50:</b> Concentration Profile of $MoO_4^{-2}$ in the Soluble, Precipitates and Adsorbed Phase for AP-Model.....	103
<b>Figure 51:</b> Soluble Phase Concentration of $Mg^{+2}$ for the P-Model and AP-Model.....	104
<b>Figure 52:</b> $Mg^{+2}$ Partitioning in Soluble, Precipitates and Adsorbed Phase for AP-Model .....	104
<b>Figure 53:</b> Average metal partitioning for wash out experiment to indicate ranking of free metal ions.....	105

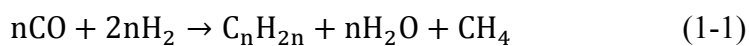
## LIST OF TABLES

<b>Table 1:</b> The role of trace elements in microbial systems <sup>b</sup> (Burgess, et al., 1999).....	8
<b>Table 2:</b> Toxic conditions of cobalt, nickel and zinc in methanogenesis processes (Fermoso, et al., 2009) .....	11
<b>Table 3:</b> Definition of Sequential extraction fractions as suggested by Filgueiras (2002).....	16
<b>Table 4:</b> Fractions for the BCR scheme with connection with to Filgueiras (2002) .....	18
<b>Table 5:</b> Repeatability (%) of the Tessier, BCR and Stover schemes (van Hullebusch, et al., 2005a) .....	21
<b>Table 6:</b> Sequential extraction fractions for the Tessier scheme with definitions as per Filgueiras (2002).....	21
<b>Table 7:</b> Fractions for the Stover scheme used in Mathir's (2013) work .....	39
<b>Table 8:</b> Model Development Overview.....	47
<b>Table 9:</b> Model Reactor and Reaction Conditions for Washout Experiment by Mathir (2013)..	50
<b>Table 10:</b> Minerals likely to precipitate under ambient conditions (-100 to 100C °, 1 atm.) in waste water (Wadley & Buckley, 1997) and PHREEQC database. ....	51
<b>Table 11:</b> Comparison between UKZiNe and Glycine Characteristics (Westergreen, et al., 2012) .....	54
<b>Table 12:</b> Results of Initial Conditions Iteration.....	62
<b>Table 13:</b> Composition of Conventional Basal Medium for methanogens (Zhang, et al., 2003)	91
<b>Table 14:</b> Initial Conditions for Anaerobic digester (Mathir, 2013).....	92
<b>Table 15:</b> Sasol Nutrient Recipe for Anaerobic Digestion of FTRW (Mathir, 2013) .....	93
<b>Table 16:</b> Experimental pH results for Anaerobic digester (Mathir, 2013).....	94
<b>Table 17:</b> Adsorption Constants for Ca <sup>+2</sup> , Cu <sup>+2</sup> and Fe <sup>+2</sup> in Sludge .....	95
<b>Table 18:</b> Experimental Results for Washout Experiment (Mathir, 2013).....	95
<b>Table 19:</b> Model Reactor and Reaction Conditions for Pilot Plant.....	96
<b>Table 20:</b> Reactions showing precipitates forming in P-Model and AP-Model.....	96
<b>Table 21:</b> Comparison of Model Results to Literature Data.....	106

# 1. INTRODUCTION

## 1.1 CONTEXT OF THE STUDY

Sasol is one of South Africa's largest producers of fuel and has been in operation since the early 1950's (Dry, 2002). The raw materials are processed in Lurgi dry-ash gasifiers, where fuel is produced from the gasification of coal in the presence of oxygen and steam at a temperature and pressure of 1200°C and 3MPa respectively (Dry, 2002). This process makes use of Fischer-Tropsch technology. Coal gasification produces a synthesis gas (syngas) composed of a mixture of CO and H<sub>2</sub>. After purification of the syngas, it proceeds into Sasol Advanced Synthol reactors where the syngas is catalytically converted over an iron-based (Fe) catalyst into methane, hydrocarbons in the range of C<sub>1</sub> to C<sub>20</sub> and water: this is where reaction water is produced. The reaction proceeds as follows (van Zyl, 2008):



The above reaction (1-1) shows that water is chemically produced as a by-product in the gasifiers and is referred to as Fischer-Tropsch Reaction Water (FTRW). The FTRW is an aqueous solution deficient in both macronutrients (N, P, etc.) and micronutrients (Van Zyl, 2008), and the organic components are principally short chain fatty acids (SCFAs) and alcohols. FTRW generally has a high organic load and is highly acidic with pH of approximately 3.8 (Van Zyl, 2008). FTRW has been found to contain trace quantities of iron due to the iron-based catalyst used in the processes upstream.

Sasol believes in the Zero Effluent Discharge (ZED) policy that has an ultimate goal of avoiding any release of contaminants to the environment, which is strongly driven by the scarcity of water in Southern Africa. This is done by treating organically polluted water and returning brine water (Wilson, 2008). In the Sasol Secunda plant, the effluent water is re-used to prevent discharging of the waste water into natural water bodies thereby resulting in their contamination.

FTRW has a high hydrocarbon content and very low pH, due to the fairly extreme conditions it cannot be directly fed into the system for reuse; it therefore undergoes treatment prior its reuse in the plant. Currently, aerobic digestion methods are employed by the Sasol Secunda plant to treat the industrial effluent water by making use of an activated sludge plant. Sasol has conducted extensive studies into anaerobic treatment of FTRW due to its potential advantages; including decreased oxygen demand costs, thereby reducing energy requirement as a result of the omission of aeration. Anaerobic digestion is known to have a lower biomass yield than aerobic digestion and this will result in a lower production of sludge during biological activity. Furthermore, energy is recovered as a result of the biogas production in anaerobic digestion.



The main concern about anaerobic digestion is its control, as anaerobic systems do not respond adequately to process fluctuations and consequently, the process may be operated within narrow boundaries of operating conditions such as pH and temperature. This results in the need for tight control for the anaerobic digesters which poses a higher risk of failure, bringing about repercussions in the effluent management systems in the Secunda plant as a whole.

In the purification process, the organic content in the FTRW is mainly broken down to CO<sub>2</sub> and CH<sub>4</sub> by microorganisms. The microorganisms use the organic content as nourishment, bringing about its decomposition.

As with any living organism, certain macro and micronutrients are necessary for survival. Nutrients are required by the microorganisms for them to function effectively, as they are essential for growth, enzymatic processes and metabolic functions (Fermoso, et al., 2009). The macronutrients necessary for the FTRW treatment microorganisms are N, P, K and S.

Sufficient micro-nutrition is needed to support all microbial genera required for the treatment process (Burgess, et al., 1999) and the micronutrient requirement for each effluent stream is specific to the nature of the waste and the ecology of the sludge (Burgess, et al., 1999). Micronutrients are in essence a mixture (recipe) of trace metals supplemented to the sludge, these include the following: Mn, Zn, Co, Mo, Ni, Cu, Va, B, Fe, I, Se, Cr and W (Burgess, et al., 1999), (Fermoso, et al., 2009).

From previous work carried out (Mathir, 2013), it has been established that in addition of the micronutrient recipe to the sludge (dosing), not all the metal ions are in a form that can be taken up and used by the microorganisms. Experimental work showed the different phases in which the metals were found; the inferred<sup>1</sup> definitions for these phases are as follows: free ions, ions adsorbed onto the exterior of cells or sludge flocs, solid inorganic precipitate, organically bound (complexed) ions, absorption of metal ions within actual cells and metals bound by chelating agents.

The Free Ion Activity Model assumes that the biological activity of a microorganism onto which a free metal has adsorbed itself has a direct relationship with the concentration of the free metal ion in solution (Brown & Markich, 2000).

In Mathir's work (2013), it was hypothesized that the presence of inorganic precipitates has a potentially strong influence on the activity and concentration of the free metal ions as presented by the solubility product. Experimental work was carried out after which a model was developed for a dynamic system describing the evolution of free metal ion concentration and the amount of

---

<sup>1</sup> An **inferred definition** is one that is brought about due to conclusions drawn from certain multiple observations and conclusive evidence.

metal ions bound in precipitates. The correlation between the experimental and model results was not strong but showed some form of relationship between the precipitates and the free metals needing to be modeled more rigorously. Furthermore, it was found that a considerable amount of metals were found in the organically bound phase, also needing consideration in the succeeding model.

## **1.2 ENGINEERING PROBLEM**

The use of micronutrients results in a major operating cost in anaerobic digestion of FTRW. The micronutrient recipe maximises the biological availability of the metals for the current digestion application. A micronutrient recipe was proposed by Du Preez and co-workers (Du Preez, 1987); however, it is not clear whether the recipe fulfils the micronutrient requirements in the most cost effective manner without the biological activity being compromised as a result of micronutrient limitation. Sasol is therefore interested in reducing the cost of micronutrient dosing, by optimisation of the micronutrient recipe, without compromising the biological activity, efficiency of the treatment process and without threatening the environment.

## **1.3 PURPOSE OF THE STUDY**

The general purpose for conducting the study was the development of a theoretical model to depict the controlling factors for the distribution of metals in anaerobic digester mixed liquor. On identification of the controlling factors, prediction of the effects of changes made to the micronutrient dosing on the concentration of free metal ions will be made, which affect metal bioavailability.

This study did not involve experimental work. It was a detailed analysis of the data produced by Mathir (2013), in order to construct a model which can be used to design experiments, for process optimisation, including a reduction in the cost of metal addition. An overview of the experimental methods is included, where needed.

## **1.4 RESEARCH OUTCOMES**

- An integrated Ionic Speciation model that can describe the fate of metals dosed in activated sludge to treat FTRW. The model would have fulfilled the requirements of the validation procedure to be used to verify its accuracy and analytical capacity.
- Possibly, potential scenario(s) Sasol Secunda can employ to their dosing strategies that will result in a decreased dosing bill without compromising process efficiency.

## **1.5 SIGNIFICANCE OF THE STUDY**

The benefits of developing the Ionic Speciation model will be the

- Availability of a tool to predict the fate of metals dosed to activated sludge during the anaerobic treatment of FTRW prior to actual dosing of the metals.
- Reduced effort and costs incurred in experimental work to predict the fate of metals dosed and their speciation within the different phases, as the model will be able to generate this data for certain inputs.

## **1.6 DELIMITATIONS OF THE STUDY**

The scope of the research was limited to the following aspects:

- The model considered continuous dosing of metals
- The experimental data was generated by making use of a sequential batch system, where the process was allowed to approach equilibrium, due to the long period where there is low biological activity.

## 2. LITERATURE REVIEW

The literature review includes essential information regarding the research required and considered in developing the model. Firstly, the role and elements of micronutrients in the biological process of water treatment was reviewed, specifically in the anaerobic process. A review of previous work was conducted where case studies were utilised to highlight previous findings in the same topic. The aspects considered in developing the models, certain trends and challenges faced were highlighted, to further aid in the construction of the new model.

### 2.1 FUNDAMENTAL CHEMICAL BACKGROUND

#### 2.1.1 ANAEROBIC DIGESTION

In a biological process, the enzymes within microorganisms catalyse the reaction by increasing the reaction rate by a factor ranging between  $10^3$  and  $10^{17}$  (Fogler, 2009). The substrate enters the active site on the enzyme; this is accompanied by a slight change in shape of the enzyme as the substrate binds to form an enzyme-substrate complex. The substrate thereafter converts into products on the active site and an enzyme-products complex forms after which the products leave and the enzyme remains with its active site, for use by another substrate (Fogler, 2009).

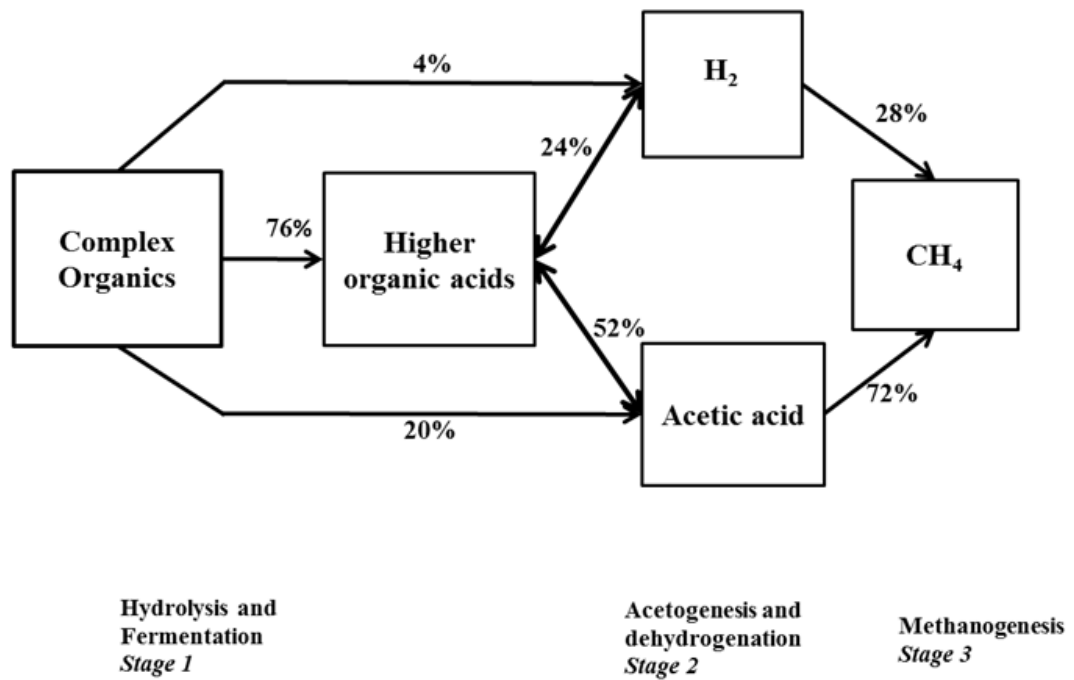
Anaerobic treatment of industrial water is a biological process where the substrate is the organic content in the water and the enzymes produced by the microorganisms catalyse the degradation thereof. It is a process whereby treatment of water, as opposed to aerobic digestion is carried out in the absence of aeration. The main aim of anaerobic digestion is the conversion of the complex organic matter in the water to final products of  $\text{CH}_4$ ,  $\text{CO}_2$ ,  $\text{H}_2$  and  $\text{H}_2\text{O}$ . The process occurs in three stages as depicted by Figure 1.

The microorganisms use the organic content as nourishment, bringing about its decomposition. This biological conversion of organics is catalysed by the presence of microorganisms that are genetically capable of processing the compound; it is desirable that the microorganisms be acclimated<sup>2</sup> to the compound (Fogler, 2009).

The complex organics are first hydrolysed by the *chemoheterotrophic non-methanogens* to form free sugars, alcohols, higher organic acids, hydrogen and carbon dioxide. The alcohols and volatile acids are thereafter converted to acetate and hydrogen by the *acetogenic* microorganisms. Finally, the methanogenic bacteria (*methanogens*) assist in converting the acetate and hydrogen into methane (Speece, 1983); they are referred to as the crucial class of microorganisms in anaerobic technology (Speece, 1983).

---

<sup>2</sup> **Acclimatization** is the process in which an individual organism adjusts to a gradual change in its environment (such as temperature, humidity or pH) allowing it to maintain performance across a range of environmental conditions.



**Figure 1: The Three Stages of Methane Fermentation <sup>a</sup>**

<sup>a</sup>: Shows the percentage flow of energy content of complex organic materials through each stage into methane, as represented by the chemical oxygen demand (McCarty, 1981)

### 2.1.2 SIGNIFICANCE OF NUTRIENTS

In biologically treated wastewater streams, there are certain macronutrients necessary for efficient treatment; moreover, there are vitamins and micronutrients necessary to stimulate biological activity (Burgess, et al., 1999).

Nutrients are required as part of the cell components of microorganisms to allow for the biodegradation processes to advance. Some of these nutrients are present in the feedstock water to the treatment process and are present within actual cell walls of the microorganisms (Zandvoort, et al., 2006). In the case where there is a deficiency in the nutrients, nutrients should be supplemented such that efficient performance of microorganisms in the biodegradation process is enhanced.

Industrial effluent wastewater streams may be deficient in necessary nutrients, both macronutrients and micronutrients (Eckenfelder & Musterman, 1994). From investigation into the composition of FTRW, it has been found that it is one such industrial wastewater stream.

**Macronutrients** are the nutrients required by microorganisms in large quantities, they are vital for the following metabolic processes: synthesis of proteins, lipids, nucleic acids and carbohydrates. There are six macronutrients required by biological microorganisms, namely: carbon, oxygen, nitrogen, hydrogen, sulphur and phosphorus, where the primary nutrients are nitrogen, phosphorus and carbon (Burgess, et al., 1999).

**Vitamins** are organic compounds required by microorganisms as vital nutrients in limited amounts. Vitamins are essential in wastewater treatment to stimulate and contribute to the growth of the activated sludge, thereby influencing cellular metabolism (Lemmer & Nitschke, 1994). It has been found that metered addition of numerous vitamins to a number of activated sludge plants resulted in a significant increase in metabolic activity and this effect was more evident in sludge with high organic loads ( (Wood & Tchobanoglous, 1975) (Singleton , 1994)).

The role played by **micronutrients** is not specifically defined as compared to the role played by macronutrients and vitamins; however, micronutrients are necessary to support microbial genera essential for the anaerobic treatment process. Addition of micronutrients stimulates biological activity within the microorganisms to bring about degradation of complex organic compounds; therefore nutrient availability has a major effect on the efficiency of the degradation process in the treatment of effluent wastewater (Burgess, et al., 1999).

It is vital that the influent contains the necessary micronutrients that bring about activation of the cellular enzymes that aid in degrading the substrate, they are necessary for microbial growth and metabolism (Burgess, et al., 1999).

The crucial role of inorganic ions, more importantly trace metals has been confirmed by developing data on inorganic nutritional requirements for anaerobic treatment, these trace metals stimulate anaerobic microbial metabolism (Speece, 1983). Lack of knowledge of the significance of trace metals in anaerobic treatment has resulted in the failure of previous studies (Speece, 1983).

### **2.1.3 MICRONUTRIENT RECIPES**

The micronutrient recipes usually include the following trace metals: Mn, Zn, Co, Mo, Ni, Cu, Va, B, Fe, I, Se, Cr and W (Burgess, et al., 1999), (Fermoso, et al., 2009). Methanogenesis has been found to be unfavourably affected when the elements: Fe, Cu, Ni and Zn are limiting (Zhang, et al., 2003). The micronutrient dosing requirements required for anaerobic digestion differ between wastewaters; this is due to the fact that the inherent micronutrient load, biological diversity and required metabolic pathways are unique to the wastewater stream in question (Burgess, et al., 1999). Burgess et al (1999) reviewed the roles played by certain trace metals in microbial systems; a summary of the findings is presented in Table 1.

The methanogens which are responsible for converting acetate and hydrogen to methane are distinct and remain in a separate class as compared to the other microorganisms; they have distinctive nutritional requirements as compared to nitrogen and phosphorus that have been traditionally considered adequate (Speece, 1983). Iron, nickel, sulphide and cobalt are essential constituents for the efficient catalysis of methanogen metabolic pathways (Speece, 1983).

**Table 1: The role of trace elements in microbial systems <sup>b</sup> (Burgess, et al., 1999)**

<b>Micronutrient</b>	<b>Requiring organisms</b>	<b>Role</b>
Iron (Fe <sup>2+</sup> , Fe <sup>3+</sup> )	Aerobic bacteria	Growth factor
	<i>Aspergillus Niger</i>	Growth factor
	<i>Chlorella pyrenoidosa</i>	Adsorbed in quantities directly proportional to the concentration available.
Fe <sup>3+</sup>	Possibly all organisms	Electron transport in cytochromes. Synthesis of catalase, peroxidase, and aconitase.
	Iron reducing bacteria	Ion reduction for floc formation.
Zinc	Bacteria	Metallic enzyme activator. Dissociable on active site of enzymes.
		Activity of carbonic anhydrase and carboxypeptidase A.
		Stimulates cell growth.
		Toxic at low concentrations (1 mg/L), especially to protozoa. Can exacerbate toxic effects of other metals and inhibit metabolism.
Cobalt	Bacteria	Metallic enzyme activator. Dissociable on active sites of enzymes.
		Activates carboxypeptidase for synthesis of vitamin B12 (cyanocobalamin), but otherwise toxic.
		Can inhibit metabolism.
Magnesium	Heterotrophic bacteria	Enzyme activator for a number of kinases and phosphotransferase
Manganese	Bacteria	Activates isocitric dehydrogenase and malic enzymes. Often interchangeable with magnesium in kinase reactions.
	<i>Chlorella pyrenoidosa</i>	Lower affinity for binding sites than other metals but still can inhibit metabolism at 1 mg/L. Adsorbed in quantities directly proportional to the concentration available.
Copper	Bacteria	Enzyme activator required in very small quantities. Can inhibit metabolism.
		Chelates other substances and reduces their toxicity.
Nickel	<i>Cyanobacteria</i> and <i>Chlorella</i>	Stimulates certain enzymes. Methane production.
	Methanogenic anaerobes	Maintenance of biomass. May inhibit metabolism.
	Activated sludge cultures	
Calcium	Aerobic bacteria	Cell transport systems and osmotic balance. Bridging anionic ECP and aiding flocculation.
	<i>Thiothrix</i> and <i>Zoogloea</i>	Increase growth rates and improve flocculation.
	All bacteria	Requirements and effects vary. Interacts with other metals.

<sup>b</sup>: Refer to (Burgess, et al., 1999) for references used in construction of the table

## **2.1.4 MICRONUTRIENT DOSING**

### **2.1.4.1 METAL BIOAVAILABILITY**

“Bioavailability” is a term which has its origin in pharmacology/toxicology; and it was developed to relate to the systemic availability of a xenobiotic after arterial or oral dosing (Naidu, et al., 2008). The definition of bioavailability has since been used in many applications and therefore depends on the environmental discipline and the receptor organism. Bioavailability in general may be termed as the extent to which a chemical compound in a feasible source is free to be taken up or for movement into an organism (Newman & Jagoe, 1992). In terms of biodegradation, bioavailability is defined as the degree to which a contaminant avails itself for biological conversion; this is known to be a function of the contaminant physicochemical properties, biological system and environmental factors ((Juhasz et al., 2000) cited in (Naidu, et al., 2008)).

Bioavailability is defined as the contaminant accessibility to microorganisms. This is with respect to the metabolism of the microorganisms, their growth ability within the contaminant chemicals, the contaminant’s ability to change the microorganism’s physiology and variation of genetic response (Sayler et al., 1998 cited in (Naidu, et al., 2008)).

In the context of this study, the contaminant is a trace metal and the metal is said to be bioavailable if it brings about biological response when available at the bio-interface ((Shargel and Yu, 1999) cited in (Fermoso, et al., 2010)). From a geochemical standpoint, the forms of metals that are considered bioavailable are those that are connected by dynamic equilibria ((Kramer et al., 1997 cited in (Meyer, 2002)). On supplementation of metals to activated sludge in the anaerobic treatment process, they are to be found in the soluble phase to allow for their bioavailability (Burgess, et al., 1999). From research conducted, it has been found that the free metal ion has been proposed to be a part of the bioavailable fraction (Meyer, 2002).

There are a number of variables affecting the bioavailability of metals in anaerobic sludge such as pH and biological responses (Burgess, et al., 1999). The total metal concentration, redox potential together with precipitation, complexation and adsorption kinetics affect metal bioavailability (Aquino & Stuckey, 2007). Zandvoort and co-workers (2006) concluded that the total metal content (concentration) does not give a sufficient representation of the bioavailability and mobility of metals within activated sludge. Fermoso and co-workers (2009) concluded that the bioavailability and mobility of metals in UASB reactors was mainly controlled by the sulphide chemistry (Fermoso, et al., 2009), due to the formation and ageing of sulphide precipitates resulting in metals being bound in the solid phase and therefore non-bioavailable (Filgueiras, et al., 2002), (Zandvoort, et al., 2006)

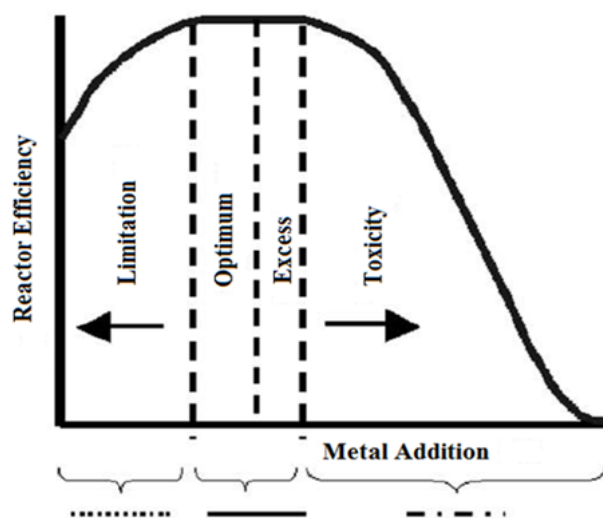


One of the approaches used to determine the bioavailability of metals within a system is the **analytical approach**; this method makes use of experimental work together with analytical determination of metal concentrations. A well-known experimental technique employed is sequential extraction (Filgueiras, et al., 2002). In sequential extraction, metal ions are extracted from the different phases they exist in, by a solvent. After extraction, the total metals concentration and the concentration of the metals in the soluble phase is analytically determined. Analytical methods do not account for metals bound in other phases and are therefore inadequate for use alone (Filgueiras, et al., 2002).

Current research points to the use of chemical speciation to indicate metal bioavailability; this would be the other approach which makes use of **chemical speciation modelling**. Chemical speciation modelling is a technique that predicts the concentration of all species in which a metal ion exists (VanBriesen, et al., 2010).

#### 2.1.4.2 METAL TOXICITY

On supplementation of metals to sludge, there exists a limiting total metal ion concentration where if exceeded can result in adverse effects on the biological process. Referring to Figure 2 below, the relationship between metal addition and reactor efficiency is depicted. From the trend, it can be seen that the reactor efficiency first increases with initial metal addition until an optimum metal concentration is reached where the reactor efficiency remains constant. Further metal addition results in an excess of metals and a constant reactor efficiency. Continuous addition of metals thereafter results in them causing a toxic environment for the microorganisms; it is shown by the drastic decrease in the reactor efficiency. The boundary conditions for metal addition to activated sludge lie between nutrient deficiency and excess that correspond to stable reactor operation and toxicity respectively (Fermoso, et al., 2009).



**Figure 2: Boundary conditions for metal addition to keep UASB reactor efficiency optimal (Fermoso, et al., 2009)**

Toxicity is brought about by an overdose of trace metals resulting in an excess of micronutrients present in the wastewater being treated which can be initially avoided by pre-calculation of dosing thresholds to allow for dosing of sufficient quantities (Burgess, et al., 1999). There are reported values for toxicity thresholds for metals in various literature sources, shown below in Table 2, is a summary of the toxic conditions of Co, Ni and Zn in various methanogenesis processes.

**Table 2: Toxic conditions of cobalt, nickel and zinc in methanogenesis processes (Fermoso, et al., 2009)**

<b>Metal</b>	<b>Organism</b>	<b>Toxic Concentration</b>	<b>Conditions</b>
<b>Co</b>	Mixed anaerobic sludge	35-400 mg/L : no detectable inhibition	The sludge was fed with nutrients and acetate or glucose as the sole carbon source under oxygen-free conditions at 35 ± 1°C.
		600-800 mg/L : 7-17% inhibition	
		950 mg/L: 100% inhibition	
<b>Ni</b>	Anaerobic granular sludge	81 mg/L : 50% inhibition of VFA degradation	The digester was acclimated in a 13.5 litre UASB reactor at 35 ± 1°C.
		440 mg/L : 50% inhibition of VFA degradation	
	Anaerobic granular sludge from lab-scale UASB reactor	118 mg/L : IC <sub>50</sub>	Granules were sample from four UASB reactors at COD loading rate of 10 g COD L <sup>-1</sup> d <sup>-1</sup> for over six months at 37 °C.
<b>Zn</b>	Anaerobic granular sludge from lab-scale UASB reactor	690 mg/L : 50 % inhibition of methanogenic activity with sludge operated at HRT 1 day	The experiment was performed at 35±1 °C Winery wastewater as substrate
		270 mg/L : 50 % inhibition of methanogenic activity with sludge operated at HRT 2 days	
	Anaerobic granular sludge from lab-scale UASB reactor	96 mg/L : 50 % inhibition of methanogenic activity	The experiment was performed at 37±1 °C. Starch synthetic wastewater as substrate.

Toxic effects in an anaerobic wastewater treatment are identified by decreased or hindered COD degradation (Burgess, et al., 1999); therefore there is an accumulation of volatile fatty acids. Accumulation of these fatty acids brings about a decrease in the pH, and a decreasing rate of methane production (Aquino & Stuckey, 2007).

Factors affecting toxicity have been found to be the influent pH and strength, the metals involved (the species, their concentration and the sequence in which they are added) together with the type and concentration of the microbial population. The extent of acclimation in the system also has an effect on toxicity (Burgess, et al., 1999). Work completed by Kuo et al (1996) suggested that microorganisms secrete soluble microbial products (SMPs) that bound excess metals by forming complexes with Ni, Zn and Cu which decreased metal toxicity brought about by these metals (cited in (Fermoso, et al., 2009)).

Research carried out by Niyogi and Wood (2004) suggested that the metal toxicity could be linked to the toxicity of free ions; furthermore, complexation brings about a decrease in metal toxicity suggesting that metal toxicity has a direct relationship with free metal ion concentration (Niyogi & Wood, 2004). The Free Ion Activity Models (FIAM) (Morel, 1983) and Gill Surface Interaction Model (GSIM) (Pagenkopf, 1983) were formulated to quantify metal toxicity. Evaluation of metal toxicity does not solely depend on the determination of the metal precipitated or sorbed in granular sludge, speciation of metals in the system as a whole is necessary to account for metal toxicity (Fermoso, et al., 2009).

#### **2.1.4.3 DOSING STRATEGY**

Micronutrient dosing defines supplementation of trace metals to the anaerobic digester sludge; it should be such that the maximum biological activity of the biomass in the anaerobic reactor is supplied, together with minimization of dosing costs and releasing of metals to the environment (Fermoso, et al., 2009). Efficient dosing of micronutrients should find a balance between the concentration corresponding to the strength of the wastewater in terms of its COD and a concentration sufficient to bring about effective execution of the metabolic pathways (Wood & Tchobanoglous, 1975).

In a study completed by Zhang and co-workers (2003), acclimated methanogens were examined making use of inductively coupled plasma mass spectrometer together with scanning probe microscopy. Under anaerobic conditions, a basal medium for the methanogens was used; its composition can be found in Table 9, Appendix A. Maekwa (one of the co-workers), found that the formation of methane increased by approximately 3.7 times when optimum trace metal concentrations were added to the medium as compared to the conventional method which makes use of the basal medium. (Zhang, et al., 2003).

The strategy used to add metals to the activated sludge impacts on the cost of metal dosing and metal losses, therefore necessitating the supplementation of an optimum metal concentration in the reactor sludge (Fermoso, et al., 2009). Having a clear understanding of the consequence of metals on the biomass and metal distribution in the reactor contents is paramount in obtaining a sound strategy for metal dosing.

An efficient dosing strategy includes determination of the following (Zandvoort, et al., 2006):

- The **metals** essential to the anaerobic treatment of the specific wastewater stream together with the **concentrations** preventing metal toxicity. Concentrations less than 1mg/L are frequently used (Burgess, et al., 1999).
- The **method** by which the metals are to be supplemented to the bioreactor, including: continuous addition, repeated pulse addition and pre-loading of metals (Fermoso, et al., 2009).

- The **timing** for metal dosing together with the duration of stable operation possible until metal supplementation is necessary. Feroso (2008a) proved that cobalt supplementation should be carried out prior to accumulation of volatile fatty acids in the effluent such that reactor acidification is prevented.
- Whether the **bioavailability** of the supplemented metals can be increased or not (Zandvoort, et al., 2006).
- The possibility of the sludge being able to self-cater for the metal requirement from the metals stored within the anaerobic biofilms or granules (Zandvoort, et al., 2006).

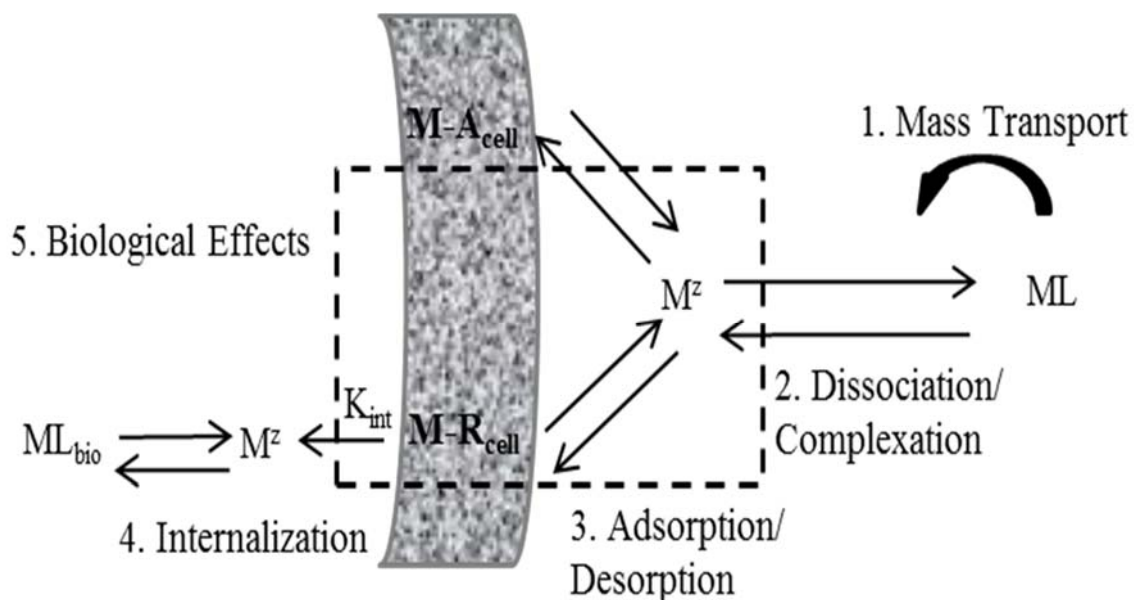
### **2.1.5 METAL UPTAKE BY MICROORGANISMS**

Once the trace metals have been dosed, the key intention is for the trace metals to be taken up by the microorganisms by transportation through the cell membrane as depicted by Figure 3. However, prior to contact between the metals and the biomass existing in a biofilm or sludge granule, there are complex biochemical processes in the reactor liquid phase to which the metals are exposed; such processes include precipitation and the formation of both organic and inorganic complexes (Zandvoort, et al., 2006). Accordingly, these biochemical processes bring about a drastic reduction in the concentration of free metals in solution.

Metals remaining in solution that are available for uptake are first bound by a transporter site and are consequently taken up. The affinity of the transporter to the metal is governed by the binding properties and the maximum rate at which metals are taken up is determined by the amount of transporter present in the sludge (Feroso, et al., 2009).

The mechanisms which result in accumulation of metals within the biofilm are the formation of complexes, metal chelation, exchange of ions, adsorption, inorganic micro-precipitation and translocation of the trace metals into the microorganism cell (Zandvoort, et al., 2006).

Different metals may be taken up by the same active site, in the case where a metal exists in excess; it negatively affects the metal-transporter interaction between other metals and the transporters. The competitive effects inhibit metal uptake, therefore decreasing metal bioavailability (Feroso, et al., 2009).



**Figure 3: Conceptual Framework of the Free Ion Activity Model and Biotic Ligand Model.  $M^z$ : free metal ion, L: ligand, ML: hydrophilic complex,  $ML_{bio}$ : metal-intracellular ligand complex,  $K_{int}$ : internalisation rate constant,  $M-A_{cell}$ : non-specific metal-surface adsorption complex,  $M-R_{cell}$ : specific metal-surface adsorption complex (Fermoso, et al., 2009)**

### 2.1.6 SPECIATION

Chemical speciation is referred to as the distribution of an element between the chemical species it may exist in, in a system. Understanding the chemical speciation of an aqueous system is important to be able to develop an understanding of the chemical toxicity, bioavailability and environmental fate and transport of metals in the various species and forms in which they exist in sludge (VanBriesen, et al., 2010).

Carrying out of direct analyses alone to obtain chemical speciation is inadequate; some methods used rely on the detection of free metal ion or total metal concentrations. Due to very low environmental concentration of metals of interest and difficulties in directly measuring different metal species, their direct measurement is further inadequate for complete chemical speciation. As a result, analytical methods are used in combination with chemical speciation models to obtain full speciation in systems (VanBriesen, et al., 2010).

## **2.2 ANALYTICAL APPROACH**

### **2.2.1 SEQUENTIAL EXTRACTION PROCEDURE**

Sequential extraction, as mentioned before, forms part of the experimental techniques used to determine metal bioavailability in an anaerobic digestion system in the analytical approach mentioned under the “metal bioavailability” section. Sequential extraction is an experimental method used to partition trace metals into the different fractions in which they exist according to their chemical nature (Divvela, 2010), after being dosed in an anaerobic digester. Sequential “selective” extraction aims at imitating the release of the selective metals into solution under numerous environments (Divvela, 2010). Sequential extraction schemes are constructed on the following principle: sorbed heavy metals can be displaced from the site onto which they are fixed by making use of suitable extracting reagents (van Hullebusch, et al., 2005b).

The sequential extraction procedure is generally comprised of four steps where in each step, different concentrations and compositions of buffers and chemicals are calculated and added to a sample to extract metal ions into the solid phase. Samples frequently used are sludge and soil (van Hullebusch, et al., 2005b). The sample to which the chemical was added, is shaken thoroughly, the leachate is thereafter digested and analysed by making use of a spectrometer. In each step, the metals concentration found in a fraction is obtained where the fractions are classified as the following: exchangeable, reducible, oxidisable and residual. The procedure ensures that all metals in the sample are extracted and therefore accounted for. A variety in metal recovered in each fraction is obtained by changing the pH, temperature and duration of each extraction step (Divvela, 2010). Various buffers are used to extract the metals within a certain fraction, depending on the purpose and limitations of the study.

Selectivity (specificity) of reagents may be explained as the ability of the reagent to only extract metals in the fractions it was intended to extract; non-selectivity (non-specificity) would therefore explain the case where metals are displaced in the targeted fraction together with metals in the other fractions. Some uncertainty in the use of sequential extraction is brought about by the lack of selectivity of the reagent chemicals used. This lack of selectivity brings about difficulty in the isolation of an extraction fraction and in finding the influence of pH, temperature, leachate time, reagent concentration, stirring system, solid particle size and the ratio of solids to extractants volume (van Hullebusch, et al., 2005b).

Redistribution of trace metals among the extraction fractions during the extraction process also poses a drawback in the sequential extraction procedure (van Hullebusch, et al., 2005a). Redistribution of metals is due to the interactions between the extractant and the solubilised metals and/or metals existing in the other fractions.

Filgueiras and co-workers (2002) studied the redistribution of Pb using synthetic model soils, by making use of two popular sequential extraction schemes, namely: the Tessier and BCR schemes. The redistribution of Pb was found to be significant, and was mostly due to the manganese oxide and humic acid extractants. In the duration of the sequential extraction experiment, the chemical binding form of Pb underwent alteration, this resulted in the re-adsorption of solubilised Pb ions onto soil matrix components; furthermore, insoluble Pb species precipitated with the extractant anion. In a sequential extraction procedure making use of MgCl<sub>2</sub> and EDTA as extractants, the following redistribution of metals was witnessed: re-adsorption of Pb during the MgCl<sub>2</sub> step, removal of adsorbed Pb and the dissolution of Pb carbonates during the EDTA step (Filgueiras, et al., 2002).

### 2.2.2 CHEMICAL SEQUENTIAL EXTRACTION FOR METAL PARTITIONING IN ENVIRONMENTAL SOLID SAMPLES (Filgueiras, et al., 2002)

Filgueiras and co-workers (2002) conducted a comprehensive review of different sequential extraction schemes in the following environmental samples: sediment, soil, sewage sludge, fly ash and many more. Of the extraction schemes reviewed, it was concluded that the Tessier and BCR (the Commission of the European communities Bureau of Reference) schemes showed the greatest degree of reproducibility.

In their study the fractions in which trace metals were fractionated were defined. A summary of the fractions and their operational definitions<sup>3</sup> proposed by Filgueiras is shown in Table 3.

**Table 3: Definition of Sequential extraction fractions as suggested by Filgueiras (2002)**

<b>Fraction</b>	<b>Description</b>
Water Soluble <sup>4</sup> (F <sub>1</sub> )	The water soluble phase comprises of trace elements that are water extractable and are the first to appear. The phase constituents have been found to be free ions and ions complexed with soluble organic matter. This is the most mobile phase and is frequently determined together with the exchangeable fraction.
Exchangeable (F <sub>2</sub> )	The exchangeable phase comprises of metals relatively weakly adsorbed on the solid surfaces by electrostatic interaction, this phase is considered the one which is the most readily released. Metals in this phase are released by co-precipitation with carbonates and by ion-exchange. A decrease in the pH and a change in ionic composition bring about remobilisation of the metals.

<sup>3</sup> An **operational definition** states something (variable, object, or term) in terms of the specific process or set of validation tests used to determine its existence and quantity. One would then define the variable or term in terms of the operations that count as quantifying it

<sup>4</sup> The fractions F<sub>i</sub> ( i =1 to 6) allows for grouping of fractions in studies to be reviewed to allow for comparison among studies as not all necessarily have a total of 6 sequential extraction fractions

Acid Soluble (F <sub>3</sub> )	This acid soluble phase includes metals which have been precipitated or co-precipitated with carbonate. The metals in this phase are mobilised by decreasing the pH by making use of an acid, they are therefore released into solution by making use of an acid.
Reducible (F <sub>4</sub> )	The reducible phase comprises of metals bound in hydrous oxides of manganese and iron by any of the following mechanisms: co-precipitation, adsorption, surface complexation, ion exchange and penetration of the lattice. Most metals have been found to occur in this phase. This phase is referred to as the reducible fraction because a reduction of Fe (III) and Mn (IV) brings about release of the trace metals bound in the hydrous oxides.
Oxidisable (F <sub>5</sub> )	This phase comprises of trace metals connected with various forms of organic matter (such as living organisms) through processes of complexation and bioaccumulation. The phase is referred to as the oxidisable phase as release of trace metals is done by oxidising of organic matter. There is possibility of metals bound by sulphides existing in this fraction.
Residual (F <sub>6</sub> )	This is the phase comprising of trace metals that are associated with silicates. In the case where the sludge has a low mineral content and low sulphur content, sulphides may be found in the residual fraction (Feuntes, et al., 2004).

These are the most general definitions of the fractions for sequential extraction schemes and further case studies to be reviewed have defined the fractions according to the descriptions given in Table 2, differences may be brought about by extractants being used to extract fractions together such as the water soluble and exchangeable fraction.

### **2.2.3 HEAVY METAL EXTRACTABLE FORMS IN SLUDGE FROM WASTEWATER TREATMENT PLANTS (Alvarez, et al., 2002)**

According to Álvarez and co-workers (2002), the main method used in Europe to dispose of used sludge is by spreading it over agricultural land allowing the recycling of the nutrients it contains. The potential environmental and health risk allied with the sludge arising from high nutrient (metal) concentrations is studied by making use of sequential extraction to establish the mobility and bioavailability of metals within the sludge. Alvarez and co-workers (2002) studied the partitioning of metals by making use of the BCR scheme as shown in Table 4. The sludge was sampled at each treatment step from a total of five municipal activated sludge plants. Thereby representing the sludge conditions of a steady state process with different residence times.

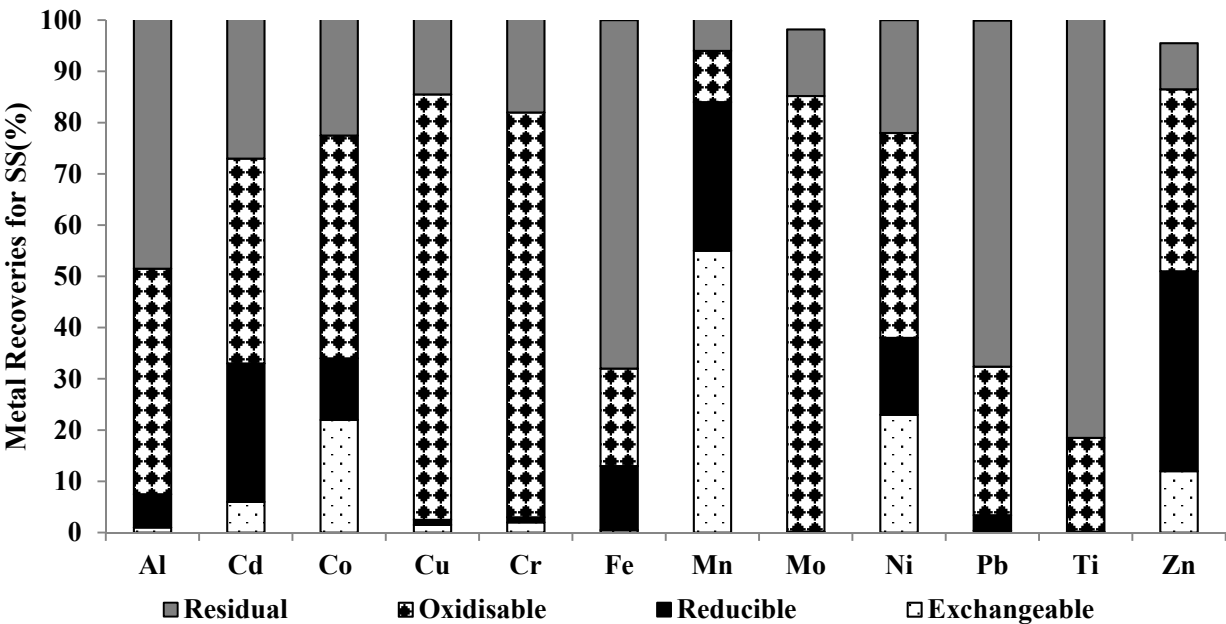


The scheme made use of four sequential extraction fractions, namely; the exchangeable, reducible, oxidisable and residual fractions. The fractions are explained in Table 3 and are shown according to the definitions of fractions in the study conducted by Filgueiras (2002).

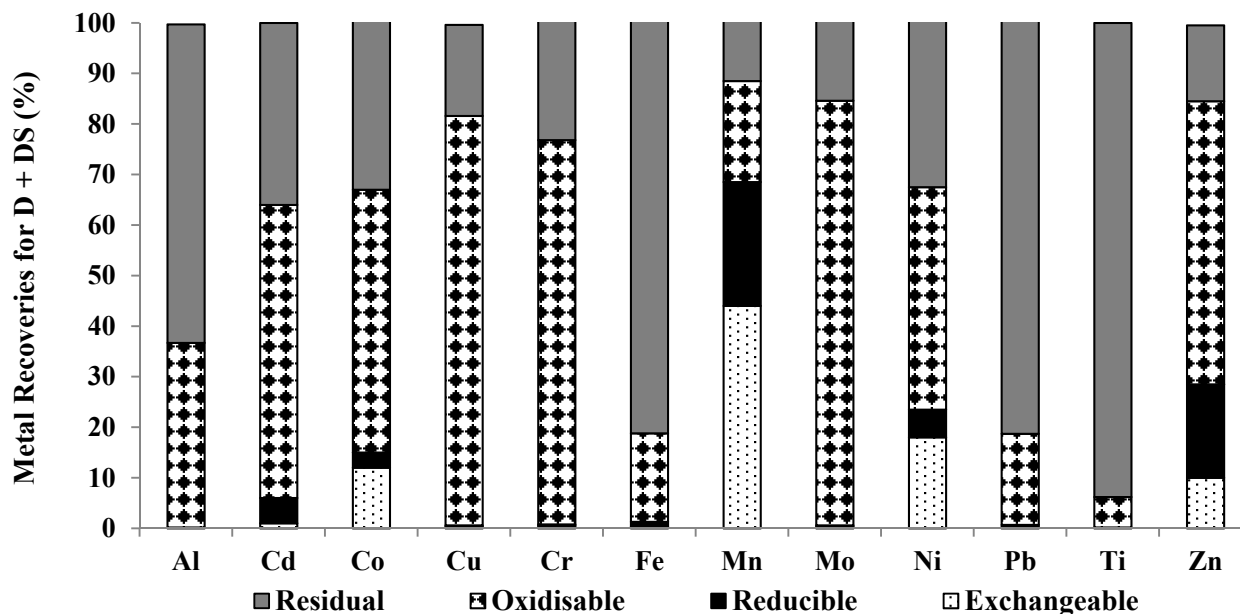
**Table 4: Fractions for the BCR scheme with connection with to Filgueiras (2002)**

Step	Fraction	With respect to Filgueiras (2002)
Step 1	<b>Exchangeable</b> fraction and fractions associated with carbonated phases	F <sub>1</sub> , F <sub>2</sub> , F <sub>3</sub>
Step 2	<b>Reducible</b> fraction or fraction associated with Fe and Mn oxides	F <sub>4</sub>
Step 3	<b>Oxidisable</b> fraction or bound to organic matter	F <sub>5</sub>
Step 4	<b>Residual</b> fraction	F <sub>6</sub>

The sludge samples taken were primary sludge (PS), secondary sludge (SS), dewatered and digested sludge (D + DS) and compost (C). The analysis of the sludges showed that SS has the highest organic load (higher concentration), and consists of biomass, which results in the microorganisms playing an integral role in the SS as a result of the activated sludge process. The SS has the largest organic mass with a pH ranging from 5.4 to 6.6, showing similar properties to the anaerobic sludge generated when treating the FTRW. The D + DS sludge is the sludge that would have undergone aerobic and/or anaerobic digestion, due to the characteristics of interest in the SS and D + DS, the results shown will be limited to the SS and D + DS. The results for the metal distribution among the four fractions for the SS and D + DS are shown in Figure 4 and Figure 5 respectively.



**Figure 4: Metal partitioning of Secondary Sludge in BCR Scheme (Alvarez, et al., 2002). Data plotted from Table 2 and Table 3 of Alvarez (2002)**



**Figure 5: Metal partitioning of Dewatered and Digested Sludge in BCR (Alvarez, et al., 2002). Data plotted from Table 2 and Table 3 of Alvarez (2002).**

From Figure 4 and 5, an increasing trend from the SS to the D + DS of metals in the oxidisable and residual fractions can be seen, depicting that as the treatment proceeds, more metals partition themselves in these fractions. The authors found that the metals found in highest quantities in the oxidisable fraction were Al, Cu, Cr and Mo. The metals found in the more potentially bioavailable fractions (exchangeable and reducible) were Co, Mn, Ni and Zn. Metals that were found to be more in the residual fraction, with the least bioavailability were Fe, Pb and Ti, with increased concentrations in the residual fraction after anaerobic digestion (in the D + DS fraction).

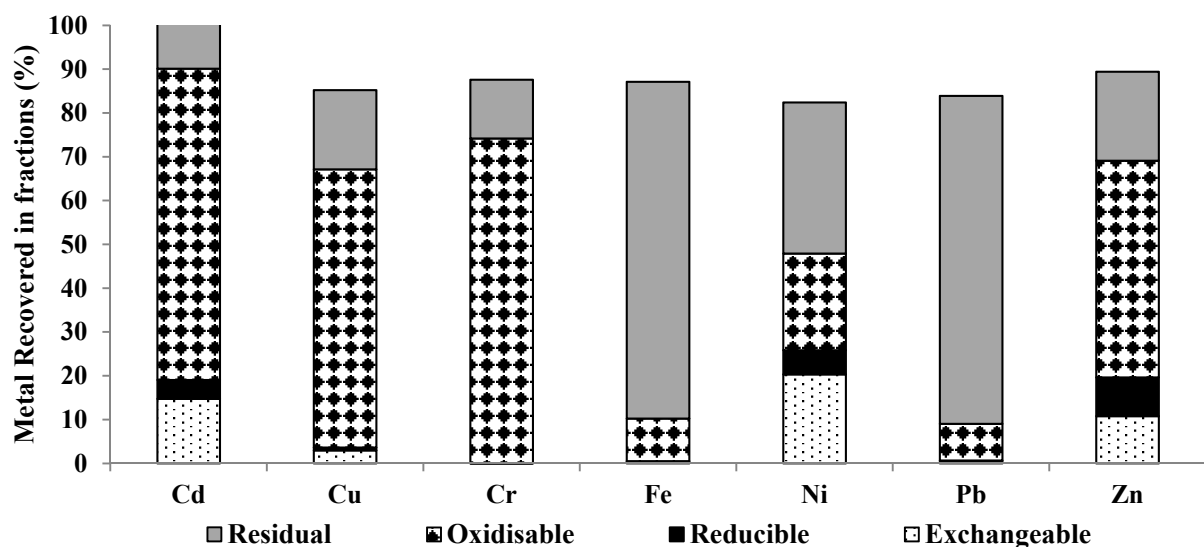
Álvarez and co-workers (2002) suggested that due to the higher water content (higher concentration) in SS than the PS, metals were found to exist in solution, in equilibrium with solids and as metals easily extracted by the extractants used, resulting in the SS having higher metals partitioned in the exchangeable and reducible fractions as compared to the other sludges. Furthermore, the presence of easily mobilisable metals was favoured, which are loosely bound to the sludge (organic matter), this is due to biological assimilation by the biomass that participates in the activated sludge process ((Fletcher and Beckett, 1987; Lake et al., 1989); cited in (Alvarez, et al., 2002)).

It was found that of all trace metals in the oxidisable fraction, Cd, Mo and Ti were found to be strongly bound by the organic and mineral matter. It was further concluded that high levels of Fe and Al were noted in the oxidisable and residual fractions and could pose negative effects on the digestion process by inhibition of bacterial activity. Due to the high levels of Zn and Mn in the most bioavailable forms (exchangeable and reducible), it was concluded that they were the most bioavailable and mobile of the metals studied.

## 2.2.4 SIMPLE AND SEQUENTIAL EXTRACTION OF HEAVY METALS FROM DIFFERENT SEWAGE SLUDGES (Feuntes, et al., 2004)

Sludge produced in waste-water treatment plants contains heavy metals; this restricts their agricultural use due to high metal concentrations that may pose environmental and health risks to the soil and plants. Feuntes and co-workers (2004) studied the distribution of heavy metals in different types of sludges to compare the bioavailability and localisation in these sewage sludges, namely: aerobic, anaerobic, unstabilised and sludge from a waste stabilisation pond.

Sequential extraction experiments were conducted, making use of simple extraction (water and DTPA) and the BCR scheme. The BCR extraction scheme comprised of four steps as shown in Table 3, and the results were limited by the simple BCR scheme as shown in Figure 6.



**Figure 6: Percent recoveries of total metals in the sequential extraction fractions for anaerobic sludge using the BCR scheme (Feuntes, et al., 2004). Data plotted from Figure 1 and Table 3 of Feuntes (2004).**

Figure 6 above depicts that most metals are found in the residual (step 4) and oxidisable (step 3) fractions and it can be further seen that the oxidisable fraction is common to all elements which is a result of the formation of stable complexes with the organic content in the sludge. Cu was found to have a high association with strong organic ligands and obstruction in minerals (quartz, feldspars, etc.) and had an accumulative recovery percentage in steps 3 and 4 of 95% (Feuntes, et al., 2004). The metals considered most bioavailable, which had the greatest mobility, in this scheme were found to be Cd, Ni and Zn that were extracted in the exchangeable fraction.

## 2.2.5 COMPARISON OF THREE SEQUENTIAL EXTRACTION PROCEDURES TO DESCRIBE METAL FRACTIONATION IN ANAEROBIC GRANULAR SLUDGES (van Hullebusch, et al., 2005a)

Various extraction schemes have been investigated to quantify the distribution of metals in sludges and soils to find the chemical nature of the metals with regard to the solid phase. van Hullebusch and co-workers (2005a) reviewed and compared three well known sequential extraction schemes, namely: modified Tessier, Stover and the revised BCR. These schemes were applied in the investigation of the distribution of the trace metals: Co, Ni, Zn and Cu together with major elements: Mn and Fe, within two dissimilar granular sludges obtained from full scale methanogenic wastewater treatment plants.

**Table 5: Repeatability (%) of the Tessier, BCR and Stover schemes (van Hullebusch, et al., 2005a)**

	Nedanco sludge						Eerbeek sludge					
	Co	Ni	Cu	Zn	Mn	Fe	Co	Ni	Cu	Zn	Mn	Fe
<b>Tessier</b>	142	108	91	165	114	104	102	109	105	107	87	118
<b>BCR</b>	100	94	93	90	95	97	98	103	97	111	101	119
<b>Stover</b>	107	92	91	92	103	40	85	103	110	108	68	67

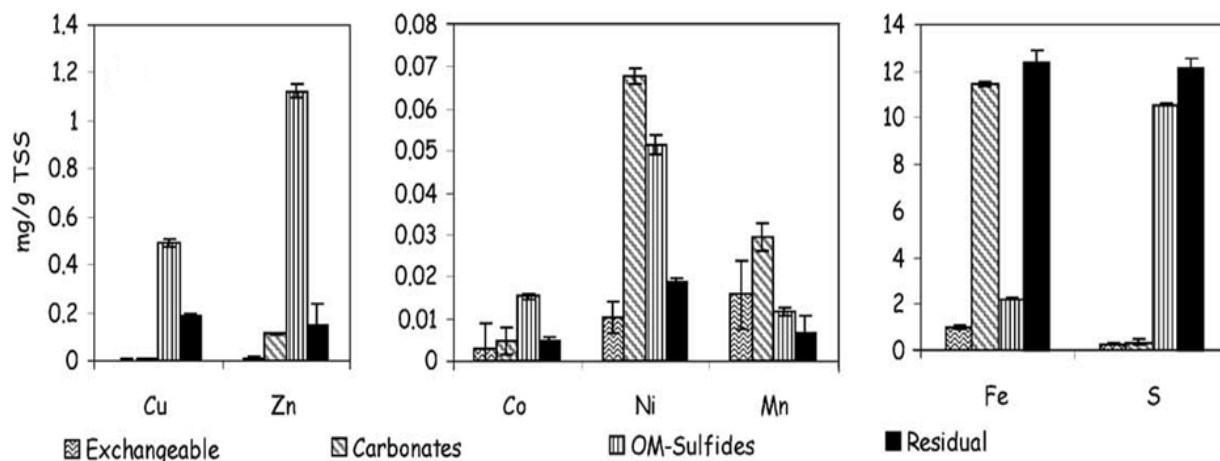
High repeatability was obtained for the Tessier and BCR schemes, the Stover scheme had the least repeatability of the three schemes, and this was due to the scheme having six fractions (as defined in the work done by Filgueiras (2002)) unlike most schemes that limit the number of fractions to four. A large number of extracted fractions lead to loss of material during centrifugation and decantation due to numerous weight, volume and analytical determinations.

The modified Tessier and Stover schemes were designed to cater for anaerobic matrices with high organic content which have relatively low iron and manganese oxides and hence do not have the reducible fraction. In the Tessier scheme, there were four extraction steps, hence four fractions extracted and are as listed in Table 5 together with the fractions according to those defined in Filgueiras (2002).

**Table 6: Sequential extraction fractions for the Tessier scheme with definitions as per Filgueiras (2002).**

Fraction	With respect to Filgueiras (2002)
Exchangeable	F <sub>1</sub> , F <sub>2</sub>
Carbonates	F <sub>3</sub>
OM (Organic Matter) -Sulfides	F <sub>5</sub>
Residual	F <sub>6</sub>

The results to be shown are limited to the Nedalco sludge as it has a higher organic matter concentration, analogous to anaerobic sludge to treat industrial wastewater with a high organic load. Furthermore, the pH is 7.2 ( $\pm$  0.1) which agrees with the resultant sludge pH for FTRW treatment. Additionally, results obtained when using the Nedalco sludge showed a greater extent of repeatability. Results obtained for the Nedalco sludge, showing the partitioning of the metals in the various fractions where the modified Tessier scheme was used, are shown in Figure 7.



**Figure 7: Metal partitioning profiles in Nedalco sludge according to the modified Tessier Sequential extraction scheme (van Hullebusch, et al., 2005a)**

As confirmed in the previous study by Alvarez (2002) together with Feutes (2003), all the metals to some degree are partitioned in the oxidisable fraction, in this case, the OM-sulfides; where the metals are bound to organic matter (OM) by bioaccumulation and complexation, and may be bound as sulfides.

Cu, due its chemistry, was found to be predominantly bound as sulfides; these sulfides do not dissolve from the solid phases in the anaerobic digester sludge. A total of 53% of Ni was extracted in the exchangeable and carbonate fractions, the labile fractions, suggesting a high bioavailability among the trace metals extracted. 42% of iron was extracted in the carbonates fraction, due to precipitation with the carbonates present in the sludge to form  $\text{FeCO}_3$ .

Across the three sequential extraction schemes studied, agreement is seen in the behaviour of Co, Zn and Cu where these metals are mostly extracted in the OM-Sulfides and residual fractions, a total ranging between 75 and 99 % of these metals were extracted in these two fractions. The high recovery of these metals in these two fractions exemplifies the controlling effect of the sulphide phase on speciation in anaerobic sludges as can be confirmed by Lake (1987) (cited in (van Hullebusch, et al., 2005a)).

A trend in the metal extractability in the oxidisable (OM-Sulfides) fraction, where the calculated average percentage of the quantity of oxidisable metal of the Nedalco sludge was investigated, the percentages ascend in the order: Mn < Fe < Ni < Co < Zn < Cu. The trend parallels the order of sequence of complex stabilities studied by Irving-Williams ((W Stumm, et al., 1996) cited in (van Hullebusch, et al., 2005a)) together with the stability of the sulfidic metal compounds.

With agreement to the studies conducted by Alvarez (2002) and Feuntes (2004), Ni, Zn and Mn show the greatest mobility and bioavailability among the fractions extracted.

#### **2.2.6 COBALT AND NICKEL BIOAVAILABILITY FOR BIOGAS FORMATION (Gustavsson , 2012)**

Cobalt and Nickel are listed as part of the trace elements essential to the performance of methanogens; the effect is usually indicated by an increase in methane production during the anaerobic treatment process. Other advantageous effects observed are an increase in process stability, more efficient use of the substrate and possible increase of the organic loading rate (OLR) of the treatment process.

Gustavsson's work (2012) included the investigation of the effects of addition of Fe, Co, Ni, Se and W on the rate of biogas (methane) production in stillage with high sulfur content. Furthermore, the bioavailability of Co and Ni by making use of speciation was investigated by sequential extraction following the well-known Tessier scheme and other techniques, namely: acid volatile sulfide (AVS) extraction and semi-continuously extracted metals (AVS-Me).

The work carried out showed that the bioavailability of Co and Ni was greatly affected by the presence of sulfides. In the case where the total sulfide level, in the form of H<sub>2</sub>S, HS<sup>-</sup> and S<sup>2-</sup>, is greater than the total concentration of Co, Ni, Cu, Fe and Zn, the metals are most likely to be precipitated in sulfide form. The effect of the sulfide chemistry is not completely avoidable in the case where the substrate is biomass-derived due to the mineralization of this organic matter.

In the case where sulfate is part of the feed or is part of the micronutrient recipe, its reduction brings about existence of sulphide ions in the system, further contributing to trace metal precipitation as sulfides. The chief source for the S species in the study for the solid phase of the sludge was found to be FeS. Another factor found to influence bioavailability was the strong complexation of metals with soluble microbial products (SMPs).

In the study conducted, Co was partitioned in the organic matter/sulfides and in the acid volatile sulfides (AVSs) with a greater amount occurring in the liquid phase as compared to the Ni occurring in the liquid phase. The high content of Co in the organic matter and sulfides was due to the complexation of Co with organic ligands or sulfides and SMPs which resulted in metals being strongly bound in the organic matter fraction. Co had a higher content in the liquid phase

due to its association with the B<sub>12</sub> vitamin (Co-corrinoids) which is a soluble organic metal complex, contributing to the conclusion made that Co is more bioavailable than Ni.

Ni, like the Co, was found to be in great association with the organic matter/sulfides fraction and AVS. An increase in biogas production as a result of Ni was observed regardless of it being bound in other phases, this suggests that Ni-sulfides potentially act as a source and storage for Ni, this factor was also proposed by Jansen et al (2007). It was found that the age and size of the Ni-sulfide precipitates further influence the bioavailability of Ni.

## **2.2.7 CONCLUSIONS ON THE ANALYTICAL APPROACH**

The interactions between the solid and liquid phases in anaerobic reactors have a great effect on the mobility and bioavailability of metals. Investigation into the mobility and bioavailability is made possible by the use of speciation, by carrying out sequential extraction experiments. Sequential extraction takes into account that knowledge of the total metal concentrations is inadequate for metal speciation. The case studies reviewed were found to successfully identify the distribution of metals (trace and main) in soils and sludge.

Sequential extraction makes use of experimental methods to find the distribution of metals in the various forms in which they exist in sludge and it also provides data to be used in conjunction with speciation modeling. From the comparison made between the three well-known sequential schemes: Stover, Tessier and BCR, the Tessier scheme showed to have the greatest reliability and reproducibility and relevance for anaerobic digestion ( (van Hullebusch, et al., 2005a); (Filgueiras, et al., 2002)).

From the case studies reviewed, it was evident that the metal mobility and bioavailability was strongly influenced by the chemical form in which the metal exists in the system. These chemical forms included metals that are fractionated as water-soluble, exchangeable, reducible, oxidisable, acid soluble and residual. The bioavailability is affected, as once a metal is bound in the reducible, oxidisable or residual fractions; it is not in a form accessible by the microorganisms in activated sludge, hence decreasing its bioavailability.

The distinction between the bioavailable and non-bioavailable fractions is therefore made by whether metals bound in the fraction of question are accessible by the micro-organisms, given the prevailing sludge conditions (temperature, pH, redox potential) in an anaerobic digester. The fractions considered bioavailable will henceforth be assumed to be the water-soluble and exchangeable fractions, due to the conditions bringing about liberation of metals in the following fractions being those anticipated in the anaerobic digester to treat FTRW.

In all case studies reviewed, it was evident that high recoveries of metals in the oxidisable fraction exist. This fraction includes metals bound by organic matter through complexation and bioaccumulation processes with a possibility of sulfides existing in this fraction. This suggested that the oxidisable fraction plays a crucial role in metal speciation in sludges and is responsible for the bulk of the metals being non-bioavailable. Careful attention was therefore paid to this fraction in speciation modeling attempts made to result in more efficient use of micronutrients.

In general, across the case studies, the most bioavailable metals were found to be Mn, Ni, Zn, Co and Cd. The study comparing Ni and Co found Co to be the most bioavailable; this was due to the fact that these metals were mainly found in the water-soluble and exchangeable fractions. The metals falling in the range of being non-bioavailable, were mostly in the oxidisable fraction, and were found to be Cu, Cr and Mo. The least bioavailable metals, where they mainly existed in the residual fraction, were Fe (mainly due to FeS formation) and Pb.



## 2.3 MODELS

### 2.3.1 INTERACTION BETWEEN METALS AND ANAEROBIC SLUDGE

Bioaccumulation is a process involving the absorption of metals, where they are transferred **onto and within** the cellular surface. This process is metabolically driven, an active process that mainly occurs within living cells (Zouboulis, et al., 1997). On the contrary, bio-sorption can be defined as the removal of metals, or metalloid species, particulates and compounds from solution by biological material (Artola, et al., 1997), these metals are adsorbed **onto** the cellular surface.

The term bio-sorption is used as a collective term for passive accumulation processes occurring in the cell walls of microorganisms of both living and dead biomass such as physical and chemical adsorption, ion exchange, complexation, chelation and micro-precipitation (Artola, et al., 1999). Bio-sorption is known to be a metabolically passive process, which is one that does not require energy (Zouboulis, et al., 1997), however, it comes about from the interactions between the metals in solution and the cell surface of the bacteria (Artola, et al., 1997). Variables influencing bio-sorption include pH, the concentration of the metals in the waste water together with the concentration, composition and characteristics of the waste water and the sludge (Artola, et al., 1997).

Another aspect to be considered is the interaction between different metallic ions. The existence of ‘competition’ for active adsorption sites amongst metals was confirmed by a study undertaken by Artola and co-workers (1999). The study focused on the adsorption of metals from a multi-metal mixture. Binary systems were investigated and results showed that the number of sites for adsorption is limited and hence the adsorption of metal ions will depend upon the availability of alternative ions and the preferences of the bacteria (Artola, et al., 1999). From the results obtained from the study, copper was found to be dominantly adsorbed onto the sludge, this was due to the desorption of the other metals (zinc and cadmium) followed by adsorption of copper metals onto the freed bonding sites as a result of a greater affinity of copper for the sludge binding sites (Artola, et al., 1999).

A study conducted by Sağ and Kutsal further confirmed the existence of ‘competition’ amongst metal ions for binding sites, they categorized this relationship as antagonistic action. Furthermore, they predicted a screening effect on the one metal ion by the second metal ion (Sağ & Kutsal, 1996).

It has been recorded that in the past 20 years, equilibrium models have been successful in describing the bioavailability of metals in environmental systems, despite the fact that environmental systems are recognized as dynamic systems that are rarely in equilibrium (Worms, et al., 2006). Widespread equilibrium models include the free ion activity model (FIAM) and the biotic ligand model (BLM).

In these equilibrium models, general assumptions are based on the belief that a reduction in available and reactive metal species as a result of competition, complexation and other mechanisms reduces the bioaccumulation of metals in microorganisms, thereby reducing biological effects that should be driven by the presence of microorganisms (Worms, et al., 2006).

### **2.3.2 FREE ION ACTIVITY MODEL (FIAM) AND EXTENDED FIAM**

The most bioavailable form in which metals can exist in sludge is of free metal ions (water-soluble fraction), where these free metal ions are chemically available for the microorganisms to take up. A model used to represent the effect of free metal ion concentration on the biological effects is the Free Ion Activity Model (FIAM).

The activity of the free metal ion is frequently used to correlate the absorption and toxicity of trace elements (Parker, et al., 2001) and environmentalists have accepted the concept of the ability of the dissociated free ion to drive a biological system (Simkiss & Taylor, 2000).

#### **FIAM**

The FIAM was originally developed to justify observations obtained as a result of experiments; its development was to formalize assumptions made on the universal importance of the activities of free metal ions on the determination of the uptake, toxicity and nutrition of cationic trace metals. In the FIAM, the central role of the free metal ion activity, which is merely a measure of the metal chemical activity at the surface of the cell, on the uptake and toxicity between metals and aquatic organisms is emphasized ((Morel, 1983) cited in (Brown & Markich, 2000)).

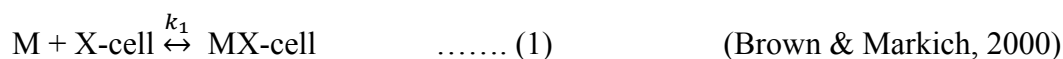
The FIAM assumes that the biological response has direct proportionality with the activity of the complex formed (M-X-cell) when the free metal ion (M) attaches to the aquatic microorganism (X-cell). Furthermore, the activity of the surface complex is directly proportional to the free metal ions concentration in solution [M] (Brown & Markich, 2000). The interaction between trace metals and aquatic organisms can be summarized in the following three steps (Campbell, 1995):

1. Diffusion or advection of the metal existing in the bulk solution to the surface of the cell membrane.
2. Sorption of the metal by surface complexation at the binding site of the cell membrane surface.
3. Transport (uptake) of the metal through the cell membrane and into the organism.

From the above process, it is evident that prior to bringing about biological response (BR), a metal must interact and traverse the cell membrane. As a result of the complexation of the metal onto the surface site, it is believed that the metal and the cell surface binding site are in rapid equilibrium.

The assumptions forming the basis for the FIAM are as follows (Campbell, 1995):

1. The primary site for interactions between the metal and aquatic organisms is the cell membrane.
2. The interaction between a free surface site on the cell membrane (X-cell) with a metal (M) is described by a surface complexation reaction as shown in equation 1:



Where the activity of the surface complex is given by:

$$\{MX\text{-cell}\} = K_1 \{X\text{-cell}\} [M] \quad \dots\dots (2) \quad (\text{Brown \& Markich, 2000})$$

Where,  $K_1$ : conditional stability constant,

$\{\}$ : activity of the surface,

$[ ]$ : activity of dissolved species.

3. There is rapid equilibrium obtained between the metals in the aqueous medium and those complexed at the surface of the cell membrane.
4. Biological response (BR) is proportional to the activity of the metal-surface complex  $\{MX\text{-cell}\}$  and the BR is derived as follows:

$$BR = k K_1 \{X\text{-cell}\} [M] \quad \dots\dots (3) \quad (\text{Brown \& Markich, 2000})$$

Where,  $k$ : constant of proportionality.

5. Within the range of metal activities bringing about a biological response, the activity of the free surface sites on the cell membrane  $\{X\text{-cell}\}$  is effectively constant and the surface complex activity  $\{MX\text{-cell}\}$  has direct proportionality to the concentration of the metal in solution  $[M]$ .
6. On exposure of the metal to the cell membrane, the nature of the cell membrane does not change.

Exceptions to the FIAM involve species such as colloidal metals, which are unable to directly react with the cell surface binding site, making them biologically inactive, together with metals that have been bound in strong complexes with organic ligands ((Anderson and Morel, 1982) cited in (Brown & Markich, 2000)). In the case of a metal complex interacting with the cell membrane, a ternary surface complex results and the BR is proportional to the activity of the metal-ligand complex. Further exceptions to the FIAM include lipophilic complexes that form as a result of inorganic and organic ligands (Campbell, 1995).

## Extended FIAM (BRT- Biological Receptor Theory)

The realisation of the possibility of a non-constant activity of the sites on the cell membrane raised concerns regarding the FIAM, these concerns were later reviewed and mathematically formalized by Brown and Markich (2000) resulting in the formulation of the extended FIAM.

Assumptions for the BRT opposing assumption 6 of the FIAM are as follows (Pfitzer and Vouk (1986) and Kenakin (1997) cited in (Brown & Markich, 2000)):

1. The maximum biological response ( $BR_{max}$ ) is reached when the total cell receptor sites concentration  $\{X-cell\}_T$  have been complexed by the metal.
2. The activity of the metal-surface complex  $\{MX-cell\}$  is small when compared to the activity of the metal existing in the aqueous solution  $[M]$ .

The BRT suggests that  $\{X-cell\}$  should decrease as BR increases, furthermore,  $BR_{max}$  follows when all the surface sites on the cell membrane have been occupied, corresponding to a surface site concentration  $\{X-cell\}$  of zero.

In the derivation of the BRT, the law of mass action is used to define the total concentration of cell receptor sites:

$$\{X-cell\}_T = \{MX-cell\} + \{X-cell\} \quad \dots\dots (4) \quad (\text{Brown \& Markich, 2000})$$

The BR is then given by the following general equation:

$$BR = \frac{BR_{max}\{X-cell\}_T^n[M]^n}{K_E^n(K_{-1} + [M])^n + \{X-cell\}_T^n[M]^n} \quad \dots\dots (5) \quad (\text{Brown \& Markich, 2000})$$

Where, n: slope value = 1, 2, 3... and does not have any physiological meaning

$K_E$  : The concentration of MX-cell bringing about half of  $BR_{max}$

$K_{-1}$ : Dissociation constant for equation 1

Equation 5 produces a graph referred to as a concentration-response curve which has a sigmoidal shape(S-shape) (Brown & Markich, 2000).

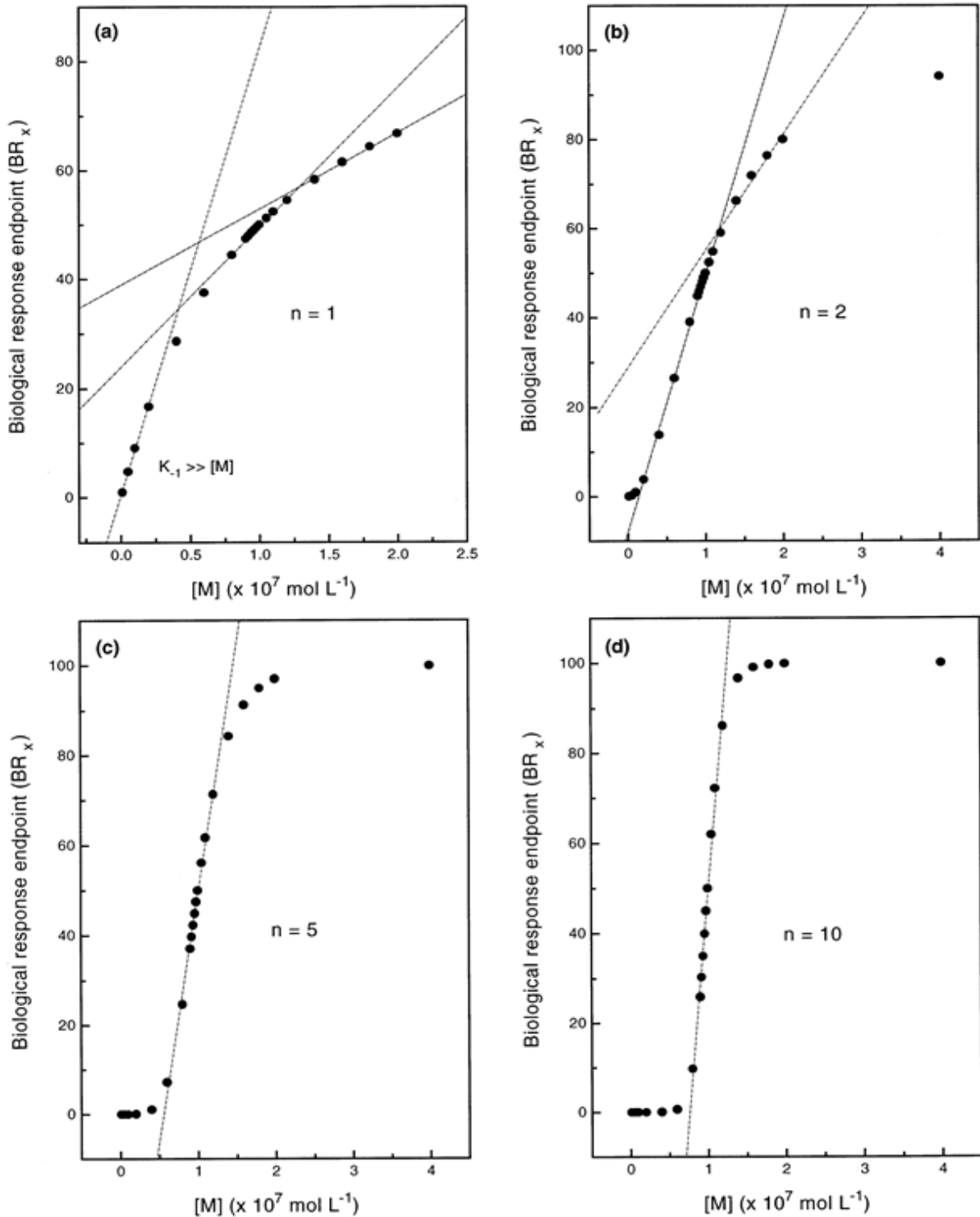
From Figure 8, it can be seen that linearity between the BR and  $[M]$  is linear within a certain region; and can therefore be represented by the FIAM, showing the direct proportionality between the BR and  $[M]$ . The FIAM implies that the proportionality between the free metal ion concentration and the BR should be evident throughout the curve, however, the experimental data in Figure 8 does not agree. Plots in Figure 8 at different slope values (n), show that the range in which a linear relationship exists increases with increasing n, therefore, the predicting ability of the BR by the FIAM increases with increasing n. Furthermore, at high n values, the FIAM has greater predictive power in regions of lower BR (Figure 8c and 8d).

In regions of direct proportionality when  $n$  is 1, the BR is given by equation 6, the best fit line of the region:

$$BR = a + bK_1\{X - cell\}_T[M] \quad \dots\dots\dots (6) \quad (\text{Brown \& Markich, 2000})$$

Where,  $a$ : intercept and  $bK_1\{X-cell\}_T$ : slope

Equation 6 is derived from the BRT and is considered a modified form of the original FIAM and is used to designate the conditions and/or region in which the FIAM effectively describes BR.



**Figure 8: Concentration-response curves for different slope ( $n$ ) values (Brown & Markich, 2000)**

The extended FIAM was concluded to have a more rigorous and mechanistic approach and successfully related the concentration of the free metal ion to the biological response, and hence its use as a method for the determination of the bioavailability of metals was confirmed.

### **2.3.2.1 The Use of Chloro-Complexation to Enhance Cadmium Uptake by *Zea mays* and *Brassica juncea*: Testing a “Free ion Activity Model” and Implications for Phytoremediation (López-Chuken, et al., 2010)**

Soils contaminated by toxic metals are successfully remediated by a well-known cost effective method termed phyto-extraction, where the toxic metals are used for the nutritional source for plants. In the study completed by López-Chuken and co-workers (2010), soil contaminated by Cd is investigated, where the uptake of the Cd by two plants, namely: *Zea mays* and *Brassica juncea*, is investigated. The increase of solubility of metals in the soil greatly optimises the phyto-extraction process; however, there exists a possibility of increasing the solubilisation of undesired metals at the same time, resulting in a decrease of Cd uptake by the plants.

The use of selective ligands to form moderate complexes with the desired metal has been studied to be a cost-effective method for increasing metal solubility. In the work carried out by López-Chuken (2010), the use of simple chloride salts ( $\text{Cl}^-$ ) to enhance Cd solubility was investigated as  $\text{Cl}^-$  is known to readily form moderate complexes with  $\text{Cd}^{2+}$ . One of the mechanisms proposed, by which  $\text{Cl}^-$  enhances Cd uptake by plants is an increase in the activity of free  $\text{Cd}^{2+}$  ions, followed by a cation exchange with the salt cation (Bingham *et al.*, 1984, cited in (López-Chuken, et al., 2010)). The effect of this mechanism and others (which are not directly relevant to this literature review) were investigated and the FIAM was used to model the uptake of Cd by plants, thereby testing its prediction viability for the uptake of free and complexed Cd species by the two plants.

In the approach used, the following assumptions were made (López-Chuken, et al., 2010):

- Sorption of free metals ( $\text{M}^{2+}$ ) or metal complexes ( $\text{MCl}^+$ ) from solution onto proposed sorption sites on the plant roots.
- The competition existing between cations and protons for sorption sites is also considered, this is an extended approach to the original FIAM.
- The transport rate of Cd from the roots to the shoots of plants is subject to the density of the occupied transport sites at the surface of the root; this rate reaches a maximum when all transport sites have been employed.
- The density of the sorption sites remains unchanged, regardless of the physiological age
- The activity of the free ion at the surface of the root remains unchanged during the period of exposure of the plant; furthermore, this activity is the same as that which is measured in the bulk solution.

The implicit definition of the FIAM used is shown in equation 7:

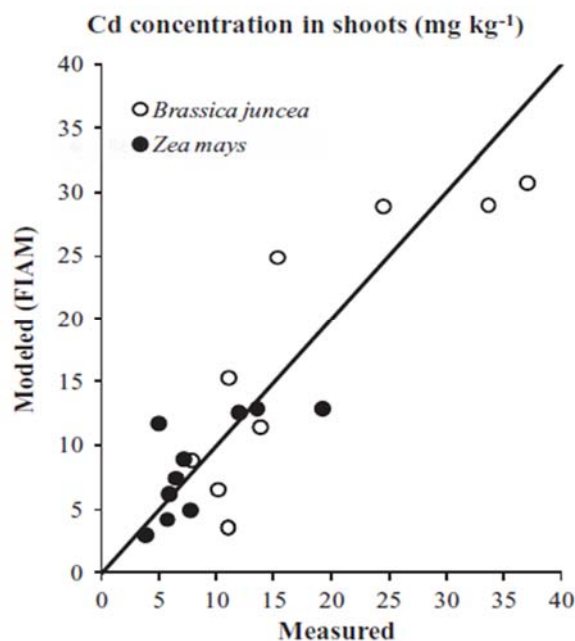
$$Cd_{\text{shoots}} = \frac{K_{\text{transfer } 1} R_{\text{tot}(1)} K_{\text{Cd}}(Cd^{2+})}{1 + K_{\text{Cd}}(Cd^{2+}) + K_{\text{Zn}}(Zn^{2+}) + K_{\text{H}1}(H^+)} + \frac{K_{\text{transfer } 2} R_{\text{tot}(2)} K_{\text{CdCl}}(CdCl^+)}{1 + K_{\text{CdCl}}(CdCl^+) + K_{\text{H}2}(H^+)} \dots \quad (7)$$

(Lo'pez-Chuken, et al., 2010)

Where,  $R_{\text{tot}}$ : total binding sites,  $K_{\text{transfer}}$ : reaction rate constant for the free ion and complex transport, (species): species concentration,  $K_{\text{H}}$ : Reaction rate constant for protonation of sites.

Equation 7 describes the uptake of Cd, by calculating the concentration of Cd in the shoots, it also incorporates the competition of  $Cd^{2+}$ ,  $Zn^{2+}$  and  $H^+$  for a single sorption site, furthermore,  $CdCl^+$  and  $H^+$  for another proposed site on the roots.

Figure 9 shows a comparison between the values that were obtained for the concentration of Cd in the shoots from the FIAM model and those obtained experimentally. It was concluded that the FIAM produces a reasonably good fit for the plants provided that there is competition by  $Cd^{2+}$  and  $CdCl^+$ , which are the most prevailing species for Cd in the soil, for sorption sites on the roots. It was further suggested that complications to the FIAM are brought about when metal-ligand complexes are predominant.



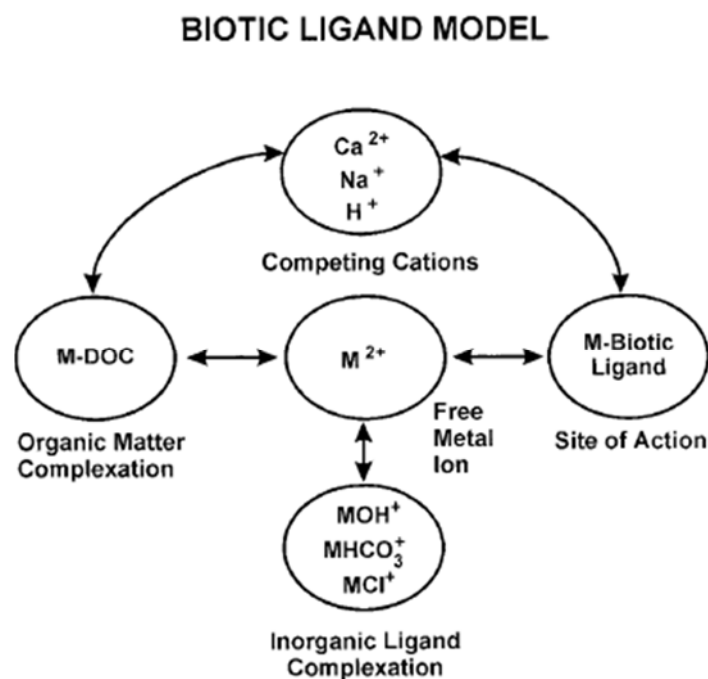
**Figure 9: Cd uptake (mg kg<sup>-1</sup>) by *Brassica juncea* and *Zea may* shoots modelled by the FIAM assuming a common absorption site for  $Cd^{2+}$  and  $CdCl^+$  ions (Lo'pez-Chuken, et al., 2010)**

### 2.3.3 BIOTIC LIGAND MODEL (BLM)

The Biotic Ligand Model is a mechanistic equilibrium model frequently used to describe the bioavailability of metals in environmental systems. It was proposed to quantitatively estimate the effects of water chemistry on the biological availability and speciation of metals in aquatic organisms. The model hypothesises that the metal-ligand complexation and competitive metal interactions at the toxic action site need to be considered (Niyogi & Wood, 2004) in assessing bioavailability of metals.

A metal cation is transported to an organic ligand (biotic ligand) by combining with a metal transporter and it is bound onto an active site on the organic ligand. The BLM extends into the incorporation of the competition between the metal cation and naturally occurring cations:  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ , and  $\text{H}^+$  (Niyogi & Wood, 2004).

Figure 10 shows a schematic summary of the BLM, clearly showing the interactions of the free metal with the hardness cations, dissolved organic matter (DOM), the biotic ligand and the inorganic ligand.



**Figure 10: Schematic diagram of the biotic ligand model (Di Toro, et al., 2001)**

Assumptions on which the BLM was developed include the following:

1. The biotic ligand (BL) is considered the site of toxic action on the organism of interest (Niyogi & Wood, 2004).
2. Equilibrium exists among all the metal ligand species; this implies reversibility for all the variables in question including the reversibility of the biotic ligand site (Bell, et al., 2002).
3. The main function of the BLM is to establish a relation between the acute toxicity and the critical metal accumulation at the BL, the  $\text{LA}_{50}$  (Niyogi & Wood, 2004).



4. Complexation by organic, inorganic ligands and dissolved natural organic matter (NOM) result in a decrease in the metal toxicity due to a reduction in the free ion activity which brings about equilibrium between the free metal ions on the BL (Bell, et al., 2002), (Niyogi & Wood, 2004).
5. The equilibrium between the free metal ions and the naturally occurring hardness cations ( $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{H}^+$ ) affects interaction with the BL resulting in them being specifically protective (Niyogi & Wood, 2004).
6. The properties of the biotic ligand remain constant irrespective of the chemistry of the water it exists in (Niyogi & Wood, 2004).

The BLM allows for incorporation of the effects of all the aspects of water chemistry on toxicity (hardness, pH, alkalinity, complexing agents, and dissolved organic matter), thereby incorporating the bioavailability concept and is therefore more flexible than other models (Niyogi & Wood, 2004).

The complexation reaction between the metal (M) and the ligand (L) is defined as follows:



Where ML is the metal bound by the ligand.

The equilibrium constant is given by:  $K = \frac{[ML]}{[M][L]} \quad \dots\dots (9) \quad (\text{Bell, et al., 2002})$

The concentrations of ML and L are expressed in terms of the total ligand concentration ( $L_t$ ) by equations 9 and 10.

$$[ML] = L_t \frac{[M]}{[M]+K^{-1}} \quad \dots\dots (9) \quad [L] = L_t \frac{K^{-1}}{[M]+K^{-1}} \quad \dots\dots (10) \quad (\text{Bell, et al., 2002})$$

The critical fraction of metal on the BL ( $C_f$ ) is correlated with a toxic response and is defined:

$$C_f = \frac{[M]}{[M]+K^{-1}} \quad \dots\dots (11) \quad (\text{Bell, et al., 2002})$$

Effectively, the toxicology properties are predicted by knowledge of the concentration of the surface bound metal and thereafter related to the concentration of the free ion [M]. Experimental data is used to obtain parameter values for the kinetic constants of the BLM to allow for use of the BLM ( (Worms, et al., 2006), (Bell, et al., 2002)). The nature or location of the ligand does not affect the prediction of toxic effects, however, as shown by equation 11, depends on the concentration of the free metal ion [M] taken up at the ligand membrane. Equilibrium is maintained as long as the flux for the diffusion of the metal through the BL's boundary is greater than the flux of metal uptake (Bell, et al., 2002).

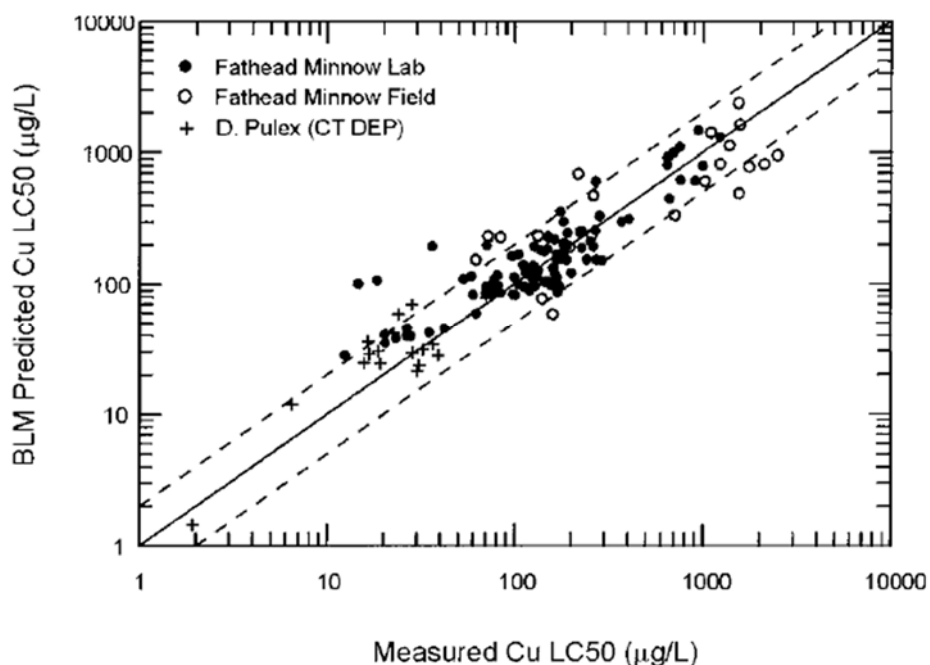
Once reaction 8 has taken place between the metal and the biotic ligand, the metal is referred to as being complexed, therefore organically bound. The concentration of free metal ions is therefore decreased by the formation of complexes.

Current BLMs have been developed on the basis of individual metals, this brings about concerns as most metals do not exist alone but rather exist in mixtures in aquatic environments and the interactions between metals is to be considered.

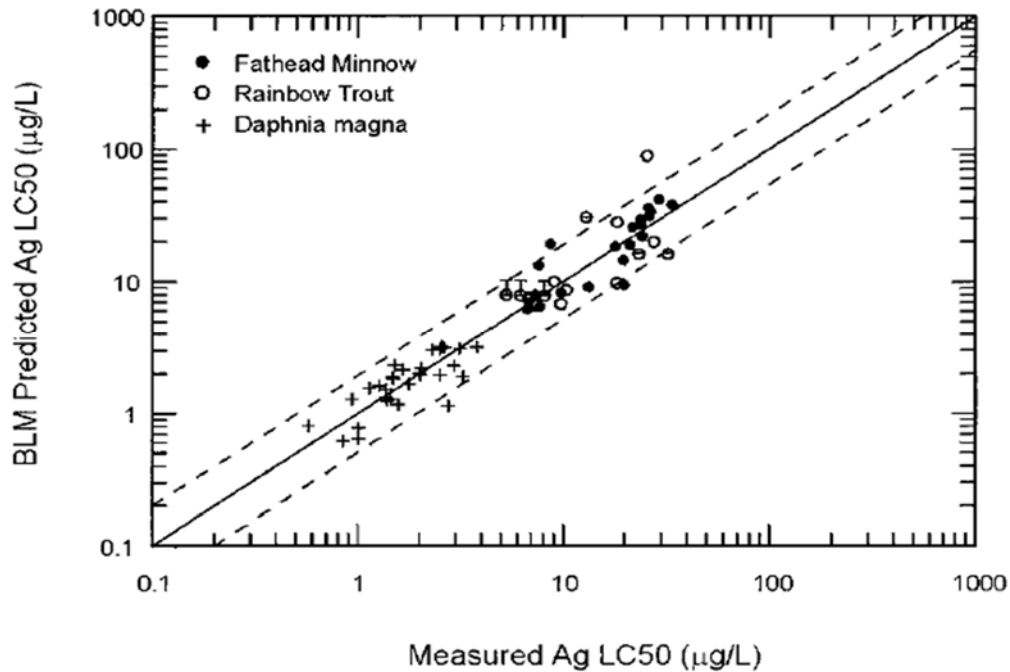
### 2.3.3.1 Biotic Ligand Model of the Acute Toxicity of Metals: Technical Basis (Di Toro, et al., 2001)

Of the applications of the BLM, computation of LC<sub>50</sub> values for a certain set of metals and aqueous ligand concentrations is the most popular and simplest. It is of great importance that a computational set is made for each range of concentrations to which the aquatic organism will be exposed due to a great variance in exposure conditions.

Work carried out by Di Toro and co-workers (2001) investigated the application of the BLM for Ag and Cu on aquatic organisms, namely: *fathead minnow*, *rainbow trout* and *daphnia magna*. The investigation was carried out by calculating the LC<sub>50</sub> values, the concentrations of the biotic ligand bringing about a mortality percentage of 50%. The mortality is believed to occur when the critical concentration of the metal-ligand complex has been reached.



**Figure 11: Comparison between measured and BLM predicted LC<sub>50</sub>s for Cu for *fathead minnow*, *rainbow trout* and *daphnia magna* (Di Toro, et al., 2001)**



**Figure 12: Comparison between measured and BLM predicted LC<sub>50</sub>s for Ag for *fathead minnow*, *rainbow trout* and *daphnia magna* (Di Toro, et al., 2001)**

Figure 11 and 12 shows the comparison between experimentally obtained and BLM obtained LC<sub>50</sub>s for *fathead minnow*, *rainbow trout* and *daphnia magna* for Cu and Ag respectively. From the observation, it is evident that the experimental data mostly lies within the linearity bounds, the dotted straight lines in Figure 11 and 12. It was concluded that the BLM can successfully predict the LC<sub>50</sub>s for the aquatic organisms studied. The BLM was shown to be predictive within a factor of two for LC<sub>50</sub>s covering the range over two orders of magnitude; deviations were brought about by water chemistry variations.

### 2.3.4 ADSORPTION ISOTHERMS

Another approach makes use of adsorption isotherm data to model the adsorption and/or complexation of metal ions on anaerobic sludge. Traditional isotherms have been used to represent experimental speciation data to a certain extent in combination with sequential extraction. The use of isotherms in modeling is mainly to investigate the sorption capacity of individual metals existing in anaerobic sludges (van Hullebusch, et al., 2005a). Use of isotherms involves the characterization of biomass such that the nature and concentration of active adsorption sites is identified (Pagnanelli, et al., 2003).

The two most popular surface adsorption models are the Langmuir and Freundlich models (Artola, et al., 1999), these are models developed for single-solute systems. The general expressions for Langmuir and Freundlich models are shown by equation 12 and 13 respectively:

$$\text{Langmuir Isotherm Model: } Q_e = \frac{Q_{max}bC_e}{1+bC_e} \quad \dots\dots (12) \quad (\text{van Hullebusch, et al., 2005a})$$

Freundlich Isotherm Model:  $Q_e = kC_e^{1/n}$  ..... (13) (van Hullebusch, et al., 2005a)

Where,  $Q_e$ : the uptake of the metal ion by biomass ( $\text{mg g}^{-1}$  TSS),  $Q_{\text{max}}$ : maximum uptake of the metal ion by the biomass ( $\text{mg g}^{-1}$  TSS),  $C_e$ : final metal concentration in the solution ( $\text{mg l}^{-1}$ ),  $b$ : Langmuir adsorption equilibrium constant,  $k$ : constant relating to the capacity of adsorption,  $n$ : constant relating to the intensity of adsorption and TSS is Total Suspended Solids.

By inclusion of the  $Q_{\text{max}}$  in the Langmuir model, it implies that the model acknowledges the possibility of the saturation of the adsorptive surface.  $Q_e$  and  $C_e$  are experimentally determined, by regression of equation 12 and 13 using experimental data, allowing for the determination of the constants  $C_e$  and  $n$  for the Freundlich isotherm and the  $Q_{\text{max}}$  and  $b$  forming the Langmuir isotherm.

It should be noted that the use of adsorption isotherms is limited, regarding speciation; this is due to the fact that they are limited, and the adsorption isotherms fail to represent interactions of metals with the solid phase. Furthermore, they are unable to determine the actual partitioning of metals in the different phases which forms the basis of speciation. Use of adsorption isotherms is therefore in conjunction with sequential extraction, and the isotherms are able to model data of metals that are already partitioned by sequential extraction.

#### **2.3.4.1 Sorption of cobalt and nickel on Anaerobic Granular Sludge: Isotherms and Sequential Extraction (van Hullebusch, et al., 2005b)**

Cobalt and Nickel play a vital role in the methanogenic activity of granular sludges and form part of the essential nutrient required for methanogens. Van Hullebusch and co-workers (2005b) investigated the retention of metals in sludges from up flow anaerobic sludge bed (UASB) reactors, the partitioning of the metals in four fractions (exchangeable, carbonates, OM/sulfides and residual) were examined individually, in a binary mixture and in competition with Fe. The results for the sequential extraction were thereafter used in conjunction with the Langmuir and the Freundlich adsorption isotherms to describe the retention capacity and strength of adsorption of the metals in the sludge.

The Langmuir was found to appropriately describe the adsorption of the Co and Ni and is shown in Figure 13 for Nedalco and Eerbeek sludge, by the agreement between the experimental data (data points) and the model predictions (solid lines). Furthermore, the isotherms fit the data to a greater extent in the range of low metal concentrations in solution.

Agreement of the Langmuir isotherm predictions of the adsorbed metal concentration ( $C_e$ ) and the sequential extraction experimental data shows the modeling capability of the Langmuir isotherm when coupled with sequential extraction.

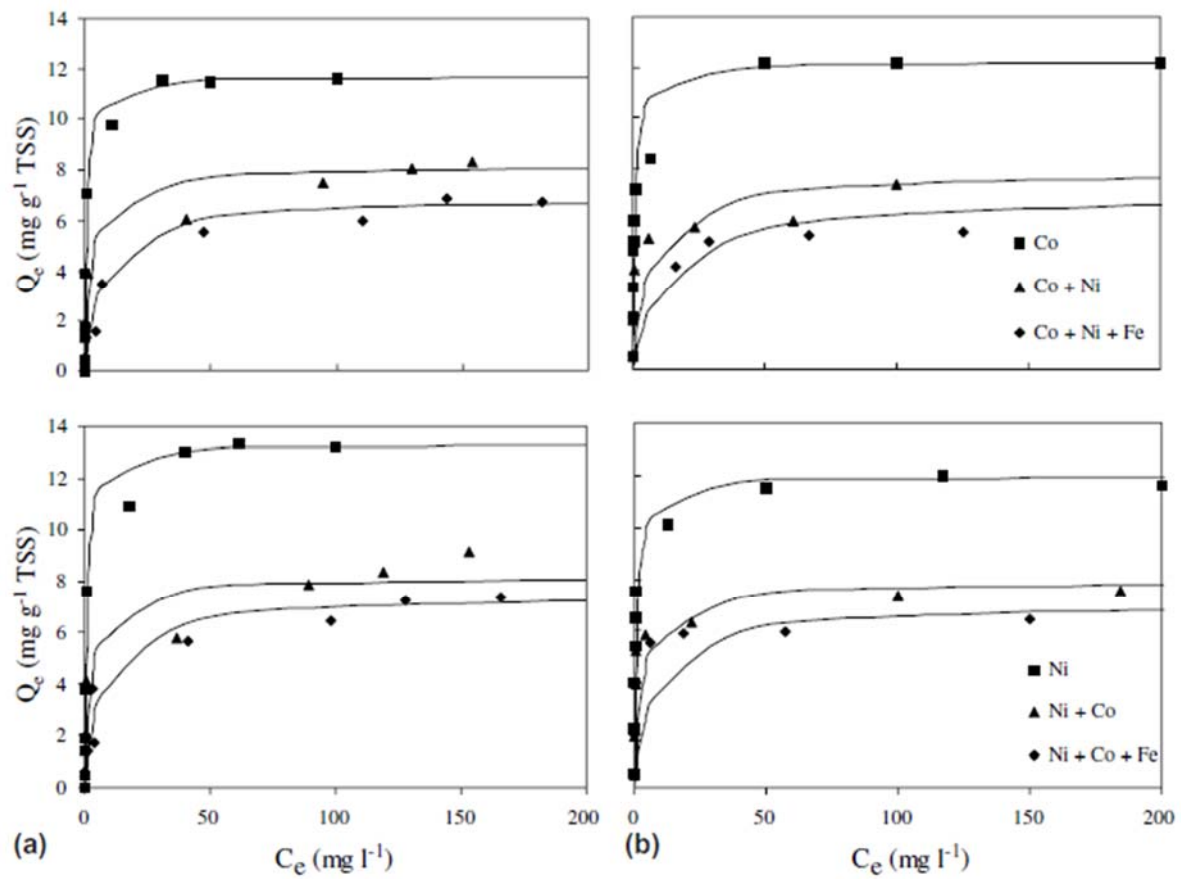


Figure 13: Nickel and cobalt sorption onto Nedalco (a) and Eerbeek (b) anaerobic granular sludges (monometal and competitive (van Hullebusch, et al., 2005b)).

### 2.3.5 ANALYSIS OF MICRONUTRIENT REQUIREMENTS FOR THE ANAEROBIC DIGESTION OF FISCHER-TROPSCH REACTION WATER (Mathir, 2013)

A recent study was completed by Mathir (2013) where sequential extraction was used together with speciation modeling of the experimentally obtained data by making use of a computer simulation package MINTEQA2, to carry out the speciation modeling. The study first involved the analysis of the micronutrient requirements for anaerobic digestion of Fischer-Tropsch Reaction Water (FTRW) followed by chemical speciation modeling which is a method used to model the distribution of an element in all the species in which it exists.

In Mathir's work (2013), it was hypothesized that the biological response is proportional to the concentration of the free metal ion. It was further hypothesized that given the existence of precipitates in equilibrium with the solution, it is possible to calculate the concentration of the free metal ion by making use of the solubility product of the precipitate. Given that hypothesis 2 was true, the fate of the metals would be predicted by modeling the evolution of precipitates over a series of SBR sequences.

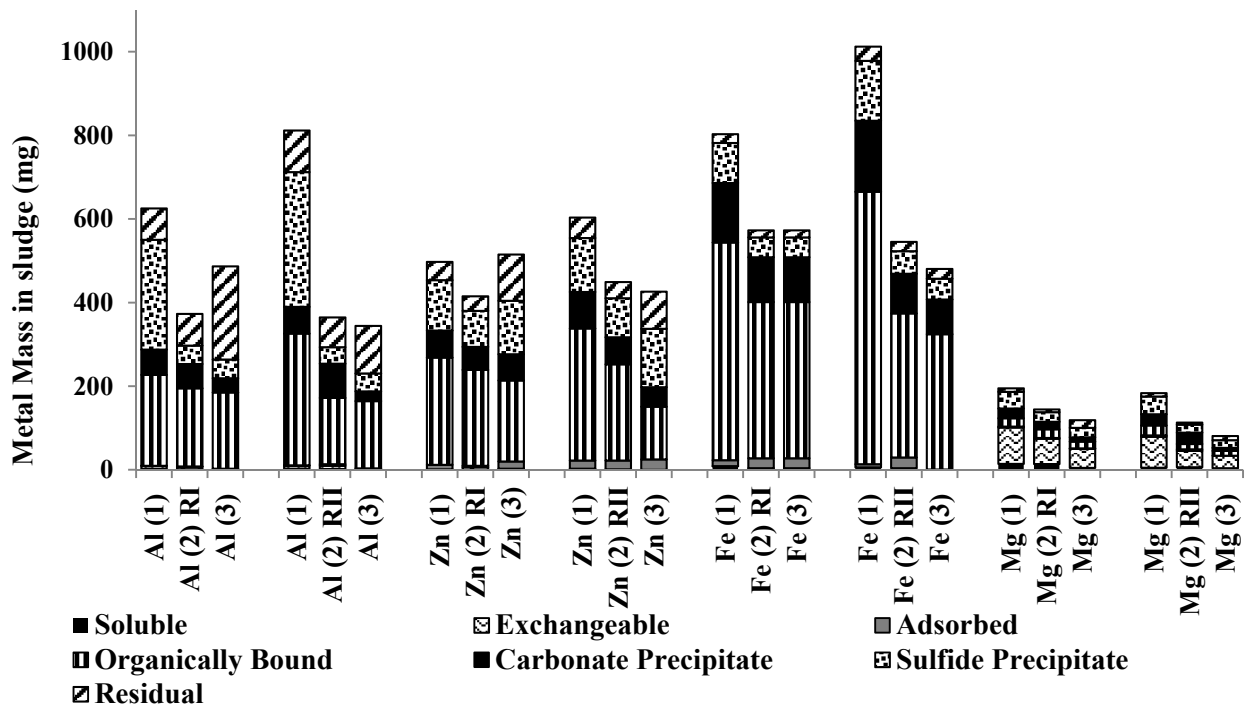
The experimental work comprised of two parts, where the one part included running of the reactors at steady state conditions (experiment A) which made use of synthetic feeds. Experiment A was completed to gain a better understanding on the speciation of metals when dosed in a system containing sludge and also to deduce whether all metals dosed can be accounted for or not. Furthermore, experiment A was completed to assess the effectiveness of sequential extraction to successfully separate the metals into the various categories and to quantify any losses from the various sequential extraction schemes performed.

Experiment B was performed using FTRW. It should be noted that this study is a re-examination of the experiment B data generated by Mathir (2013), and hence the experimental method is explained fully. The sequential extraction experiments were completed by making use of the scheme proposed by Stover (cited in (van Hullebusch, et al., 2005b)). The fractions used are shown in Table 7:

**Table 7: Fractions for the Stover scheme used in Mathir's (2013) work**

<b>Fraction</b>	<b>With respect to Filgueiras (2002)</b>
Soluble	F <sub>1</sub>
Exchangeable	F <sub>2</sub>
Adsorbed	F <sub>5</sub>
Organically Bound	F <sub>5</sub>
Carbonate Precipitate	F <sub>3</sub>
Sulfide Precipitate	F <sub>5</sub>
Residual	F <sub>6</sub>

Distribution of metals in the respective fractions is depicted by Figure 14; this would be the experimental speciation data of the metals dosed.



**Figure 14: Experimental Metals Speciation Results for Sequential Batch Reactor for Experiment A (Mathir, 2013)**

As depicted by Figure 14, it was evident that the fate of the metals was found across the different phases and it was also seen that the organically bound phase has a high metal content. Mathir (2013) stated that Al, Zn and Fe were found mainly in the organically bound fraction and Mg was mostly found in the exchangeable fraction.

One of the limitations exposed as a result of completing experiment A, was the fact that concentrations of the micronutrients in the various fractions was close to the calibration lower limit of 0.01 mg/l, bringing about significant errors (Mathir, 2013). As a result of such anticipated errors, the sequential extraction procedure was omitted in experiment B, however, the analysis of metals in the supernatant liquid was used to quantify the bioavailable fraction and the sludge metal analysis was used to quantify the potentially bioavailable and non-bioavailable fraction (Mathir, 2013).

Figure 16 depicts the changes of the metals in the precipitates. It was noted that all metals exist in the precipitates fraction as of the beginning of the experiment. Two of the minerals;  $MnHPO_4$  and  $Ca_5(PO_4)_3OH$ , de-associate, resulting in a decrease in mineral content with the advancement of the experiment.

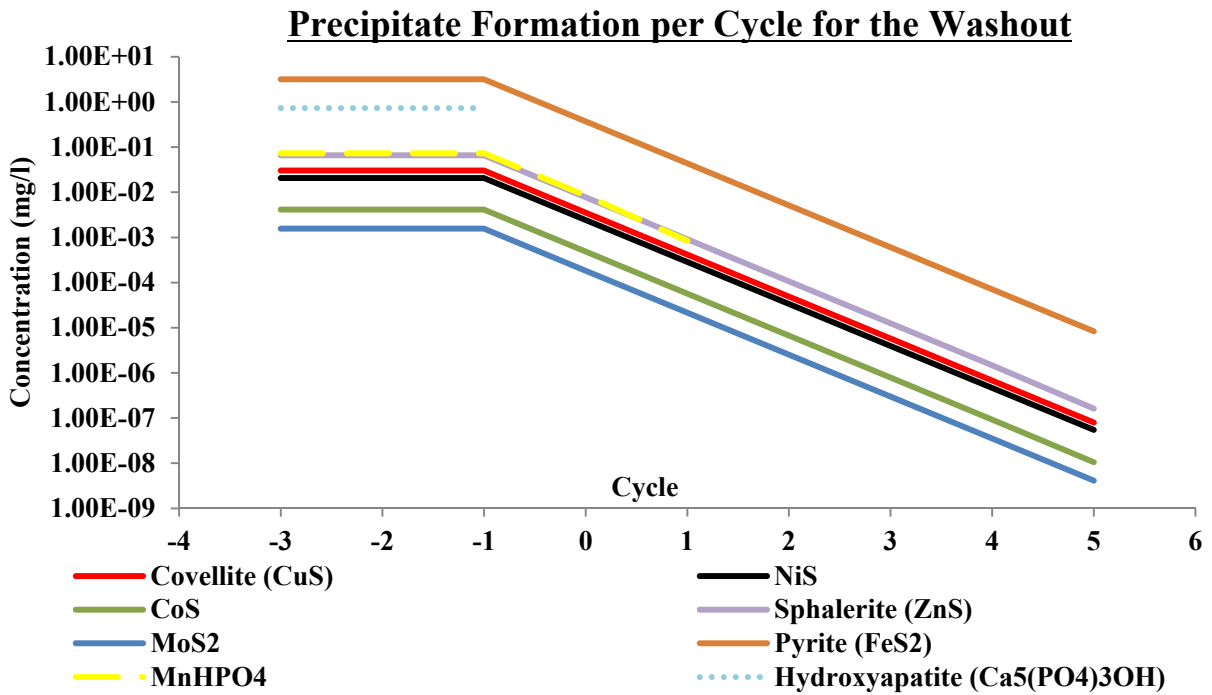


Figure 15: Concentration of precipitated minerals as predicted by model for each successive cycle in Experiment B (Mathir, 2013)

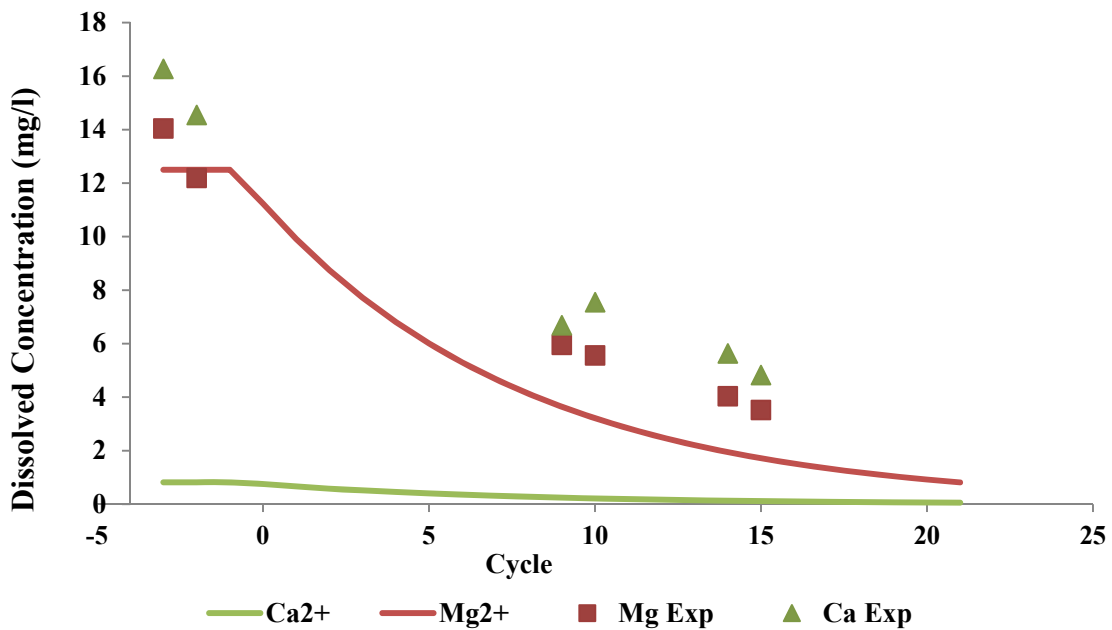
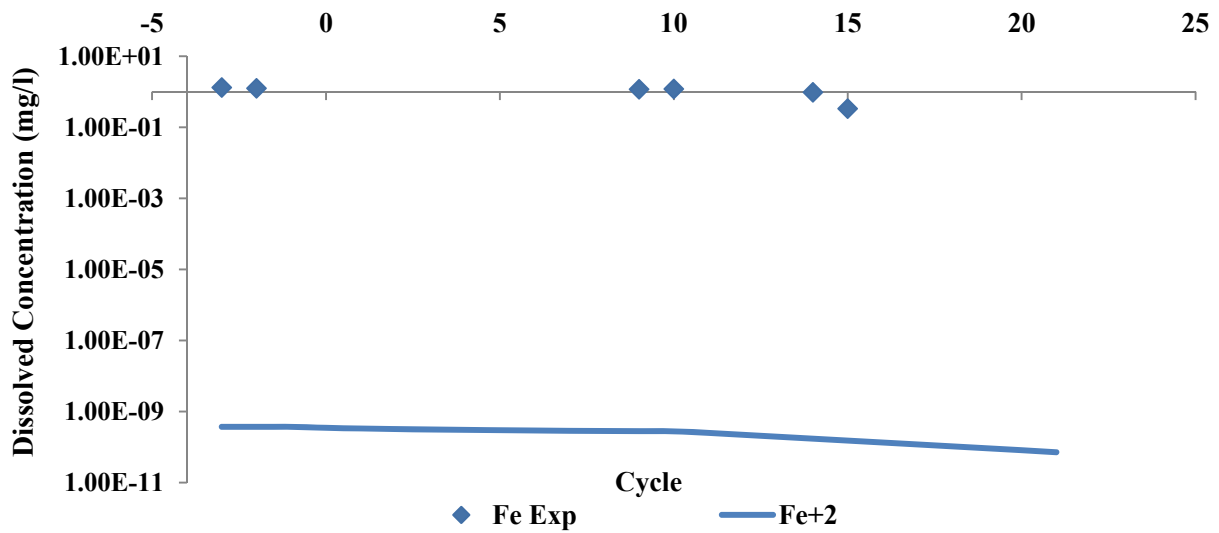


Figure 16: Concentration profile of Ca<sup>2+</sup> and Mg<sup>2+</sup> in the dissolved phase (mg/l) including comparisons to experimental data in Experiment B (Mathir, 2013)

Changes in the metals in the soluble phase in successive cycles is depicted in Figure 17, together with comparison between the model results and the experimentally obtained results in for Mg and Ca. Mathir suggested that the experimental and model results are comparable for Mg and not so comparable for Ca. Figure 17 shows the comparison between model predicted and experimental concentration of dissolved Fe<sup>2+</sup>, the same trend can be seen between the two sources of data, making them comparable.





**Figure 17: Concentration profile of Fe<sup>+2</sup> in the dissolved phase (mg/l) including comparisons to experimental data in Experiment B (Mathir, 2013)**

The concentration of the metals in the soluble phase were calculated by making use of the precipitates-controlled model and compared to the experimental data (Figure 15 and 17) and some degree of agreement was suggested. Partially successful representation of the speciation model for the precipitates fraction was therefore suggested by Mathir.

The inclusion of the adsorbed, organically bound, carbonate and sulphide precipitates and the residual phases in the precipitates resulted in an over-prediction of the amount of precipitate formed by the model. Mathir concluded that the formation of precipitates brings about a decrease in the metal bioavailability by sequestering trace metals which had been added. The model constructed was able to predict the effect of the formation of precipitates on metal bioavailability, even though it is a simplified model.

Mathir suggested that inclusion of the organically bound phase (falling in the oxidisable fraction) might result in an improvement in the correlation between experimental and model-predicted data.

### **3. SYNTHESIS OF LITERATURE**

#### **3.1 LITERATURE REVIEW SUMMARY**

Studies on the nutritional requirement of microorganisms taking part in anaerobic treatment of waste water show the importance of micronutrients in the form of trace metals for stimulation of enzymatic and biological responses. Studies have shown that the existence of micronutrients in the anaerobic treatment process bring about an increase in the rate of methanogenesis.

In the case where metals in the biofilms of micro-organisms existing in the anaerobic sludge used to treat waste water anaerobically is insufficient, when trace metals are supplemented (dosed). Careful dosing is to be ensured to prevent toxicity of the environment, which brings about adverse effects on the treatment process. Not all the dosed metals are in a form that the organisms can readily take up, and the concept of bioavailability was introduced. Bioavailability may be defined as the extent to which metals in sludge are available for biological uptake by the microorganisms to bring about a biological response. Knowledge of the bioavailability of metals in sludge allows for determination of toxicity levels and whether metals are available for the microorganisms to stimulate biological response or not.

Sequential extraction techniques were used to investigate the manner in which metals are partitioned in the various species and fractions in which they exist. From the extraction results, the partitioning of metals in the bioavailable fractions were shown. The sequential extraction results showed that the metals are liberated into the soluble phase when acidic (for the acid-soluble fraction), and oxidative (for the oxidisable fraction) conditions prevail. Since the likelihood of such conditions in the anaerobic digesters is minimal, the bioavailable fractions in anaerobic sludge are limited to the water-soluble and exchangeable fractions.

It was therefore established that sequential extraction schemes successfully describe the bioavailability and mobility of metals in the various species in which they exist in anaerobic sludge. Of the sequential extraction schemes studied, the Tessier scheme and BCR schemes showed to be most applicable to anaerobic digestion, where the Tessier showed the greatest reproducibility ( (van Hullebusch, et al., 2005a); (Filgueiras, et al., 2002)).

Speciation models allow for determination of the free metal concentration, which was found to be proportional to the biological response, without the need for experimental procedures. Sequential extraction experimental data was used to validate the FIAM, BLM and Adsorption isotherms. It was found that the FIAM and BLM successfully model the free ion concentration of metals and are both mechanistic approaches. The BLM takes aquatic effects into consideration and competition among metals for complexation sites, which has an effect on the bioavailability of metals. The BLM therefore proved to be the most robust model in determination of speciation of metals.

The speciation models have been validated by making use of sequential extraction results, which allows for the calculation of the concentrations of the free metal ions while taking into consideration the effect of the other phases (precipitates, organically bound, etc.) on the concentration of the free metals. The concentration of the free metal is related to the biological response which indicates metal bioavailability, since the metal bioavailability was defined to be the extent of accessibility of the metal to the microorganism to bring about a biological response. The models can therefore be utilised to predict the bioavailability of metals in anaerobic sludge.

Throughout the case studies, it was observed that a large proportion of the total metal content is present in-between the soluble and precipitated or residual phase, in which metals have been bound by organic matter by complexation and bioaccumulation processes, this observation, was further concluded by Mathir (2013). This observation points to the consideration of this phase in question for inclusion in a speciation model.

Mathir showed that modelling of the concentration of the free ions while taking into account the controlling effect of precipitation increased the agreement between experimental and model-predicted results. Mathir further suggested that inclusion of the organically bound phase is assumed to show a further increase in the agreement between experimental and model data.

### **3.2 GAP ANALYSIS**

The literature review shows that models have been successfully fitted to sequential extraction data, especially the BLM; however, not much work has been completed where the models have been used to predict the metal speciation. Zandvoort (2006) and co-workers also state that little knowledge exists on the relationship between metal speciation and metal bioavailability in anaerobic bioreactors. Mathir's work is one of the few studies completed where a model was used to predict the metal speciation in systems containing sludge, thereby giving an indication of the bioavailable metals.

Mathir (2013) was successful in describing some of the effects of metal washout from a sequencing batch reactor. In Mathir's model, it was assumed that there were two phases into which the metals partition themselves, the soluble and precipitates phases. The prediction of the concentration of the metals in these phases was not entirely accurate due to the over prediction of the solid phase. The study was successful in directing the development and was able to form a basis onto which improvements may be made as it was incapable of accurately predicting the fate of **all** important metals (e.g. Ca, Cu, Fe). Due to this shortfall, it cannot be utilised as yet to recommend changes in the strategy used to dose metals.

The available literature suggests the importance of an intermediate phase existing between the soluble and precipitates phase, introducing an opportunity for the extension of the precipitate-controlled model to the inclusion of such a phase or a portion thereof to model the metal speciation in anaerobic sludge.

### **3.3 RESEARCH QUESTIONS**

Can a stoichiometric formulation of an adsorbed phase improve the correspondence between model and data for soluble metal ions and metal ions immobilised by a solid phase?

### **3.4 RESEARCH METHODOLOGY**

In the research, equilibrium models were developed to predict the micro-nutrient dosing requirements in anaerobic sludge. Inclusion of an intermediate phase between the soluble and precipitates phase was completed, as suggested by the literature review and the gap analysis. Effectively, the research included the extension of the Biotic Ligand Model (BLM) where a single phase, behaving like an adsorbed phase, was added. In the study, it was found that the intermediate phase comprises of more than a single adsorbed phase; however, the scope of the study is limited to addition of the adsorbed phase.

The approach used was best described by the BLM allowing for incorporation of the biotic ligands onto which the metals complex and there is competition (equilibrium) among metals for active sites.

According to given inlet conditions (influent FTRW COD, nutrient recipe, temperature, etc.), the developed model was created to predict process variables sufficiently, including the pH, methane production, effluent COD, etc. The ionic speciation chemistry carried out within the model aided in locating the following important phases that indicate the extent to which the metals were effectively distributed between:

- Precipitates of minerals that are calculated to be supersaturated at the dosing conditions,
- Metal ions organically bound in particulates as dictated by the analysis of existing and new experimental data phases associated with sludge flocs and granules in the anaerobic digester mixed liquor suspended solids (ADMLSS).

### **3.4.1 HYPOTHESIS**

A stoichiometric formulation of an adsorbed phase will improve the correspondence between model and data for soluble metal ions and metal ions immobilised by a solid phase.

### **3.4.2 HYPOTHESIS TESTING**

The study hypothesis was to be tested by constructing speciation models on PHREEQC, where the one model represents a scenario where the only controlling phase is the precipitates phase and the second model is one where it has been extended to incorporate an adsorption phase. The precipitates phase model was to be compared to the precipitates and adsorbed phase model, to distinguish the benefit of the addition of the adsorbed phase. Adsorption of metal ions on the biomass surface was to be modelled by making use of complexation reactions between the metals and biomass. Due to the simulation package requiring definitions of species being in their ionic forms, an ionic representation of the biomass is necessary which may bring about the need to alter certain complexation reactions in the simulation package database. To test the comparative accuracy, the model generated concentrations of the free ions were to be compared to experimental data.

#### 4. MODEL DEVELOPMENT AND DESIGN

As suggested in the “Hypothesis Testing” section, an equilibrium model taking precipitation controlling effects only, followed by a model taking into account precipitation and adsorption controlling effects was to be constructed. On construction of the models, they were to test whether addition of adsorption controlling effects on the existing precipitate-controlled model improves the prediction of free metal ion concentrations in anaerobic sludge.

There are numerous processes that occur within an anaerobic sludge reactor, as was seen in Figure 1, the volatile fatty acids go through various stages till they are converted into methane ( $\text{CH}_4(\text{g})$ ); such as hydrolysis and methanogenesis. Taking the processes which occur as a result of the presence of micronutrients adds further complications to the model, as all the processes occur simultaneously in the reactor. The broader model is therefore sectioned to simplify constituent models.

The model is sectioned into two main parts, the first part is where the biological conversion of the volatile fatty acids in the FTRW to methane is carried out, resulting in an increase in the initial pH and the biomass. The second part calculates the speciation of the metals in the sludge, where the fate of the metals, in terms of the phase in which they are likely to be found, is quantified.

An overview of the two sections with corresponding inputs and outputs for the development of the micronutrient speciation model is outlined in Table 8.

**Table 8: Model Development Overview**

Model Section	Section Input	Section Outcome
<b>Biological Conversion</b>	Experimental pH.	Model pH (1)
	Initial reactor conditions (mol/litre)	Total biogas production (litres)
	Temperature ( $^{\circ}\text{C}$ ), Pressure (atm), Reactor Volume (litres)	Reactor composition after biological conversion
<b>Speciation</b>	Nutrient concentration as per Sasol recipe (mol/litre)	Model pH (2)
	Remaining Acetate <sup>-</sup> , Propionate <sup>-</sup> and $\text{CO}_3^{2-}$ concentrations (mol/litre)	Precipitated and adsorbed metal per cycle
	Model pH (1)	Free metal ion concentration (mg/litre) per cycle

## **4.1 MODELLING OF WASHOUT EXPERIMENT BY Mathir (2013)**

It should be noted that no experimental work was done in this investigation. The washout experimental data produced by Mathir (2013) was examined in detail and used to evaluate improvement to the model which Mathir used. The term ‘washout’ refers to feed with no micronutrients.

### **4.1.1 EXPERIMENTAL DATA**

Construction and execution of experimental work was unnecessary as the data used in the study was generated by Mathir (2013) in the study conducted to analyse the micronutrient requirements for anaerobic digestion of Fischer-Tropsch Reaction Water. The experiment was carried out in two anaerobic digesters treating FTRW by making use of the Sasol micronutrient recipe over a total of 19 cycles. A single cycle comprised of the following (Mathir, 2013):

1. Addition of  $\text{NaHCO}_3$  buffer to the feed beaker with FTRW and micronutrients followed by charging into the reactor.
2. Allowing the reactions within the digester to take place
3. Collection of the total biogas produced for analysis
4. Decanting of 1 litre of the resultant supernatant liquid, which is a mixture of the unreacted FTRW contents together with metals in the liquid. The supernatant liquid was sent for analysis (pH, metal composition, etc.).
5. Addition of 1 litre of fresh feed as mentioned in ‘1’, the total cycle time was 48 hours.
6. Cycle -3 to -1 represented the addition of FTRW, micro and macronutrients and cycles 0 to 15 represented the addition of FTRW and macronutrients only.

Due to the novel nature of the experimental set-up, limited sources exist wherein the anticipated trends for  $\text{CH}_4$  produced, metals precipitated and adsorbed and free metal ions may be obtained besides that which has been produced by Mathir (2013). From the analysis of the data, the pH per cycle data produces the most reliable trend; this is also due to the technique and accuracy of pH measurement as compared to the measurement of total biogas. A best fit of the experimental data provides the anticipated model pH which is utilised for the biological conversion set-up to generate a set of results that are precisely the experimental pH.

### **4.1.2 BIOLOGICAL CONVERSION**

The ‘biological conversion’ section is important in the construction of the model, this is due to the fact that within an anaerobic digester, the volatile fatty acids undergo a biological process with intermediate steps to a final product of methane gas, carbon dioxide and trace amounts of water vapour ( $\text{CH}_4(\text{g})$ ,  $\text{CO}_2(\text{g})$  and  $\text{H}_2\text{O}(\text{g})$ ). This section serves a purpose of replicating the biological portion of what happens in an anaerobic digester, explicitly showing the extent to

which the volatile fatty acids are converted and how much biomass is generated with the biological conversion.

Even though the processes occurring in the digester may take place simultaneously, it has been assumed for the sake of simpler modelling that the biological conversion occurs separately, after which the distribution of metals (speciation) is modelled.

Due to the existence of a steady state model depicting the anaerobic digestion process with acetate being the main organic contributor, the biological conversion is carried out by a steady state model constructed on Matlab<sup>5</sup> (Brouckaert, 2012). The model makes use of stoichiometry and the extent of the reaction, which is found iteratively such that the calculated model pH agrees with the experimental pH, to output concentrations of the remaining reactants and products (CH<sub>4</sub>, H<sub>2</sub>O (g), CO<sub>2</sub> and biomass) as suggested by Figure 18.

### **4.1.3 INITIAL AND FEED CONDITIONS**

During the construction of a model, it is of utmost importance for the model to represent a real life situation as close as possible. With this in mind, the reactor conditions for the very first cycle (initial conditions) are to represent a real life situation. The initial conditions in the anaerobic digesters were generated by using a model, developed on WEST<sup>6</sup> (Lees, et al., 2012) carrying out the anaerobic digestion of FTRW in a continuous membrane reactor and are cited in Mathir's work (2013), refer to Table 14 in Appendix C. As depicted by Figure 18, the product of the biological conversion for a current cycle is the initial reactor conditions for the succeeding cycle.

The feed composition is calculated by making use of the Sasol recipe found in Table 15, Appendix C. The composition varies with the increase in the amount of NaHCO<sub>3</sub> buffer added across cycles and also between cycle -3 to -1 and cycle 0 to 15 due to the omission of micronutrients for cycles 0 to 15. A fuller explanation and display of results is shown in section 5.2.1 as more work was done on the initial conditions quoted in Appendix C.

### **4.1.4 REACTOR AND REACTION CONDITIONS**

A summary of the conditions in the reactor and for the reaction are listed below in Table 9. The reactor conditions are identical to the anaerobic digesters used in Mathir's experimental work. For the reaction conditions, a stoichiometric reaction with extent is used due to the construction of the Steady state model and due to the presence of experimental data allowing the determination of the extent by iteration. The two main contributors to the volatile fatty acids are narrowed down to acetate and propionate due to their generally higher compositions as compared to valerate and butyrate.

---

<sup>5</sup> Matlab is a simulation package with a user friendly language, it is the most widely used computing language in engineering.

<sup>6</sup> WEST is an open source waste water treatment modelling software



**Table 9: Model Reactor and Reaction Conditions for Washout Experiment by Mathir (2013)**

<b>Reactor Conditions</b>		
Volume	litres	9
Headspace Volume	litres	0.5
Supernatant Liquid Volume	litres	5
Sludge Volume	litres	3.5
Total Cycle Time	hours	48
Reactor Type	-	UASB (Up-flow Anaerobic Sludge Bed)
Temperature	°C	35
Pressure	atm	1

<b>Reaction Conditions</b>		
Volatile Fatty Acids	-	Acetic (CH <sub>3</sub> COOH) and Propionic (CH <sub>3</sub> CH <sub>2</sub> COOH) acid.
Reaction type	-	Stoichiometric with extent of reaction

## 4.2 SPECIATION MODELING

Chemical speciation is referred to as the distribution of an element between the chemical species it may exist in, in a system. Furthermore, analytical methods are used in combination with chemical speciation models to obtain full speciation in systems (VanBriesen, et al., 2010).

Mass balances written out for speciation modelling equate the total concentration of each component in the system, which is normally analytically measured, to the sum of the concentration of the component in each species it exists in. These mass balances have been incorporated into PHREEQC, a programme by which the speciation modelling was done, allowing for all metals to be accounted for in the respective species in which they exist. The mass balances are presented in Appendix B.

### 4.2.1 PHREEQC

The speciation model developed was constructed on PHREEQC, a simulation package used to simulate various reactions and processes in natural waters or laboratory experiments. It was established to allow for the calculation of 'real world' hydro-geochemistry, and carries out the simulation as close as possible to the physical state.

PHREEQC makes use of an input file where the problem statement is specified by making use of KEYWORDS and data blocks. A database file is first read when the simulation is run, this database contains the specification of elements, molecules, protonation, various phases and many other reactions occurring in the water system. The basic output for a simulation is the pH, solution composition of various elements, species distribution (in terms of molality) and the saturation indices of various species.

Various studies carried out on anaerobic digestion, suggest the existence of Fe in its ferrous state, which corresponds to a valence state of 2: Fe<sup>+2</sup>, instead of its ferric state; Fe<sup>+3</sup> (Ogunremi, et al., 2007). Due to PHREEQC having a default coding of the relationship between Fe<sup>+2</sup> and Fe<sup>+3</sup>, where equilibrium is ensured between the two valence states, disequilibrium between the two states was introduced to prevent the automatic oxidisation of Fe<sup>+2</sup> in the model as it would misrepresent the real life system.

The phenomenon of introducing disequilibrium between valence states on PHREEQC is not new. It was implemented in the creation of a speciation model in which disequilibrium between nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>) in an aquifer was necessary, due to equilibrium between the valence states occurring to a negligible extent (World, 1998).

#### 4.2.2 PRECIPITATION MODELLING IN PHREEQC

PHREEQC carries out the mechanisms by which certain minerals precipitate out of solution when they have reached conditions of super-saturation. This is done by equilibrating the solution with “Equilibrium Phases” which are recognized by PHREEQC as the minerals expected to precipitate from the solution.

A careful selection of the minerals likely to precipitate was made, with the aid of MINTEQA2’s CHEMICAL SPECIATION SELF-STUDY WORK MANUAL (Wadley & Buckley, 1997). This manual suggests thermodynamically possible precipitates, in the form of minerals that may form in the temperature range of -100 to 100 °C and at a pressure of approximately 1 atmosphere. A summary of the minerals expected to precipitate in the current study is shown in Table 10. All minerals were allowed to precipitate in the model, of which minerals precipitated were as a result of precipitation requirements being fulfilled.

**Table 10: Minerals likely to precipitate under ambient conditions (-100 to 100C °, 1 atm.) in waste water (Wadley & Buckley, 1997) and PHREEQC database.**

<b>Mineralogical name</b>	<b>Chemical Formula</b>	<b>Comments on occurrence of formation</b>
Covellite	CuS	Produced by treating Copper (II) salts with hydrogen sulphide HS <sup>-</sup> in the following formation reaction: Cu <sup>+2</sup> + HS <sup>-</sup> = CuS + H <sup>+</sup>
Halite	NaCl	Analytically formed by evaporation of a highly saturated saline solution. Ionically formed by reaction Na <sup>+</sup> and Cl <sup>-</sup> in the following formation reaction: Na <sup>+</sup> + Cl <sup>-</sup> = NaCl
Hydroxyapatite	Ca <sub>5</sub> (PO <sub>4</sub> ) <sub>3</sub> OH	Analytically prepared by precipitation from solutions of calcium salts with the addition of ammoniacal phosphate solutions. Ionically formed by reaction Ca <sup>+</sup> ions with phosphate ions in the following formation reaction: 5Ca <sup>+2</sup> + 3PO <sub>4</sub> <sup>-3</sup> + H <sub>2</sub> O = Ca <sub>5</sub> (PO <sub>4</sub> ) <sub>3</sub> OH + H <sup>+</sup>

CoS	CoS	Produced by treating Cobalt (II) salts with hydrogen sulphide HS <sup>-</sup> in the following formation reaction: $\text{Co}^{+2} + \text{HS}^- = \text{CoS} + \text{H}^+$
MnHPO <sub>4</sub>	MnHPO <sub>4</sub>	Analytically produced by adding known concentrations of Mn <sup>+2</sup> to a saturated solution of monopotassium phosphate (MKP) with reaction temperatures ranging between 20 and 60 °C. Ionically formed by reaction Mn <sup>+</sup> ions with phosphate ions in the following formation reaction: $\text{Mn}^{+2} + \text{PO}_4^{-3} + \text{H}^+ = \text{MnHPO}_4$
MoS <sub>2</sub>	MoS <sub>2</sub>	Produced by treating Molybdate (II) ions with phosphate ions in the following formation reaction: $\text{MoO}_4^{-2} + 6\text{H}^+ + 2\text{HS}^- + 2\text{e}^- = \text{MoS}_2 + 4\text{H}_2\text{O}$
Nickel Sulphide	NiS	Produced by treating Nickel (II) salts with hydrogen sulphide HS <sup>-</sup> in the following formation reaction: $\text{Ni}^{+2} + \text{HS}^- = \text{NiS} + \text{H}^+$
Portlandite	Ca(OH) <sub>2</sub>	Formed on addition of water to CaO. Another way is to heat aqueous solutions of calcium salts with alkalis.
Sphalerite	ZnS	Sphalerite is the main form of zinc found in nature. It is ionically produced by treating Zinc (II) salts with hydrogen sulphide HS <sup>-</sup> in the following formation reaction: $\text{Zn}^{+2} + \text{HS}^- = \text{ZnS} + \text{H}^+$
Vivianite	Fe <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> :8H <sub>2</sub> O	It is found with iron, copper and tin ones. It may occur in clay, and especially in bog iron-ore. It is ionically formed by reaction Fe <sup>+2</sup> ions with phosphate ions in the following formation reaction: $3\text{Fe}^{+2} + 2\text{PO}_4^{-3} + 8\text{H}_2\text{O} = \text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$

#### 4.2.3 ADSORPTION MODELLING IN PHREEQC

The main drive of the current study lay in the addition of an adsorption phase in the existing precipitate-controlled speciation model, as mentioned in the “Gap Analysis” section. From Mathir’s study, the experimental data suggested that there was another phase, besides the precipitates, into which the metals are bound. PHREEQC is able to allow for the addition of an adsorption phase by allowing metals to react with an ionic surface, therefore, an ionic representation of the biomass surface onto which the metals may adsorb (“react”) was essential. Furthermore, the interaction between the ionic representation of biomass and metals in the form of complexation needed to be modelled adequately.

#### 4.2.3.1 IONIC REPRESENTATION OF BIOMASS IN SLUDGE

A study was conducted by Artola (1997) and co-workers where the well-known glycine-metal system was compared to the sludge-metal system. The bio-sorption process as previously stated deals with the cellular wall; it has been found that amino acid groups existing in the cell wall are the most active binding sites in the sludge. Furthermore the common abundant amino acid in the cell wall is glycine (Artola, et al., 1997), this supports the use of glycine in the study undertaken to compare it with sludge.

Westergreen et al (2012) did a follow-up study on the work carried out by Artola and co-workers (1997) by determination of the effect of biomass on the buffer capacity of a solution comprised of mixed ions, using a **glycine** equivalent. In this study, a model was constructed where the biomass was represented by glycine, and the hydrogen affinity was modelled as a function of pH. The model was compared to titrations of strong acid against a solution containing anaerobic sludge, but the model and experimental data were found to differ significantly.

Thereafter, a new component, UKZiNe (pr. You-ka-zeen) was formulated to describe the acid adsorption characteristics of the sludge. This was effectively an ionic representation of the sludge surface and was found to describe the data well when it was formulated to consist of two carboxylic acid groups, a phosphate group and an ammonium group per amount of volatile solids (Westergreen, et al., 2012).

Westergreen's work made use of mixed salts and prepared solids from municipal anaerobic digester sludge. The mixed salts were calcium, magnesium, ammonia, carbonate, phosphate and acetate. The pH and conductivity were measured and were compared to model predicted data, where the UKZiNe formulation was incorporated. The model included 13 ionic components for the representation of the solution composition, including  $H^+$ ,  $Na^+$ ,  $K^+$ ,  $Ca^{+2}$ ,  $Mg^{+2}$ ,  $NH_4^+$ ,  $Cl^-$ ,  $Ac^-$ ,  $Pr^-$ ,  $CO_3^{-2}$ ,  $SO_4^{-2}$  and  $PO_4^{-3}$ . This meant that the resultant pH was also indicative of the interaction of these compounds as they were included in the model solution composition.

From the titration curves constructed, it was evident that the UKZiNe fitted closely to the biomass experimental data in the pH range of 5 to 8.5 (Westergreen, et al., 2012), which is the range of interest in anaerobic processes. This study indicated that glycine did not represent the proton affinity of sludge well across a range of pH values, but UKZiNe did. The model included the above stated components in the solution composition, which are analogous to components in the nutrient recipe, to predict pH and the conductivity made use of in the UKZiNe formulation. The UKZiNe formulation was incorporated in the current study. Details of the UKZiNe molecule are explained in Table 11:

**Table 11: Comparison between UKZiNe and Glycine Characteristics (Westergreen, et al., 2012)**

<b>Group</b>	<b>Glycine mmol/g VS</b>	<b>pKa (at 25°C)</b>	<b>UKZiNe mmol/g VS</b>	<b>pKa (at 25°C)</b>
COOH1	13.146	2.350	0.170	4.350
COOH2	-	-	0.170	5.650
NH <sub>4</sub> <sup>+</sup>	13.146	9.778	0.076	9.244
HPO <sub>4</sub> <sup>2-</sup>	-	-	0.021	7.198

The UKZiNe ionic representation of biomass was incorporated into PHREEQC by making use of its characterisation as shown in Table 11. It provided an ionic acceptor for the positively charged metal cations, thereby allowing the formation of complexes between the biomass (organic content) and the metal ions.

#### **4.2.3.2 COMPLEXATION BETWEEN IONIC REPRESENTATION OF BIOMASS AND FREE METALS**

Westergreen's literature survey showed that an anaerobic sludge generally includes the carbonate, phosphate, ammonia, acetate and water weak acid-base subsystems (Westergreen, et al., 2012). Westergreen's study also revealed the existence of common functional groups between UKZiNe and glycine, justifying the initial comparison of biomass with glycine. The UKZiNe formulation was fitted to the experimental data by adjusting the concentrations of the components representing the functional groups in the biomass (Westergreen, et al., 2012).

Due to glycine and UKZiNe having the same ionic functional groups and complexation taking place between the ionic functional groups (most active binding sites in sludge) and metals, equilibrium constants used for complexes between the metal ions and the functional groups were that of glycine. Furthermore, thermodynamic data for glycine aqueous chemistry for both protonation and **complexation** reactions is well established. The equilibrium constants between metals and glycine ionic functional groups have been listed in Appendix D, Table 17. The intention was to regress for the equilibrium constants to better represent complexation reactions of metals with UKZiNe against experimental data; however, this was not achieved. This is further explained in the results section.

The complete model was constructed by using both Matlab and PHREEQC, and the model algorithm is discussed in detail in the next section – "Model Algorithm".

### 4.3 MODEL ALGORITHM

The washout experiment carried out by Mathir, involved dosing of FTRW with a micronutrient recipe for cycles -3 to -1, and omission of the nutrient recipe from cycles 0 to 15. For each cycle, the mixture was allowed to equilibrate, after which a litre of effluent was decanted and replaced with a litre of fresh feed. Chemical extraction of metals in each phase was carried out to quantify the metals partitioning in the soluble and additional phases. Each cycle of the experiment was therefore made up by biological conversion of the organic material to form products as well as metal speciation in the sludge. The model was constructed in two parts – the biological conversion section which was completed by making use of a Matlab model and a metal speciation section which was completed using PHREEQC.

The biological conversion section carried out in Matlab, made use of the following inputs; initial reactor conditions (mol/l), temperature, pressure, reactor volume and the experimental pH. The model was constructed to allow for the biological reaction to take place at a certain extent of reaction, which was the variable adjusted to fit the resultant model pH to the experimental pH. The experimental pH gave a more accurate and fuller experimental set, to which the model could be compared for the best representation of the experimental set-up conditions. Once the difference of the experimental and model pH per cycle was minimal, the biological conversion section of the model produced the composition and conditions of the resultant stream: concentrations of reactants and products (mol/l) as well as the pH.

It should be noted that in the biological conversion section of the model, the effect of the addition of the  $\text{NaHCO}_3$  buffer was considered by reflecting the concentration added in a cycle in the feedstock, such that the  $\text{NaHCO}_3$  formed part of the reactants.

In this biological conversion section, the metals in the beginning and the end of each cycle remained unchanged as there was no speciation taking place in the Matlab section of the model. Cycles were interlinked in the biological conversion section of the model to reflect the decanting of 1 litre of the supernatant liquid from the previous cycle (cycle  $i$ ) and replacement of the 1 litre with fresh feed of the following cycle (cycle  $i + 1$ ). This biological conversion algorithm is shown by the **MATLAB** part of Figure 18 and produced a total of 19 stream compositions for cycles -3 to 15, which served as inputs into the metal speciation part of the model.

PHREEQC was used to carry out the metal speciation section of the model with the inputs being the molalities of the resultant biological conversion section for reactants, products and metals as well as the pH. In the PHREEQC model, a list of “equilibrium phases” is introduced, which comprises of minerals likely to precipitate out of solution if the conditions allow. Formation of the equilibrium phases essentially meant formation of precipitates, bringing forth the precipitates phase model (P-Model). The model was extended to include an ionic representation of the sludge

as well as adsorption (complexation) reactions of metals with ionic functional groups on the sludge, allowing for the representation of the effect of adsorption of metals on the sludge. The extended model brought about the Adsorbed and Precipitates phase model (AP – Model). The data produced by the PHREEQC model was a resultant speciation data set of metals per cycle for both the P-Model and AP-Model for the soluble phase, precipitates phase and the adsorbed phase.

Figure 19 shows the algorithm used to test the hypothesis. It should be noted that Mathir's experimental work was used for the direct comparison with model generated data, the speciation results from other sources were merely used to give direction as they were used to put the literature review together and form the main driver of the study. A directional comparison was therefore given by the work of these authors for more surety of the conclusions to be drawn from the study.

The comparisons to answer the hypothesis thereafter included diagrams showing the experimental data per cycle, P-Model data per cycle and AP-Model data per cycle. Due to the best and fuller experimental data for the speciation model being the metals left in the sludge as opposed to the supernatant liquid, the model results of metals in sludge were compared to the experimental results. In the model results, the metals in sludge comprised of precipitated metals and adsorbed metals. The results section therefore compared the trends of the experimental sludge metal concentrations, P-Model sludge metal concentrations (precipitates) and AP-Model sludge metal concentrations (precipitates and adsorbed metals) to answer the hypothesis. A full set-up of results is shown in the next section titled the Results section.

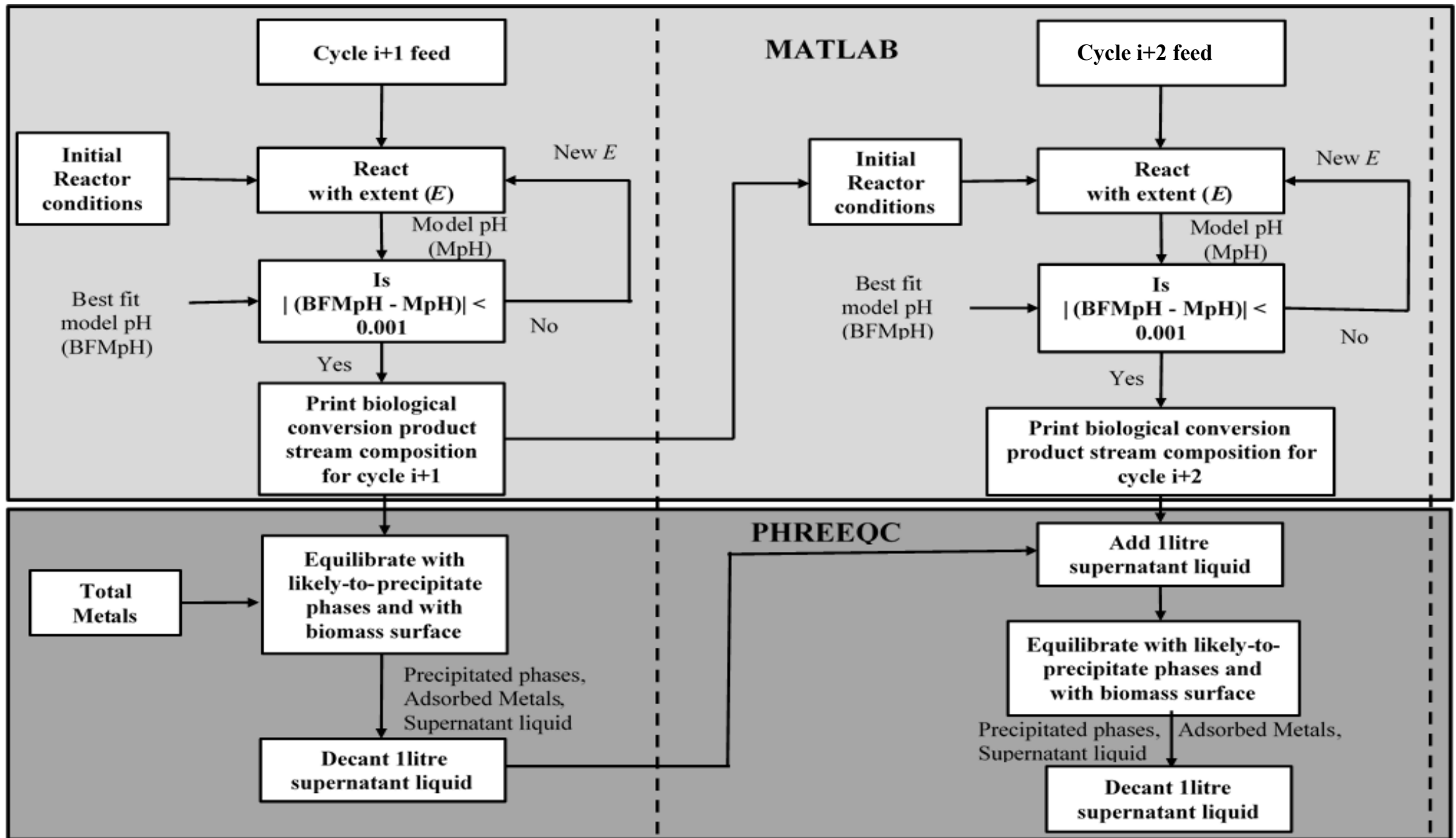


Figure 18: Overall Speciation Model Algorithm



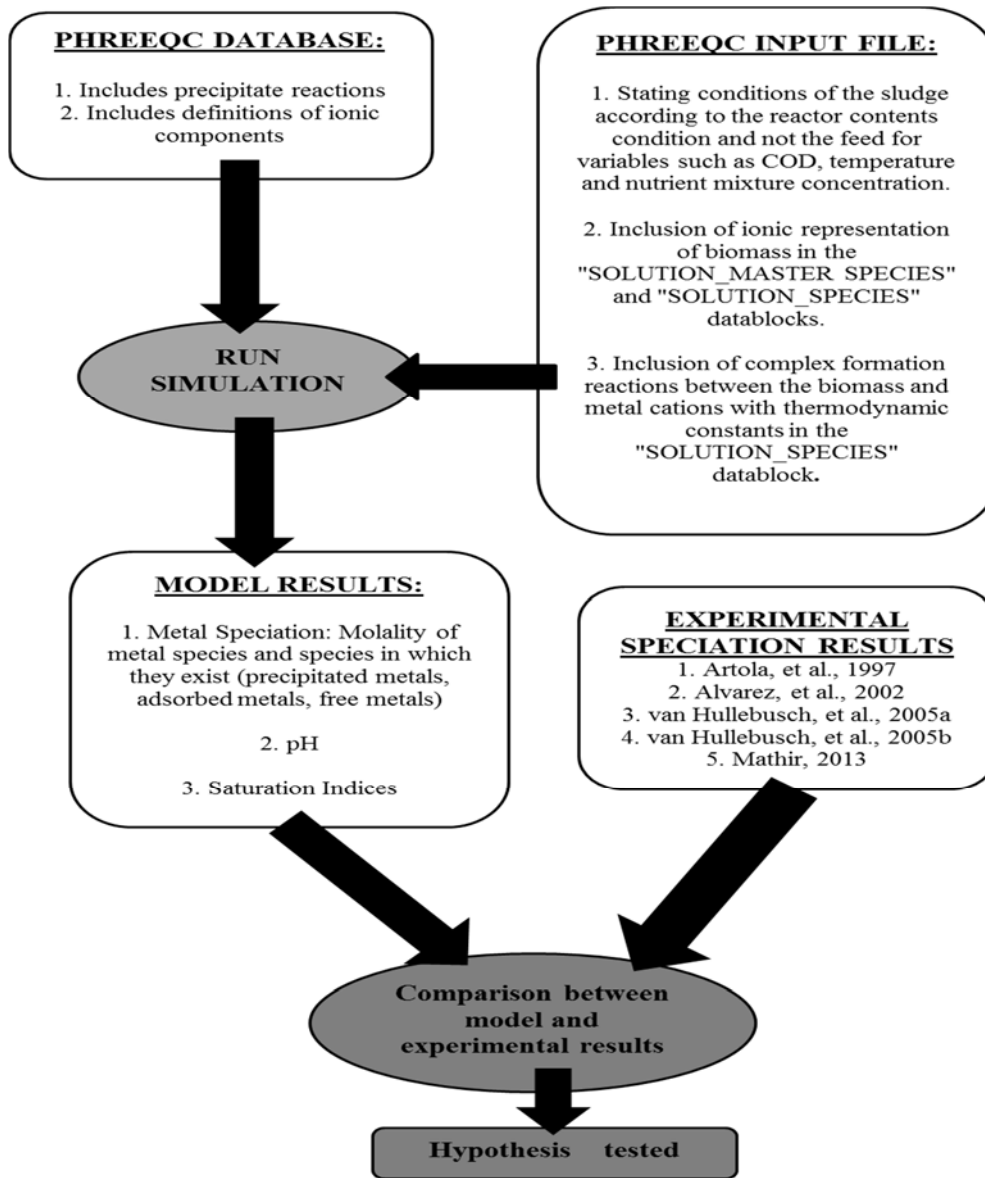
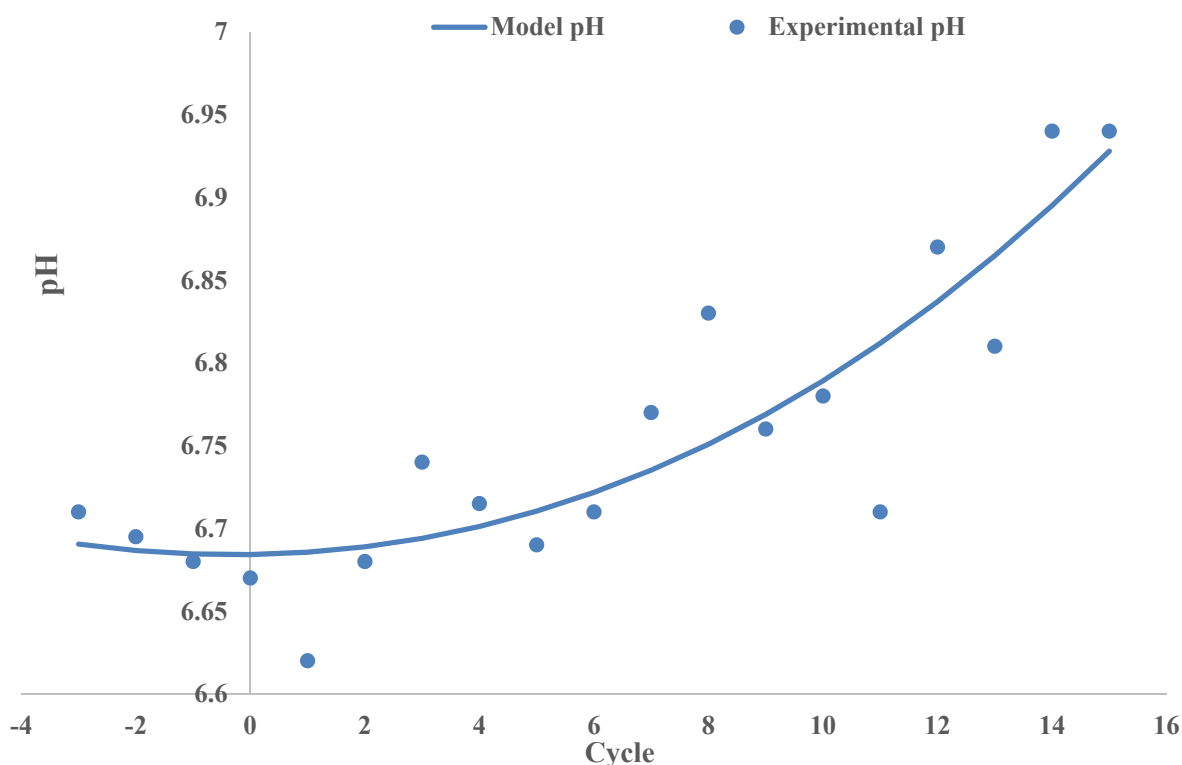


Figure 19: Model construction algorithm for AP-Model and Hypothesis Testing

## 5. RESULTS

### 5.1 BIOLOGICAL CONVERSION OF ORGANIC CONTENT IN FTRW

As indicated in the model algorithm section, a portion of the model was implemented in Matlab, where the biological conversion of the organic matter (Acetic Acid and Propionic Acid) to  $\text{CH}_4$  was carried out. At every cycle, the model pH was iteratively matched to the experimental pH (Table 16, Appendix C) by changing the extent of the reaction to generate Acetate<sup>-</sup> and Propionate<sup>-</sup> concentrations. Figure 20 shows the model and experimental pH, where a general increasing trend in the pH can be seen which is to be expected; as the anaerobic digester contents become less acidic the more the acetic and propionic acids were biologically converted into  $\text{CH}_4$ .

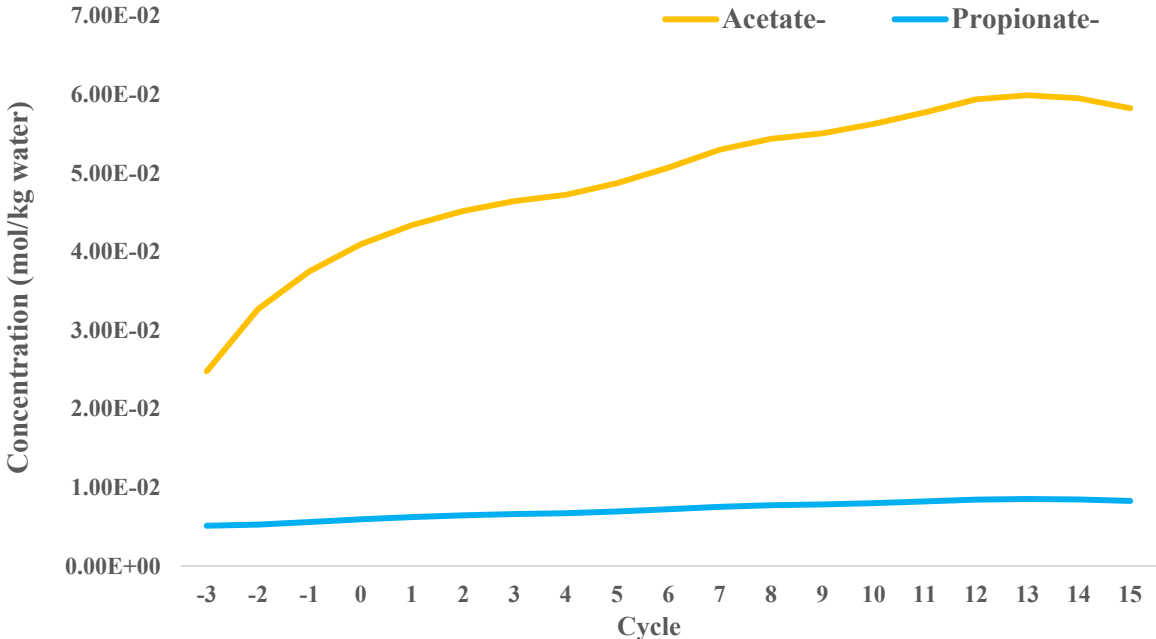


**Figure 20: Experimental and Model pH per nutrient washout experiment cycle**

The prediction of the percent conversion of Acetate<sup>-</sup> and Propionate<sup>-</sup> depends on the experimental pH at the end of each cycle. These concentrations are increased at the beginning of each cycle by addition of the feed, therefore during every cycle there is biological conversion that takes place. Figure 21 depicts the trend followed by model predicted acetate and propionate concentrations. From Figure 21, an increase in the amount of Acetate<sup>-</sup> and Propionate<sup>-</sup> in the system is seen due to a decrease in the amount of acids biologically converted from one cycle to the next.

Due to an increase in the amounts of acid in the system, a decrease in the pH is anticipated as the environment becomes more acidic. However, the decreasing concentration profile gradients are less steep with each cycle and their overall effect is not reflected in the pH profile, which shows a continuously increasing pH trend.

The continued pH increasing trend is mainly due to the increasing  $\text{HCO}_3^-$  content, which provides the system with alkalinity and is more dominant than the slight decreasing trend of Acetate and Propionate acids towards the end of the experiment.



**Figure 21: Concentration profile of Acetate<sup>-</sup> and Propionate<sup>-</sup> during the nutrient washout experiment**

## 5.2 EFFECT OF ADDITIONAL PHASES ON SOLUBLE PHASE

Initial conditions, have a great effect on the representation of the behaviour of the metals in the system, and it was necessary to have the same initial reference point between the model and experimental data, to allow for extensive comparison between the two. This was the first optimisation step taken in modelling of the washout experiment.

### 5.2.1 SLUDGE CONCENTRATIONS – Initial Conditions

The initial conditions were first set-up by allowing equilibrium to form between the anaerobic digester conditions quoted in Table 14, Appendix C together with the first feed of the nutrient recipe shown in Table 15, Appendix C. In Mathir's work, referring to Table 18, Appendix E; a fuller experimental data set existed for measurement of metals remaining with the sludge biomass as compared to those in the soluble phase due to most soluble phase concentrations being below detectable limits (Mathir, 2013). Availability of experimental data for metals in sludge as opposed to soluble phase concentration warranted consideration of sludge concentration as a comparison to the model, to aid in finding the initial conditions of the system as a whole.

A precipitate forms in a system when a solution is supersaturated with respect to certain minerals and the conditions are such that the crystallisation process can be induced. Since the concentration of metals in the soluble phase underpin the saturation and unsaturation of a specific metal; for metals saturated in the soluble phase in cycle -3 and given sufficient sulphide and phosphate in the system, minerals form in this initial cycle.

It was expected to find precipitated metal deposits together with metals adsorbed onto the biomass surface in the sludge. The model sludge concentrations were therefore obtained by addition of metals found in the precipitates with adsorbed metals for each cycle. In the first step of iteration, the model was allowed to form initial precipitates and adsorbed metals, without any limitations enforced on the metal speciation after which a comparison between model and experimental results for cycle -3 was done. Deviations between experimental and model results were found to range between 60% and 70%. Such a high deviation called for a continued iteration procedure to be carried out to allow for alignment between the experimental and model sludge conditions.

The final initial conditions were thereafter obtained by fitting the model results to the experimental results, and this was carried out by adjusting the **precipitates** forming in the beginning of the experiment such that the initial sludge concentrations match the experimentally measured concentration.

The precipitates forming in the first cycle of the system were regressed for by adjusting the concentration of the anions with which they react to form precipitates in succession – particularly the sulphide ( $\text{HS}^-$ ) and phosphate ( $\text{PO}_4^{3-}$ ) ions.

**Table 12: Results of Initial Conditions Iteration**

<b>Initial Conditions</b>			
	<b>Experimental (mg/L)</b>	<b>Model (mg/L)</b>	<b>Deviation (%)</b>
$\text{Co}^{+2}$	0.186	0.192	3.19%
$\text{Mn}^{+2}$	1.471	1.229	16.49%
$\text{Zn}^{+2}$	3.936	3.568	9.33%
$\text{Fe}^{+2}$	13.859	7.128	48.57%
$\text{Cu}^{+2}$	0.225	0.181	19.29%
$\text{Ca}^{+2}$	3.388	3.073	9.30%
	<b>Average</b>		<b>17.70%</b>

The average deviation between experimental and model for cycle -3 is 17.7%, with  $\text{Co}^{+2}$ ,  $\text{Zn}^{+2}$  and  $\text{Ca}^{+2}$  metals being lower than 10% as shown by Table 12 above. Compared to initial deviations of between 60 and 70%; a deviation of less than 20% qualified as a minor deviation. These minor deviations between experimental and model metal concentrations in sludge warranted the continuation with regressed initial conditions. This allowed for the extension of the model to represent the system soluble, precipitates and adsorbed phase conditions for subsequent cycles given sufficient alignment of the initial conditions.

An interesting and pivotal aspect of the study was found during obtaining of the initial conditions: the model predicts that a large proportion of the metals found in the sludge are in the form of precipitates, particularly sulphide precipitates. Concentration of sulphide brings about the precipitation of metals to form sulphide minerals which greatly displace the metals from the soluble phase. Due to a significant effect of sulphide, a sensitivity analysis of sulphide on the soluble, adsorbed and precipitates phases was carried out.

### 5.3 SULPHATE-SULPHIDE SYSTEM

From studying the nature of the system in question, the following suggests considerable uncertainty about the presence of sulphide in the system:

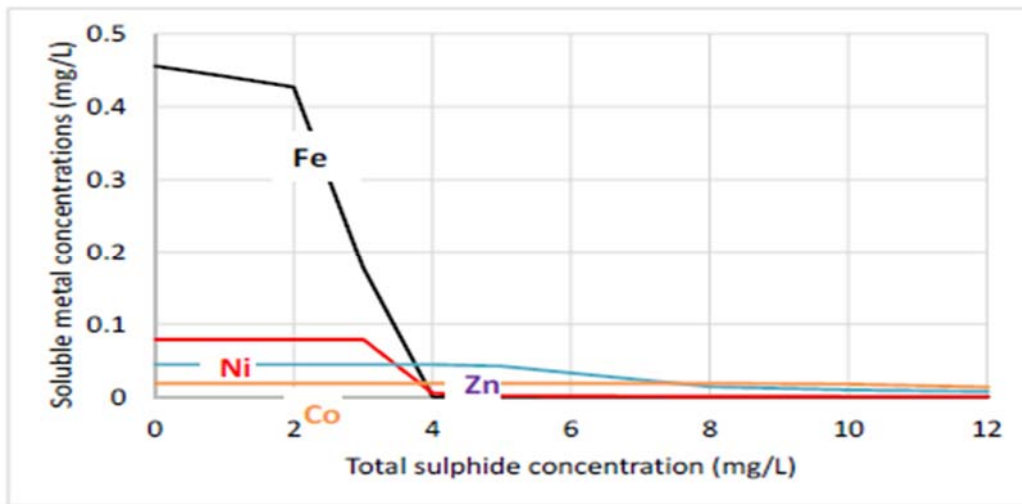
- $\text{SO}_4^{2-}$  was dosed into the system, and in order for sulphide to occur, sulphate reducing bacteria were required to be present. If present, sulphate reducing bacteria compete with the methanogens for acetate. Methanogens only require sufficient acetate concentration compared to the sulphate reducing bacteria that requires sufficient acetate and sulphate concentration.
- High initial conversion of acetate and propionate to methane,  $\text{CO}_{2(g)}$  and  $\text{H}_2\text{O}_{(g)}$  is shown by model generated data (using experimental pH values), suggesting that the methanogens are favoured instead of the sulphate reducing bacteria. Experimental data implies low sulphide concentrations during cycle -3 (due to prioritisation of methanogens) and the model predicts sulphide precipitates to form in the same cycle, suggesting opposing behaviours.
- PHREEQC has a default reaction of sulphide formation from sulphate, however, in the case of the current experimental set-up; a biological reaction; this reaction forms when sulphate-reducing bacteria is present and thrives. The presence of sulphate-reducing bacteria needs to be experimentally determined.

#### 5.3.1 SENSITIVITY OF SOLUBLE, PRECIPITATES AND ADSORBED PHASE TO SULPHIDE CONCENTRATION

The behaviour of the sulphate-sulphide system was investigated by making use of the model by studying the behaviour of the soluble, precipitates and adsorbed phase at various concentrations.

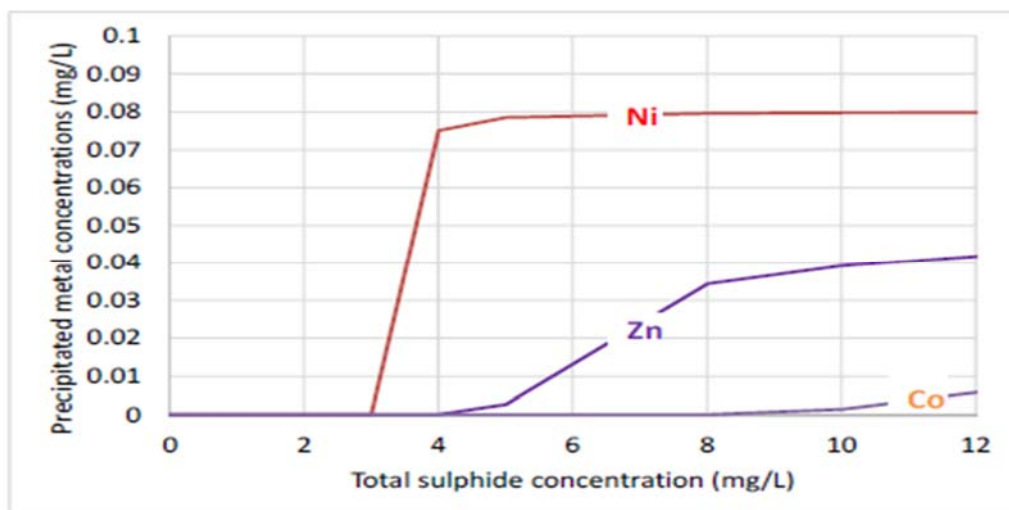
Figures 22, 23 and 24 show the relationship between soluble phase concentration, precipitate formations and adsorption of the metal sulphide precipitates. The concentration of sulphide has a great impact on the formation of Ni, Co and Zn precipitates (Figure 23). The precipitation of the trace metals is determined by the solubility product of the metal ion and the sulphide ion. The solubility product for iron is the lowest for the base metals and hence FeS is normally the first ion to precipitate. Iron precipitation limits the increase in sulphide concentration until most of the iron is precipitated. Nickel is the next to start precipitating, while there is still some iron left as depicted by Figure 22.

Figure 24 shows that as the concentration of sulphide in the system increases, the extent of adsorption of Ni, Co and Zn decreases. This effect is a result of the formation of precipitates, which essentially displace metals from the soluble to the precipitates phase. The reduction of concentration in the soluble phase causes desorption.

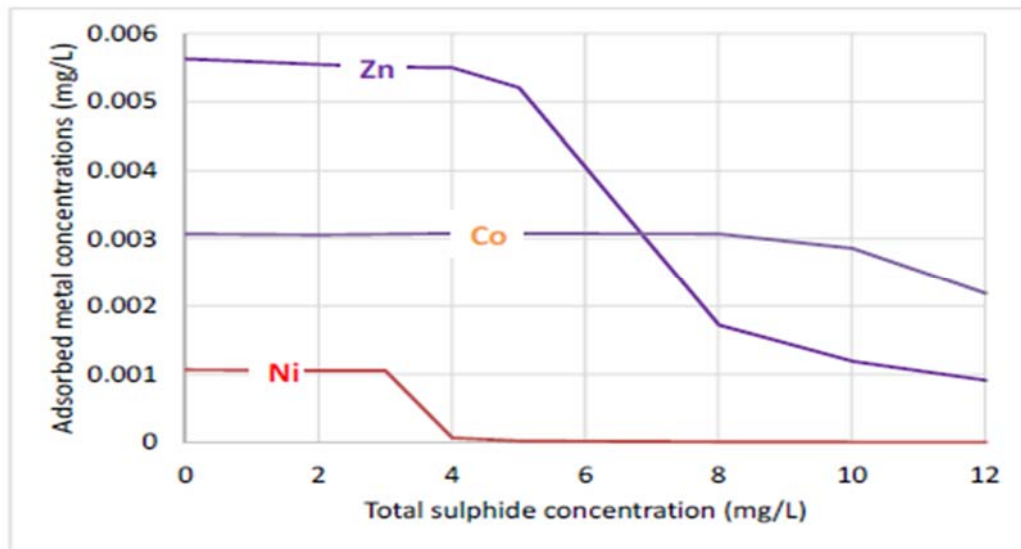


**Figure 22: Model predictions of soluble metal concentrations for the Pilot Plant (Ntuli & Brouckaert, 2014)**

Due to the formation of the precipitates and adsorption of the metals affecting the metal concentration in the soluble phase, as shown by Figure 22, the concentration of the sulphide in the system therefore impacts the soluble phase concentration of the metals. The significant change in soluble metal concentration as a result of the sulphide concentration clearly points to the dependence of the bioavailability of the metals on the concentration of sulphide in the system.



**Figure 23 : Model predictions of precipitated metal concentrations for the Pilot Plant (Ntuli & Brouckaert, 2014)**



**Figure 24: Model predictions of adsorbed metal concentrations for the Pilot Plant (Ntuli & Brouckaert, 2014)**

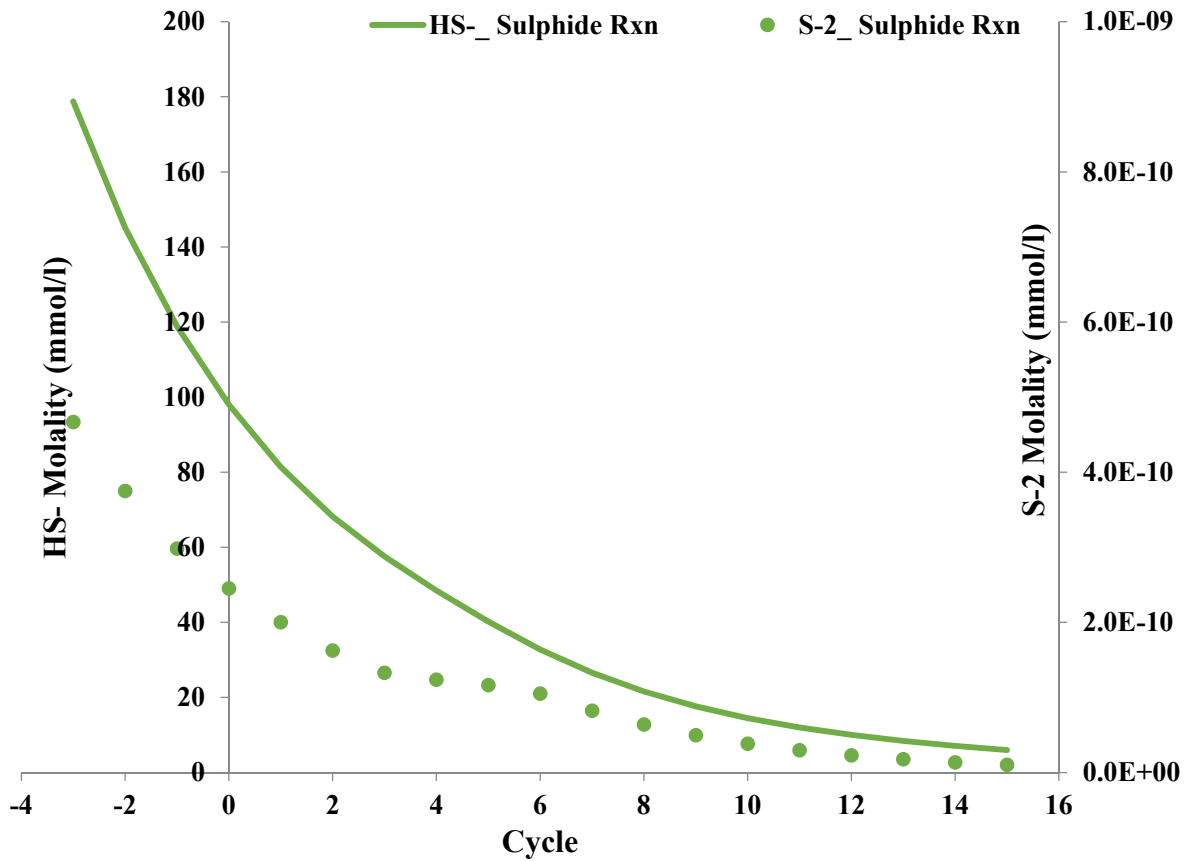
### 5.3.2 WASHOUT EXPERIMENT AT DIFFERENT SULPHIDE CONCENTRATIONS

Due to the sulphide concentration having an impact on the bioavailability of metals in the system, the effect of the sulphide concentration **on the washout experiment** was tested. The AP model with equilibrium conversion of sulphate to sulphide shows the one extreme, where the sulphide concentration is generated by the reduction of the sulphate dosed with the micronutrient recipe as per PHREEQC reaction referenced in Appendix F, reaction E-2.1. For this case, no restrictions were placed on the amount of sulphide formed as a result of the reduction of sulphate present in the system. The sulphide concentrations were not capped, to allow for representation of the metal partitioning when the model is allowed to freely predict sulphide concentration as per equilibrium concentrations.

To represent the opposite extreme, total sulphate and sulphide in cycle -3 is represented as sulphate and the conversion of sulphate to sulphide was prohibited in the model. In the extreme of no sulphide ion formation; the only precipitates that form are phosphate and carbonate precipitates.

It is to be noted that PHREEQC has a distinction between components which are used to specify the material content of the solution, and species which are the chemical forms actually present in solution. The graph below shows the distinction between the molalities of the HS<sup>-</sup> component and the S<sup>-2</sup> species; the S<sup>-2</sup> species is responsible for bringing about precipitation of some metal ions out of solution. Figure 25 depicts the underlying sulphide (S<sup>-2</sup>) concentration for the one extreme calculated by the speciation routine; with sulphide present in the system.

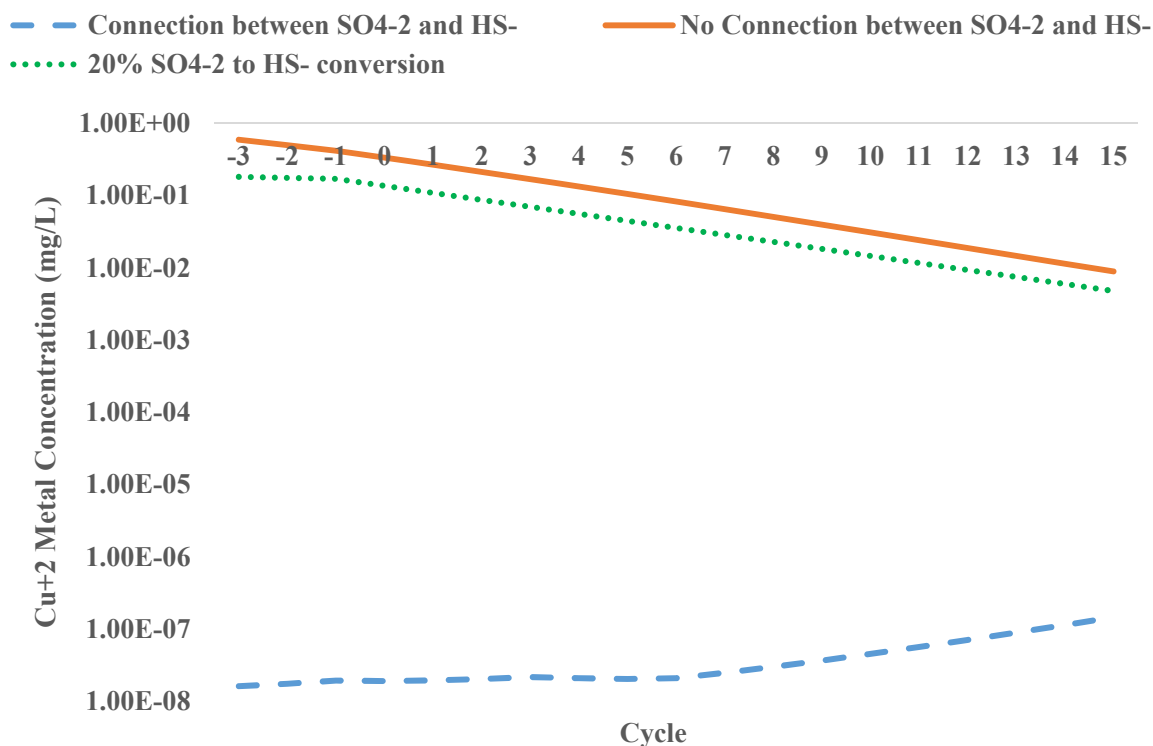




**Figure 25: Concentration of HS<sup>-</sup> and S<sup>-2</sup> when the PHREEQC reduction reaction is permitted**

The next two Figures depict the behaviour of the **soluble phase** concentration for the two sulphide concentration extremes as well as a trend where the conversion of sulphate to sulphide is restricted to 20%. It is expected that there will be a greater extent of precipitation of metals when there is sulphide present in the system, correlating to a lower soluble phase concentration.

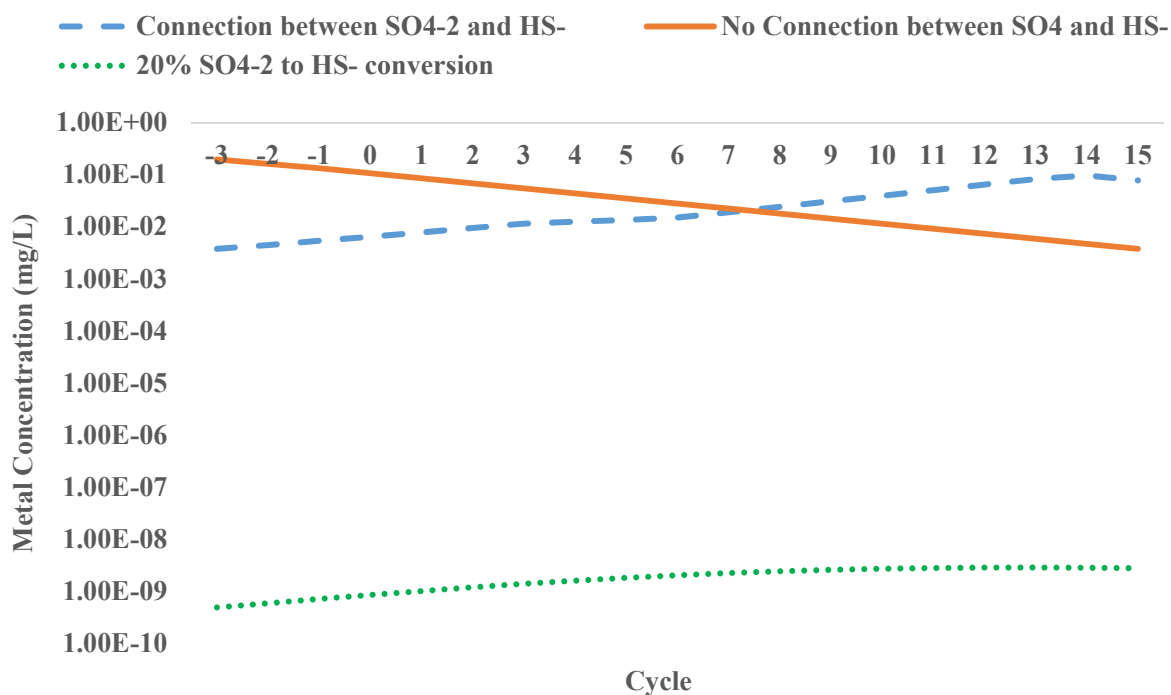
Figure 26 below, shows soluble Cu<sup>+2</sup> would decrease as from cycle -3, with a steeper decrease as from cycle 0 which correlates to the cycle where there is no addition of the micronutrient recipe. It is expected that the soluble phase concentration will be considerably higher for the model with no sulphide as no metals are trapped in the precipitates phase, resulting in increased bioavailability as per FIAM suggestions.



**Figure 26: Soluble Cu<sup>2+</sup> concentration in the AP-Model at different conversions of SO<sub>4</sub><sup>2-</sup> to HS<sup>-</sup>**

Comparing the two extremes, a total opposite direction of soluble phase concentration is seen where sulphide is present in the system; the sulphide precipitates appear to be a reservoir from which the metals re-solubilise into the soluble phase, resulting in a gradual increase in the soluble phase concentration. A logarithmic scale is used to depict this as the concentrations are all minute for the micronutrients, showing the re-solubilisation to have a minimal affect in comparison to the metals being entrapped due to precipitation. For Cu<sup>2+</sup>, the sulphide-less model soluble phase concentration is higher for the whole washout experiment, showing greater partitioning of Cu<sup>2+</sup> in the soluble phase when there is no sulphide present in the system.

Figure 27 shows a prediction of cobalt concentration, when there is no sulphide in the system. There are opposing relationships depicted in the soluble phase concentration when there is no sulphide in the system as the trends cross each other, as shown above. The two extremes depicting significantly different trends further emphasises the importance of knowing the sulphide concentration in the real life system, allowing for correct modelling of the sulphate-sulphide relationship.



**Figure 27: Soluble Co<sup>2+</sup> Concentration in the AP-Model at different conversions of SO<sub>4</sub><sup>2-</sup> to HS<sup>-</sup>**

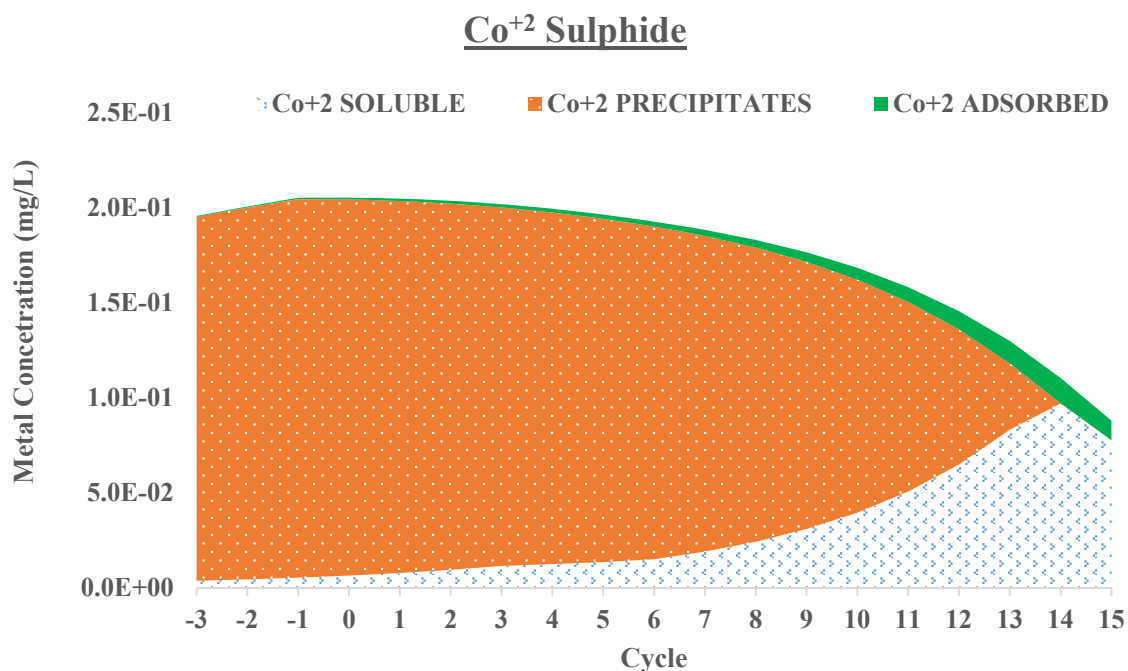
The trends for 20% conversion of sulphate to sulphide for Cu<sup>2+</sup> and Co<sup>2+</sup> do not show consistency in terms of their position with respect to the equilibrium soluble phase concentrations. The equilibrium soluble phase concentrations are essentially the soluble concentrations where the relationship between sulphate and sulphide is calculated by PHREEQC, which assumes equilibrium at each cycle. PHREEQC takes redox equilibrium into consideration, which for the current study, the reduction of acetate to methane has an impact of redox equilibrium.

The redox potential due to the conversion of acetate is important, giving direction to redox equilibrium which has an effect on equilibrium conversion. No redox potential measurements were made during the experimental work to offer guidance on the modelling of redox conditions in the solution. PHREEQC assumes redox equilibrium for all reactions and species, which may not necessarily be the case for industrial processes. Furthermore, there are some redox reactions that may not reach equilibrium as assumed by PHREEQC.

To accurately account for redox equilibrium and the impact it has to the calculation of redox potential, it is essential for all redox reactions in the system to be quantified and experimentally determined. In Appendix F-2, it can be seen that the reduction of sulphate to sulphide is a redox reaction, one of which plays a crucial role in the current study. Successful prediction of redox potential will aid in correct prediction of equilibrium sulphate and sulphide concentrations in the system. Clear quantification of the sulphate prior cycle -3 and throughout the washout experiment will be obtained, assisting in resolving uncertainties around existence of sulphate in the system, or lack thereof.

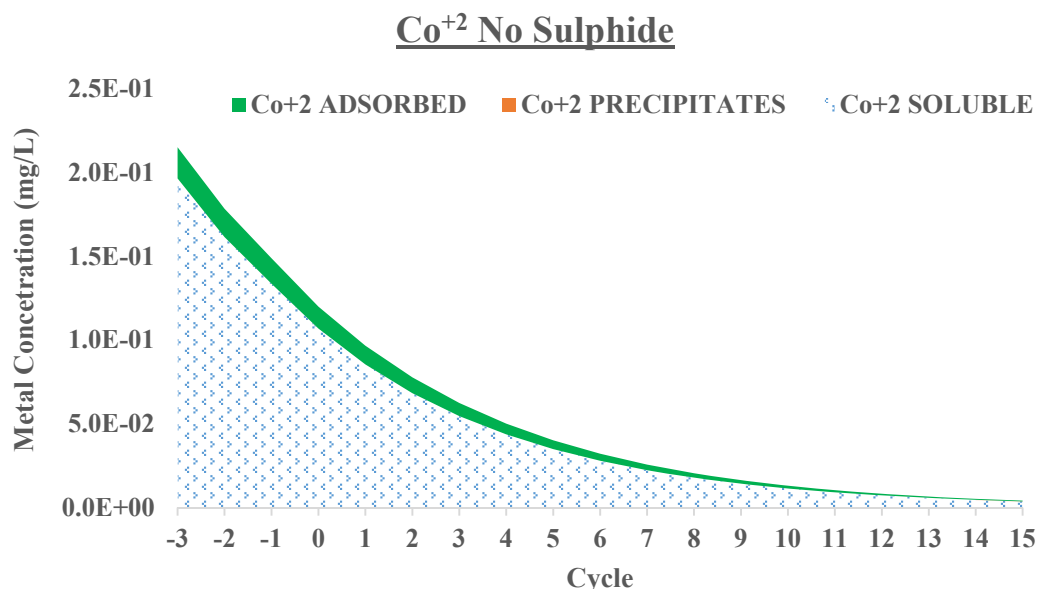
### 5.3.3 METAL PARTITIONING AT THE TWO SULPHIDE CONCENTRATION EXTREMES

This section serves to show the significance of the presence of sulphide and lack thereof; on the **partitioning** of the metals in the various phases in which they exist. Correct partitioning of metals in anaerobic digestion is the main objective of this speciation model and if a significant change is brought about by the status of the sulphide in the system; the lack of any knowledge of the sulphide in the system qualifies as a major limitation of the model to represent metal partitioning in the current system. The sulphide extremes correlate to the trends shown in Figures 25 and 26 in section 5.3.2.



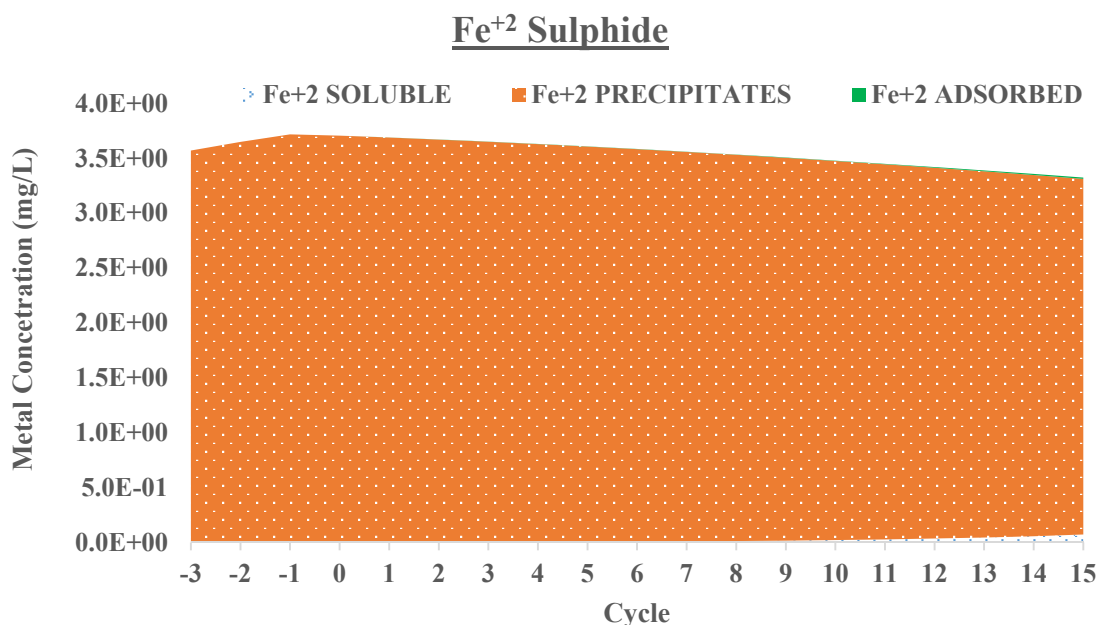
**Figure 28: Model prediction of Partitioning of  $\text{Co}^{+2}$  in the Soluble, Precipitates and Adsorbed Phases with Sulphide present in the system**

Figure 28 and 29 show there is significantly more  $\text{Co}^{+2}$  in the soluble phase for the model without sulphide in the system as also shown in the previous figures. Furthermore, when there is no sulphide present in the system, no sulphide precipitate forms and there is a significant increase in the metals adsorbed onto the sludge surface at the beginning of the experiment. The total metal concentration decreases steeply when there is no sulphide in the system, this is due to more metals being in the soluble phase, from which the decanted medium is taken. This results in a greater effect of the decanting on the system as the metals are predominantly in the soluble phase.



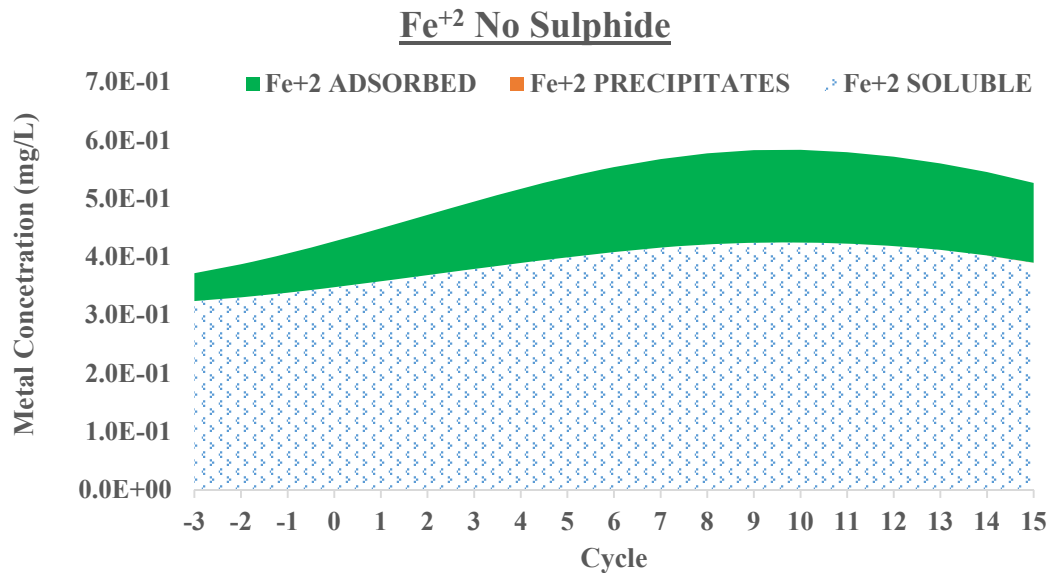
**Figure 29: Model prediction of Partitioning of Co<sup>+2</sup> in the Soluble, Precipitates and Adsorbed Phases with no Sulphide present in the system**

In the model where there is sulphide in the system, the precipitates; particularly the sulphide precipitates are the controlling factor, however, in the model where there is no sulphide in the system, the adsorbed phase has a significant controlling factor for metals displaced from the soluble phase. Appendix G shows the precipitates phase to be the controlling phase for all metals when there is sulphide present in the system; where the precipitates gradually re-solubilise into the soluble phase resulting in an increase in the soluble phase concentration.



**Figure 30: Model prediction of Partitioning of Fe<sup>+2</sup> in the Soluble, Precipitates and Adsorbed Phases with Sulphide present in the system**

Figures 30 and 31 show a similar trend of the shift from the precipitates to the adsorbed phase being the controlling phase when there is no sulphide in the system. Furthermore, Figure 30 shows the great extent to which the adsorbed  $\text{Fe}^{+2}$  increases when there is no sulphide in the system. With  $\text{Fe}^{+2}$  having higher concentrations in the nutrient recipe as well as its presence due to use of an  $\text{Fe}^{+2}$  catalyst; it is expected to have a significant amount of  $\text{Fe}^{+2}$  partitioned in the soluble and adsorbed phases, as seen in Figure 31.



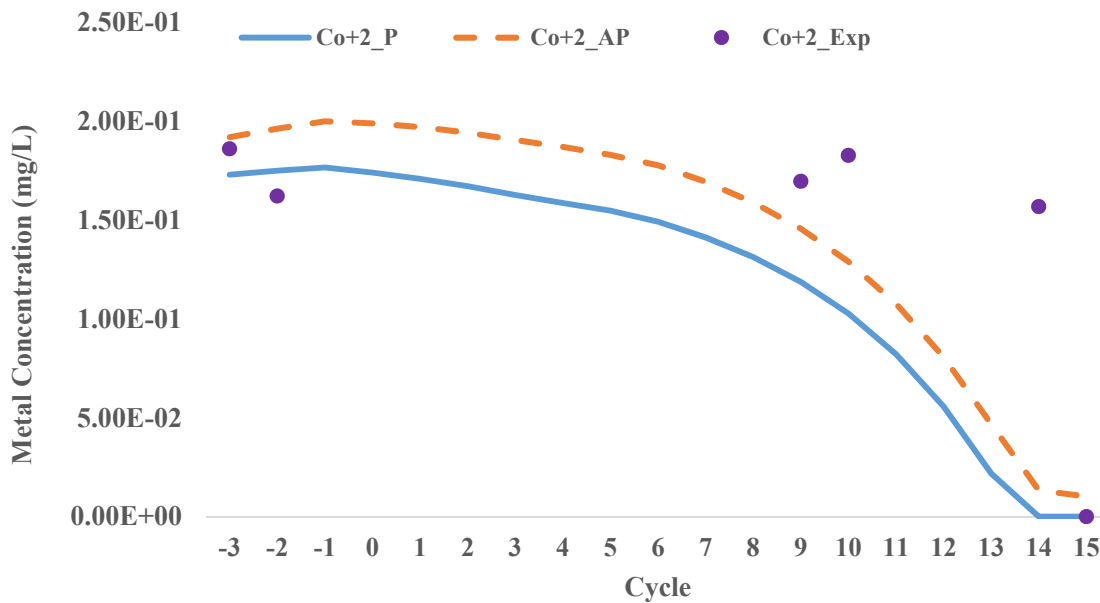
**Figure 31: Model prediction of Partitioning of  $\text{Fe}^{+2}$  in the Soluble, Precipitates and Adsorbed Phases with no Sulphide present in the system**

Figure 31 shows  $\text{Fe}^{+2}$  to increase in concentration for the adsorbed and soluble phase till cycle 9 after which it plateaus and decreases. This is an indication of the behaviour for a metal in a continuous anaerobic washout experiment where dosing is initially done in higher quantities.

### 5.3.4 COMPARISON BETWEEN MODEL AND EXPERIMENTA DATA

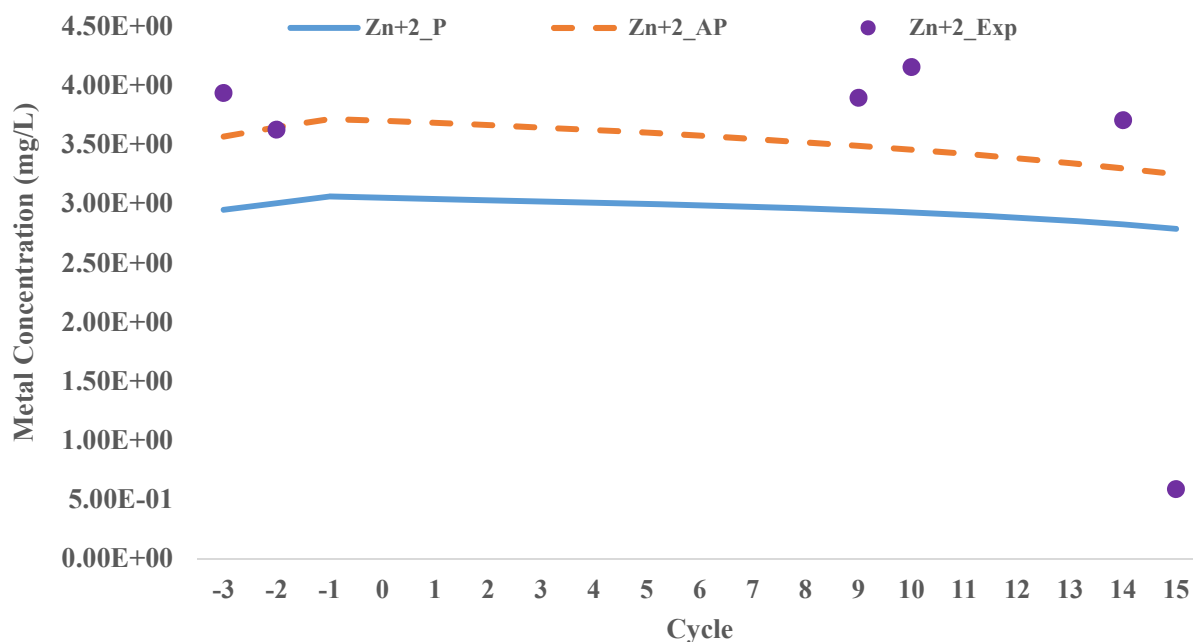
The limitation of the information of what is present in the system has resulted in the inability to efficiently predict the speciation of metals in anaerobic digester sludge. This makes it difficult for the model to realistically portray metals trapped in the sludge phase; particularly the sulphide precipitates. This inability to account for sulphide precipitate ion concentration is due to the uncertainty pertaining the generation, if present, of sulphide in the system. The conversion of sulphate to sulphide was allowed, to maintain consistency with the method used to obtain the initial conditions; which were obtained by comparison with the same experimental results and allowing sulphide ion formation. Model results were compared to the experimental results to understand the extent of uncertainty brought about by the presence of sulphide in the system in comparison to reality and the impact on apportionment of metals amongst the phases.

In the context of the main aim of the micronutrients, which is to sustain the microorganisms, it is desirable to maximise the availability of metals in the sludge, for enhanced treatment of FTRW. The model portrays the behaviour when metals are extensively trapped in the sludge, particularly as phosphate and sulphide precipitates. Most metals are shown to precipitate out of solution to form sulphide precipitates.



**Figure 32: Co<sup>+2</sup> Concentration in sludge for washout experiment**

The models were used to assess the effect of ions in the precipitated phase (sludge); the P-Model and AP-Model. It can be seen that the addition of an adsorbed phase reduces the deviation between experimental and model results for all metals. The requirement of the addition of an adsorbed phase into the speciation modelling of the washout experiment is therefore a step forward. Figure 33 depicts the behaviour explained for the Co<sup>+2</sup> in the sludge, as the AP-Model is much closer to the experimental data points.

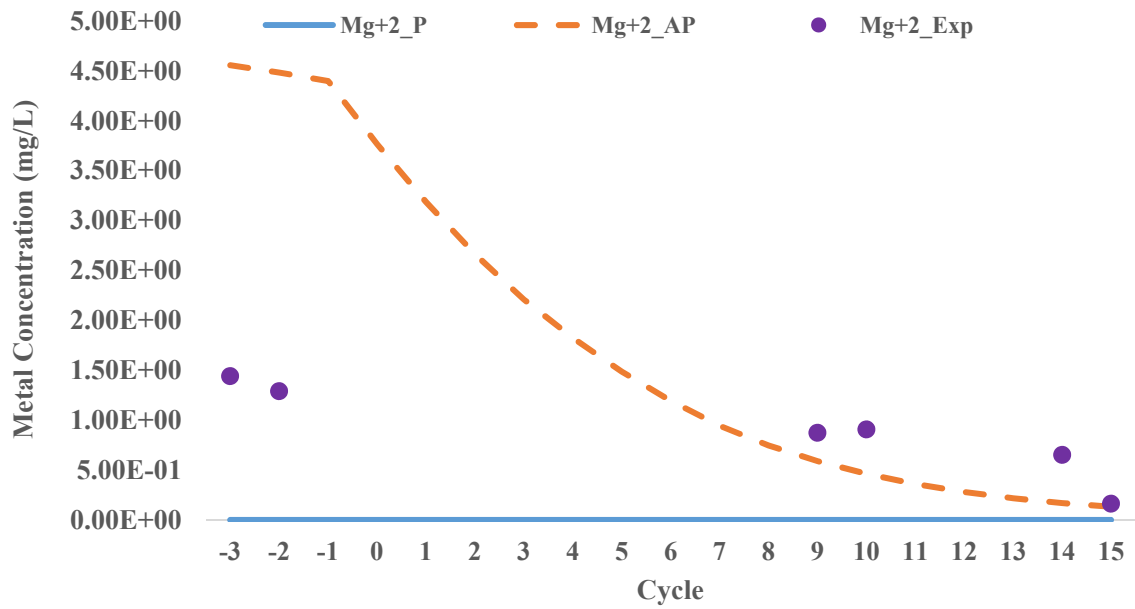


**Figure 33: Zn<sup>+2</sup> Concentration in sludge for washout experiment**

Correlation between the model and experimental, for Zn<sup>+2</sup> retained in the sludge is shown in Figure 33, where the last experimental point indicates a sharp decrease between cycle 14 and 15. This sharp decrease is indicative of full re-solubilisation of ZnS precipitate to the soluble and adsorbed phases, which for the calibration is reliant on the assumption taken up by PHREEQC of the equilibrium sulphide concentration. However, the model does not exhibit full re-solubilisation, and since there is no data post cycle 15 proving this, cycle 15 is considered an outlier for purposes of correlating sludge metal concentrations for metals showing a steep decrease in cycle 15.

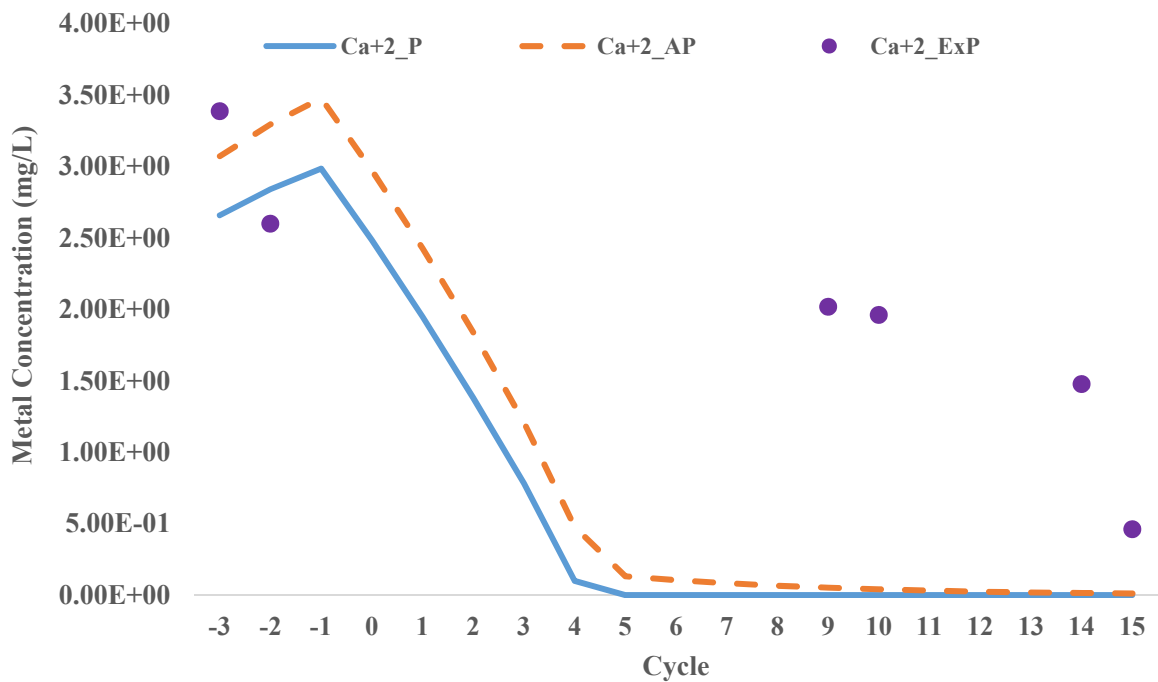
Figure 34, indicates that the model overstates the amount of Mg<sup>+2</sup> retained in the sludge, furthermore, the concentration of Mg<sup>+2</sup> is also overstated in the soluble phase. With Mg<sup>+2</sup> only existing in the soluble and adsorbed phase, the overstated sludge concentrations are due to overstated number of adsorption sites on the ionic sludge creation, resulting in a considerable amount of adsorbed Mg<sup>+2</sup> ions compared to the experimental set-up, depicted by the higher difference between experimental and model results from cycles -3 to 6.





**Figure 34: Mg<sup>2+</sup> Concentration in sludge for washout experiment**

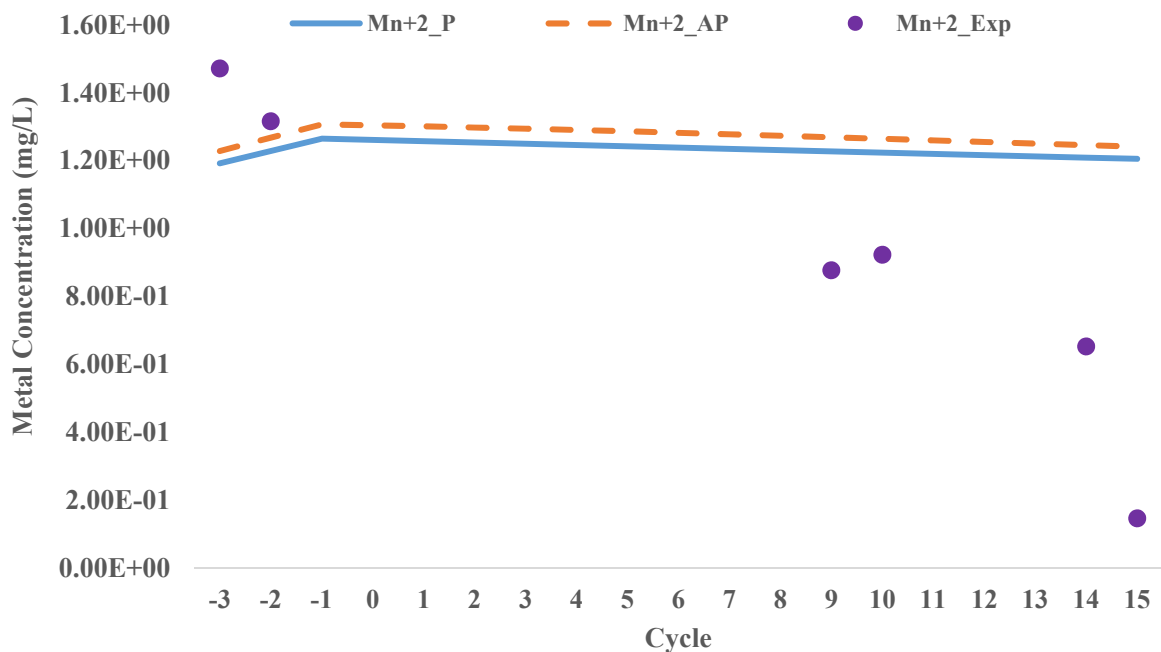
The method for obtaining the initial conditions did not favour Mg<sup>2+</sup>; hence, re-adjustment solely for the Mg<sup>2+</sup> initial concentration will also assist in reducing the initial deviation between experimental and model results from cycles -3 to 6. Furthermore, regression of complexation (equilibrium) constants between the ionic functional groups and metals is required as per the intended aim in section 4.2.3.2, which calls for experimental data for metals particularly in the adsorbed phase.



**Figure 35: Ca<sup>2+</sup> Concentration in sludge for washout experiment**

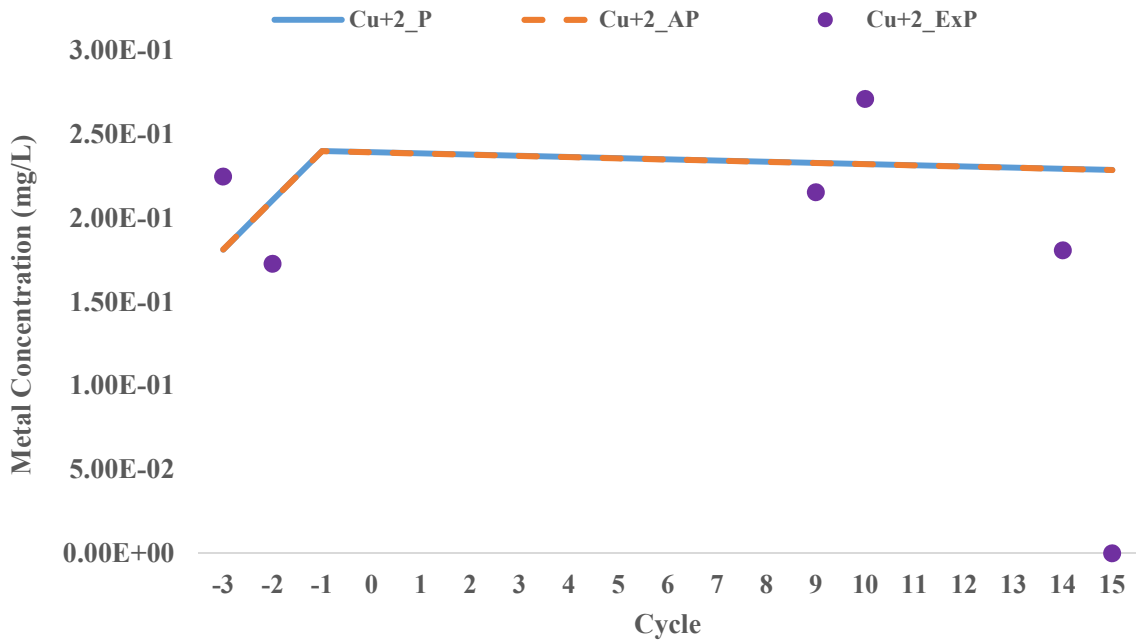
Referring to Figure 35, the model data for  $\text{Ca}^{+2}$  concentration in sludge traverses through the experimental  $\text{Ca}^{+2}$  concentration results, however there is a great deviation between the experimental and model from cycle 8 to 15. The experimental data suggests retention of  $\text{Ca}^{+2}$  in the sludge to a greater extent, which is due to  $\text{Ca}_5(\text{PO}_4)_3\text{OH}$  re-solubilising to a lesser extent than predicted by the model or retention of  $\text{Ca}^{+2}$  to a greater extent by the adsorbed phase.

In Figure 35, the model does not predict the rapid fall in  $\text{Mn}^{+2}$  concentration in the sludge. With  $\text{Ca}_5(\text{PO}_4)_3\text{OH}$  and  $\text{MnHPO}_4$  cations reacting with the same anion, Figures 34 and 35 show that the experimental conditions favour the precipitate formation of the anion with  $\text{Ca}^{+2}$  more than  $\text{Mn}^{+2}$ . If this effect were to be factored into Figures 35 and 36, the deviation between the model and experimental results would be reduced for  $\text{Ca}^{+2}$  and  $\text{Mn}^{+2}$ .



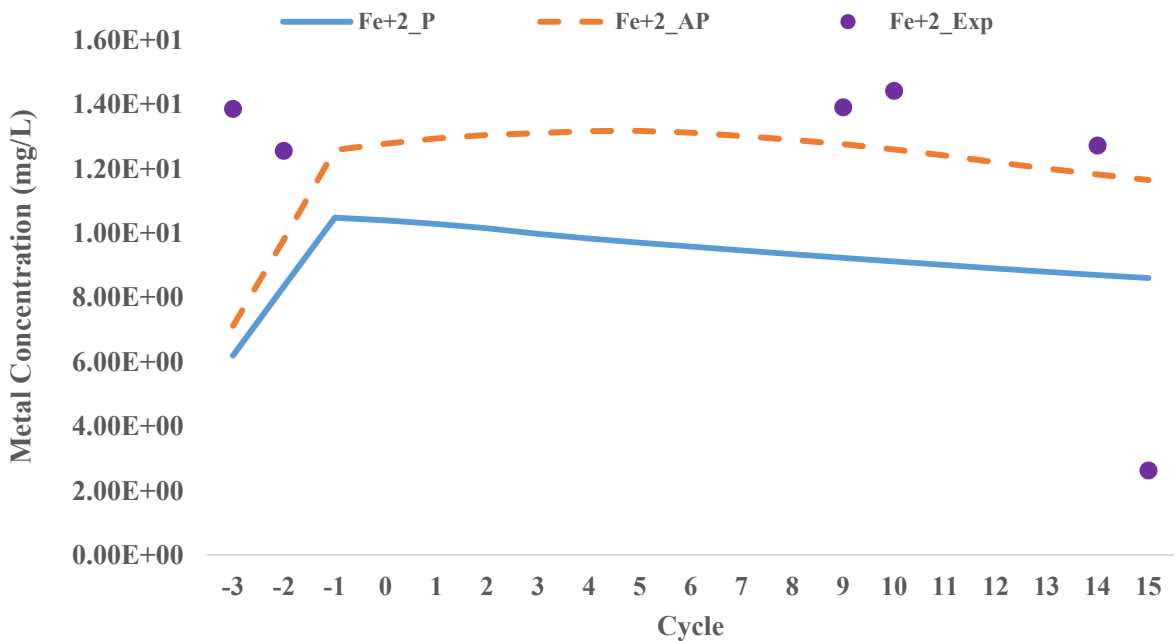
**Figure 36:  $\text{Mn}^{+2}$  Concentration in sludge for washout experiment**

Figure 37 shows the sludge concentrations for  $\text{Cu}^{+2}$ ; the model data traverses between the experimental data for the bulk of the washout experiment, except cycle 15 which has already been categorised as an outlier.



**Figure 37: Cu<sup>+2</sup> Concentration in sludge for washout experiment**

Figure 38 below confirms the closer correlation between model and experimental results with the addition of an adsorbed phase for Fe<sup>+2</sup>. The AP model greatly depicts the behaviour of Fe<sup>+2</sup> in the precipitates and adsorbed phases. In the first three cycles, the FeS<sub>2</sub> concentration increases due to the sulphide concentration present in the system, which is in question due to the great dependence of the model on the concentration of sulphide; as shown by the sulphide sensitivity.



**Figure 38: Fe<sup>+2</sup> Concentration in sludge for washout experiment**

There is a strong correlation between the AP-model predicted sludge concentrations and the experimental sludge concentrations. The experimental sludge concentrations gave an indication of a shift of the metals from the sludge to the soluble phase; however, it is still essential to understand the ternary relationship between the phases. The ternary relationship aids in confirming whether the sludge concentrations are due to precipitates (sulphide and/or phosphate), the adsorbed phase or a combination of the two phases. A considerable amount of metals are found in the sludge and the model suggests these metals are to a large extent trapped in the precipitates, specifically sulphide precipitates.

Throughout the comparison between model and experimental results, it was noted that in as much as the model may predict the sludge concentration, there is still uncertainty of whether the precipitates (which are linked to sulphide concentration) or the adsorbed phase is controlling.

Experimental results for metals entrapped in sludge provided **total** concentrations, i.e. the sum of metals trapped in sludge by precipitation and adsorption. Without the apportionment of metals between the adsorbed phase and the precipitates phase, the correct modelling of the adsorbed phase in isolation was unsuccessful. Furthermore, due to the inability of the model to represent the precipitates adequately as a result of the uncertainty of the sulphide concentration, the precipitates phase was not modelled correctly. With the inability to model the precipitates phase correctly, the adsorbed phase could not be quantified by subtraction, therefore the ternary relationship between the phases to best represent the experiment was not quantified. Knowledge of the sulphide concentration will therefore greatly aid in identifying the ternary relationship between the phases, and to furthermore identify the controlling phase.

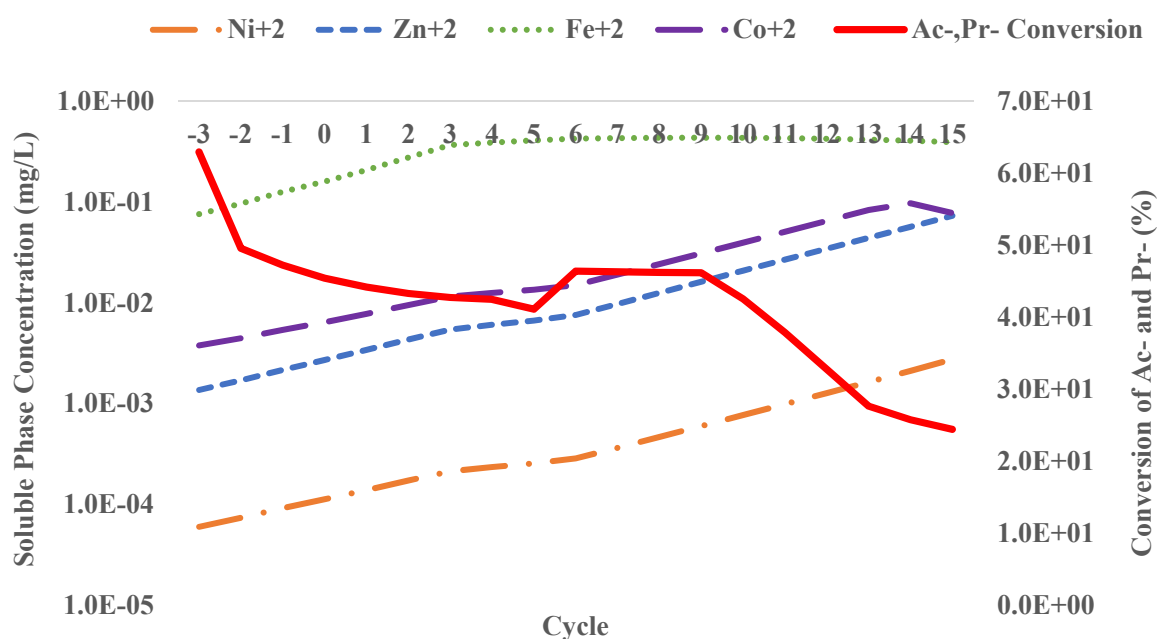
### **5.3.5 RELATIONSHIP BETWEEN CONVERSION, BIOAVAILABILITY AND METAL CONCENTRATION AT DIFFERENT SULPHIDE CONCENTRATIONS**

According to the FIAM, the soluble metal concentration is the best indicator of metal bioavailability, which is the extent of chemical availability of metals in sludge for biological uptake by the microorganisms to bring about a biological response. This is due to the metals being the most mobile when in the soluble phase. With this high metal mobility, an uptake of metals by microorganisms has the greatest opportunity when metals are in the soluble phase than the precipitates and adsorbed phases.

A positive correlation between soluble metal concentration, bioavailability and conversion is therefore expected to exist, where if one increases or decreases, the others follow an increasing or decreasing trend as well. In the context of the study and interpretation of the next two Figures; as the concentration of metals in the soluble phase decreases; the amount of metals available to bring about biological response decreases, reducing the conversion of organic content (acetate

and propionate) to CH<sub>4</sub>, H<sub>2</sub>O (g) and CO<sub>2</sub>. Furthermore, conversion is calculated per cycle as a percentage of the difference in organic content before and after the biological reaction.

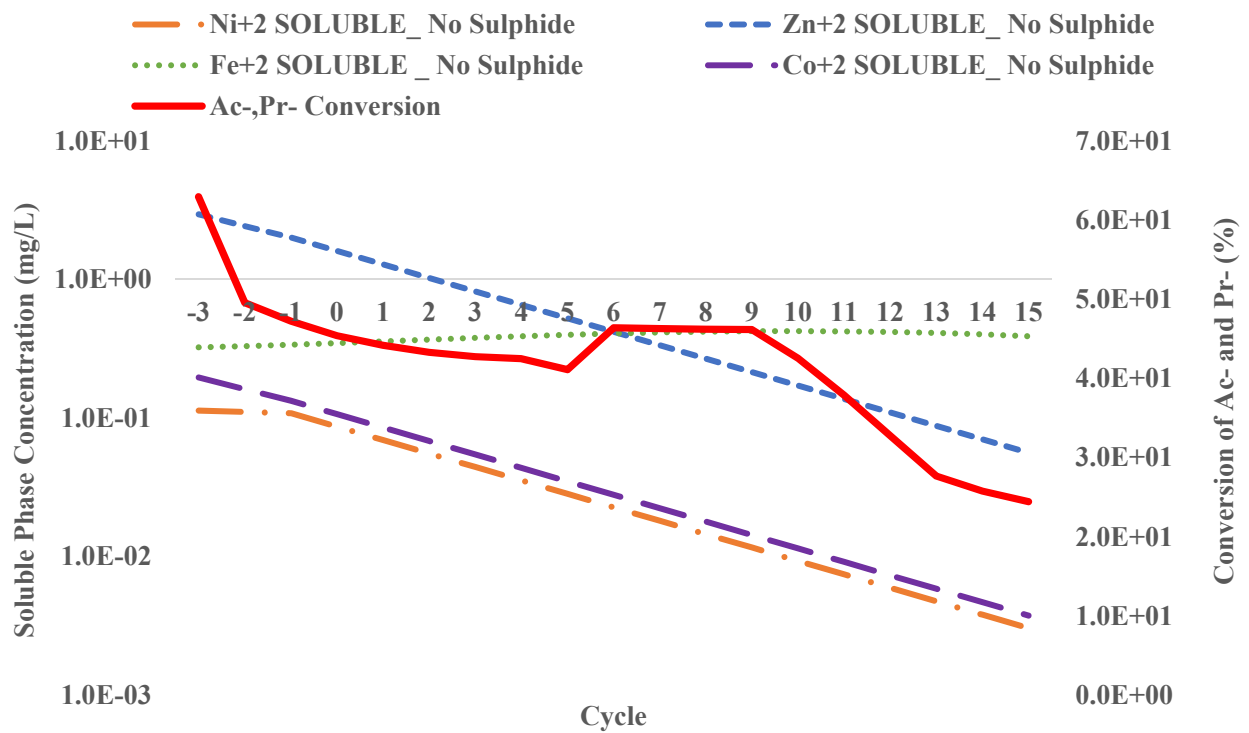
Figures 39 and 40 show the concentration of Fe<sup>+2</sup>, Ni<sup>+2</sup>, Co<sup>+2</sup> and Zn<sup>+2</sup>, of which the first three metals are essential constituents for the efficient catalysis of methanogen metabolic pathways. The graphs below compare % conversion of Ac<sup>-</sup> and Pr<sup>-</sup> and soluble concentration (logarithmic scale), however, directionally depict a relationship between the conversion and concentration per cycle. Due to the uncertainties with the model representation of the soluble phase as a result of the sulphide concentration, two sulphide scenarios are depicted with the relationship between soluble metals concentration, conversion and bioavailability assessed in each scenario.



**Figure 39: Relationship between conversion and Ni<sup>+2</sup>, Zn<sup>+2</sup>, Fe<sup>+2</sup> and Co<sup>+2</sup> soluble Concentration with Sulphide present in the system**

Figure 39 indicates that the bulk of the reaction takes place in the beginning of the washout experiment; cycle -3. The soluble phase concentrations shown are of metals as predicted by the model with sulphide present. A constant increasing trend for the soluble phase concentration is seen, which in the presence of sulphide is explained as metals re-solubilising into the soluble phase. The increase in soluble phase concentration is meant to be accompanied by an increase in conversion, which is not the case for the model in which sulphide is present in the system.

Figure 40 depicts the soluble phase concentrations **in the absence of sulphide** in the system and the same conversion trend as Figure 39. It can be clearly seen that the soluble phase concentrations are highest at the beginning of the washout experiment, cycle -3. With respect to the conversion, this is the cycle at which the bulk of the reaction takes place; depicting the expected relationship of positive correlation of the soluble phase concentration, bioavailability and conversion.



**Figure 40: Relationship between conversion and Ni<sup>2+</sup>, Zn<sup>2+</sup>, Fe<sup>2+</sup> and Co<sup>2+</sup> soluble Concentration with no Sulphide present in the system**

With different concentration of sulphide in the system, different behaviours are shown by the soluble, precipitates and adsorbed phase concentration trends. As a result, the sulphide concentration has a significant impact on the system as a whole and given the effect shown by different sulphide conditions on the speciation, it is evident that the sulphate-sulphide system needs to be accurately quantified as an extension of the washout experiment.

Directionally, when there is no sulphide in the model, the concentration trends agree with the conversion trend, however, this model needs to be compared to a full experimental data set, which clearly quantifies sulphide prior cycle -3. An overview of the findings of the study together with their significance to answering the research questions, fulfilling the study objectives and aiding in solving the engineering problem are highlighted in the next section of this paper; the discussion.

## **6. DISCUSSION**

The main driver for the study is to aid in the dosing mechanisms employed by Sasol to treat their FTRW prior its reuse. With dosing of micronutrients being the highest variable cost in this anaerobic treatment process, it is vital for the most efficient dosing mechanism to be employed. Throughout the discussion, the various findings and learnings of the study are pointed out, together with their significance. In the beginning of the paper, clear objectives and outcomes were defined; fulfilment or lack thereof of stated objectives and outcomes is discussed.

### **6.1 MODEL DEVELOPMENT AND RESULTS**

The method used to develop the model is discussed fully in section 4.3; of which the principle advance was the addition of the adsorbed phase by making use of the ionic representation of the sludge surface as well as the adsorption constants for glycine. The construction of the adsorbed phase was carried out in line with the BLM to incorporate competition between metals for adsorption sites; this was successfully represented by the value of the adsorption constants.

There were significant challenges, limiting the realistic representation of the anaerobic system which affected the final conclusions of the study. The first challenge faced was the misalignment of the initial conditions calculated using the initial solution added and the experimental initial conditions due to little knowledge about the conditions prior dosing in the first cycle. As a result of the novel nature of the experimental set-up, limited sources exist wherein the anticipated trends for CH<sub>4</sub> produced, metals precipitated and adsorbed and free metal ions may be obtained besides that which have been produced by Mathir (2013). The limiting factor of experimental results had to therefore be worked around to allow for model creation and comparison with available experimental data.

This was overcome by iteratively calculating the initial conditions by altering the precipitates phase such that the sludge experimental concentrations are met. This method was not the most accurate manner of finding initial conditions as only one sludge phase contributor – the precipitates, was being adjusted. In reality, there may be a significant amount of adsorbed metals on the sludge, requiring the adjustment of both the precipitates and adsorbed phases to match the same experimental initial conditions. Therefore the adjustment of one phase for obtaining the initial conditions aligned to the experimental conditions may have introduced error.

The second challenge, which was coupled with the initial experimental conditions, was the sulphide-sulphate conversion prior cycle -3 as well as measurement throughout the experiment. In the results section, it was highlighted that the sulphide concentration is the most significant factor affecting bioavailability or lack thereof for metals in the system as sulphide precipitates are greatly formed given the presence of sulphide in the system.

With sulphide present in the system, precipitates have a controlling effect as the sulphide results in the displacement of metals from the soluble phase to form sulphide precipitates. The Model with conversion from sulphate to sulphide indicates the highest concentration of sulphide precipitates in the first cycle, resulting in metals not being bioavailable for subsequent cycles.

Limitation of anaerobic treatment of wastewater by sulphide concentration is not a new phenomenon. The literature survey highlighted findings of Feroso and co-workers (2009); pointing out that the bioavailability and mobility of metals in UASB reactors was mainly controlled by the sulphide chemistry due to the formation and ageing of sulphide precipitates resulting in metals being bound in the solid phase and therefore non-bioavailable (Filgueiras, et al., 2002), (Zandvoort, et al., 2006).

Taking into consideration the fractions existing in anaerobic digestion together with their operational definitions as in Table 3; it was highlighted that there is a possibility of metals bound by sulphides existing in the oxidisable fraction. The oxidisable fraction essentially comprises of trace metals connected with various forms of organic matter (such as living organisms) through processes of complexation and bioaccumulation together with metals bound by sulphides. With the literature survey showing metals being partitioned to the oxidisable phase to a great extent; the possibility of metals being trapped in the oxidisable phase as a result of sulphide precipitation exists.

Furthermore, the Literature survey highlighted the existence of Cu as bound to sulphides due to its chemistry, of which Cu-sulphides do not dissolve from the solid phases. With the existence of sulphide in the system; Appendix G, Figure 44 shows little difference of CuS with the inclusion of an adsorbed phase. Figure 53, Appendix G also shows Cu to be one of the least bioavailable metals which form sulphide precipitates, aligning to observations made by previous studies.

$\text{Ni}^{+2}$  and  $\text{Co}^{+2}$  are two metals essential to methanogenesis, which increase in concentration by  $10^2$  in the absence of sulphide in the system. Bearing in mind that nutrients are in the form of micro-metals, with the corresponding minute concentrations, an increase of this magnitude is significant enough to consider the sulphate-sulphide concentration as controlling at this point of the study.

The study shows two scenarios for sulphide concentrations to display the effect on partitioning of metals. In reality, the true conditions that prevail are un-known and may lie in-between the two scenarios provided. A comparison between model generated data and literature sequential extraction data obtained by sequential extraction of metals in various types of sludge is shown in Table 19, Appendix F. For the two sulphate-sulphide scenarios presented; it can be seen that when there is no sulphide in the system, there is a significant increase in the metals entrapped in the adsorbed phase.



In comparison to the literature data in Appendix H, Table 21, the model generated data when there is no sulphide corresponds to literature data which suggests a significant amount of metals being found in the oxidisable fraction. The literature data points to a considerable amount of metals in the oxidisable fraction, corresponding to both adsorbed metals and metals displaced by sulphide. In comparison to the set-up of the current study, which segregates the adsorbed metals and the metals trapped by sulphide, experimental data segregating the adsorbed (complexed) and sulphide precipitated metals is essential. Furthermore, the sulphate-sulphide relationship, in the form of concentrations, needs to be adequately quantified with the progress of the washout experiment.

Another dominant theme of the study is the relationship between metal concentration and bioavailability. As pointed out in the study, a metal is said to be bioavailable if it brings about biological response when available at the bio-interface. The reaction brings about a decrease in the amount of acetate and propionate in the system, with a corresponding increase in the amount of biogas produced. The results section shows how the relationship between bioavailability and soluble metal concentration is affected by speciation of metals, which is impacted by the sulphate-sulphide relationship.

Additional challenges were faced in modelling of the adsorbed phase to best represent metals adsorbed in the sludge, mainly due to the form in which the experimental data was provided. The experimental data provided total sludge metals concentrations, depicting the combined effect of the precipitated metals and adsorbed metals. Looking at the adsorbed phase in isolation, the aim was to use glycine equilibrium constants to model the adsorption of metals onto sludge, followed by detailed regression of equilibrium constants to best represent the complexation (adsorption) reactions between UKZiNe and free metal ions. This was not achievable due to the lack of data for adsorbed metals only and the inability to quantify the adsorbed metal concentrations by subtracting precipitated metals from total metal concentrations as a result of the sulphate-sulphide relationship uncertainty.

The soluble metals concentration data was provided, however, could not be effectively used for all metals due to an incomplete experimental data set. Furthermore, the use of measurement equipment with high precision particularly for the soluble phase, which has shown to have low detection limits, was not used. Knowledge of the full experimental set for soluble phase would have been very beneficial in determining the expected bioavailability and biological activity trends as this is the phase with greatest metal mobility.

At this point of the study, the speciation of metals is greatly impacted by the amount of metals entrapped as precipitates and adsorbed onto the surface. As a result, the sulphate-sulphide system

which impacts on metal speciation will also have an impact on the displayed relationship between bioavailability and metal concentration and can only fully be quantified once the gaps identified in metal speciation have been fully closed out. Furthermore, true measurement of the relationship between conversion, metal concentration and bioavailability lies with plotting of CH<sub>4</sub> generation and soluble metal concentration.

From the speciation model results shown for the P-Model, it can be seen that the precipitates have a controlling effect on the soluble metals, given the formation of the precipitates. The precipitates form if there are sufficient anions with which they precipitate, making them the controlling phase. The precipitates phase is controlling if there is sufficient sulphide, phosphate and carbonate present in the system.

## 6.2 RESPONSE TO THE RESEARCH QUESTION

The purpose for the model was to carry out metal speciation for anaerobic treatment of FTRW and reduce the deviation between experimental and model results. With a lack of accurate experimental results, it cannot be confirmed that the current model adequately represents the speciation for anaerobic treatment of FTRW.

The research question asked was whether a stoichiometric formulation of an adsorbed phase improves the correspondence between model and experimental data for soluble metal ions and metal ions immobilised by a solid phase. From comparison of the **sludge metal concentrations** between the model and experimental, a decrease in deviation between experimental and model was seen, however, it cannot be proved that the decrease in the deviation is solely due to the addition of the adsorbed phase as there are a number of gaps yet to be closed out for a more compact and representative model.

Unfortunately, it was not possible to prove the existence of an adsorbed phase with the data available. It should be mentioned that a better correlation between experimental and model is also reliant on the sulphide concentration as well as the initial conditions of the anaerobic digester and nutrient recipe.

## 7. CONCLUSIONS

The general purpose for conducting the study was to develop a theoretical model to depict the controlling factors of the distribution of metals in anaerobic digester mixed liquor to successfully treat FTRW. Further to this, the model was to be used to provide potential scenario(s) Sasol Secunda can employ to their dosing strategies to result in a decreased dosing bill without compromising process efficiency.

An integrated Ionic speciation model was developed, incorporating both precipitated and adsorbed phases in the speciation of metals in anaerobic digester mixed liquor. The validation of the model required the assessment of the accuracy by which it depicts reality, which aligns to a decrease in the deviation between experimental and model results.

The speciation model developed, endeavoured to define the distribution of metals between the precipitated and adsorbed forms, by making use of total concentrations in sludge; which were the only available measurements for purposes of determining speciation. On successful quantification of the precipitates phase via equilibrium modelling, it was hoped that the adsorbed metals could be estimated by difference. In the findings of this study, it became very evident that the precipitation modelling is highly dependent on the possible presence of sulphide in the system. Without the experimental measurement of sulphide concentration in the system, the estimation of the amount of metals in precipitates was not possible.

It is concluded that a series of further experimental work needs to take place, which will be followed by further model optimisation as a result of the findings of the experimental work. The requirements to be fulfilled by the experimental work have been outlined in the “Next Steps”.

## **8. NEXT STEPS**

Throughout this dissertation, it is evident that there are pivotal points to the study that need further work and clearer quantification. These will aid in answering the biggest questions raised in the study and will allow for obtaining of in depth knowledge of the expected result from the model. Further work to be done for this study has been categorised into the experimental and model section as well as the Engineering problem section.

### **Experimental and Model**

The generic recommendation is to carry out more washout experiments to gain a greater source of experimental data. The experimental should aim to provide:

- Clear quantification of the initial conditions (feed concentrations, metal concentration per phase, pH, sulphate concentration, sulphide concentration).
- Sulphate and sulphide concentration throughout the experiment.
- Redox equilibrium throughout the experiment.
- A fuller experimental data set, with increased accuracy is required and can be achieved by making use of measurement equipment with high precision particularly concentrations of low detection limits.

The advances to be made on the model largely depend on the success of the experimental work and the following:

- Incorporation of the experimentally observed sulphate sulphide relationship
- Regression for equilibrium constants to reflect the adsorption via complexation between UKZiNe and free metals
- Carrying out the biological conversion together with the metal speciation on one modelling program. In an anaerobic biological reactor, the distribution of metal species changes as biological conversion proceeds, Furthermore, the biological conversion is expected to increase with an increase in bioavailability of metals which is suggested by soluble metal concentration as obtained via speciation. A single code incorporating the biological reaction and speciation mechanism will clarify the relationship between biological conversion and metal speciation.

### **Engineering Problem**

Taking the assumptions of the BLM, which takes into account the equilibrium between the phases and the adsorption sites on the sludge surface, it could be beneficial to have a cation

existing in the system which has a great affinity to form complexes with the adsorption sites and sulphide precipitates than other essential metals – a donor cation. This will allow for metals essential for the decomposition of acetate and propionate to be kept in the soluble phase, the most bioavailable phase while the donor cation takes up adsorption sites and sulphide anions. It is essential that the donor cation does not in any way inhibit the biological reaction, which is the main objective of the anaerobic setup.

### **Further Study**

In addition to the research questions posed at the beginning of the current study, the research questions that arise from this study are:

“Does sulphate reducing bacteria exist in the anaerobic treatment system of FTRW? If there is sulphate reducing bacteria; does it bring about the formation of sulphide, resulting in the precipitates phase controlling the concentration of the free metal ions?”

## 9. BIBLIOGRAPHY

Alvarez, E. A., Mochon, M. C., Sanchez, J. C. J. & Rodriguez, M. T., 2002. Heavy Metal Extractable Forms in Sludge from Wastewater Treatment Plants. *Chemosphere*, Volume 47, pp. 765-775.

A, M., 2013. *Analysis of Micronutrient Requirements for the Anaerobic Digestion of Fischer-tropsch Reaction Water*, Durban: University of Kwa-Zulu Natal.

Amer, S. I., 2004. Simplified Removal of Chelated Metals. *Metal Finishing*, 102(4), pp. 1-5.

Aquino, S. F. & Stuckey, D. C., 2007. Bioavailability and Toxicity of Metal Nutrients during Anaerobic Digestion. *The Journal of Environmental Engineering*, 133(1).

Artola, A., Balaguer, M. D. & Rigola, M., 1997. Heavy Metal Binding to Anaerobic Sludge. *Water Research*, 31(5), pp. 997-1004.

Artola, A., Balaguer, M. D. & Rigola, M., 1999. Competitive Biosorption of Copper, Cadmium, Nickel and Zinc from Metal Ion Mixtures using Anaerobically Digested sludge. *Process Metallurgy*, Volume 9, pp. 175-183.

Bell, R. A., Ogden, N. & Kramer, J. R., 2002. The Biotic Ligand Model and a Cellular Approach to Class B Metal Aquatic Toxicity. *Comparative Biochemistry and Physiology Part C*, Volume 133, pp. 175-188.

Brouckaert, C. J., 2012. *SANI Steady State Anaerobic Digestion Model*. Durban: University of Kwa-Zulu Natal, Pollution Research Group.

Brown, P. L. & Markich, S. J., 2000. Evaluation of the Free Ion Activity Model of Metal-Organism Interaction: Extension of the Conceptual Model. *Aquatic Toxicity*, Issue 51, pp. 177-194.

Burgess, J. E., Quarmby, J. & Stephenson, T., 1999. Role of Micronutrients in Activated Sludge-Based Biotreatment of Industrial Effluents. *Biotechnology Advances*, pp. 49-70.

Campbell, P. G. C., 1995. Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model. In: T. A. a. T. D. R., ed. *Metal Speciation and Bioavailability in Aquatic Systems*. Chichester: Wiley, pp. 45-102.

Coetzee, P. P., 1993. Determination and Speciation of heavy Metals in Sediments of the Hartbeespoort Dam by Sequential Chemical Extraction. *Water SA*, 19(4), pp. 291-300.

Di Toro, D. M. et al., 2001. Biotic Ligand Model of the Acute Toxicity of Metals: Technical Basis. *Environmental Toxicology and chemistry*, 20(10), pp. 2383-2396.

Divvela, P., 2010. *Sequential Extraction Procedure*, Kelso, WA: Lab Science News, ALS Environmental.

Dry, M. E., 2002. The Fischer-Tropsch Process: 1950- 2000. *Catalysis Today*, Issue 71, pp. 227-241.

Du Preez, 1987. Growth Kinetic Studies of *Acetobacter calcoaceticus* with special reference to acetate and ethanol carbon sources.. *Internal Sasol Report*.

- Eckenfelder, W. W. & Musterman, J. L., 1994. Treatment and Pretreatment Requirements for Industrial Wastewaters in Municipal Activated Sludge Plants. *Water Science & Technology*, 29(9), pp. 79-88.
- Fermoso, F. G., Bartacek, J., Jansen, S. & Lens, P., 2009. Metal Supplementation to UASB Bioreactors: From Cell-Metal Interactions to Full-Scale Application. *Science of The Total Environment*, Issue 407(12), pp. 3652-3667.
- Fermoso, F. G. et al., 2010. Dosing of Anaerobic Granular Sludge bioreactors with Cobalt: Impact of Cobalt Retention on Methanogenic Activity. *Bioresource Technology*, Volume 101, pp. 9429-9437.
- Feuntes, A. et al., 2004. Simple and sequential extractions of heavy metals from different sewage sludges. *Chemosphere*, Volume 54, pp. 1039-1047.
- Filgueiras, A. V., Lavilla, I. & Bendicho, C., 2002. Chemical Sequential Extraction for Metal Partitioning in Environmental Solid Samples. *J. Environ. Monit*, Volume 4, pp. 823-857.
- Fogler, H. S., 2009. *Elements of Chemical Reaction Engineering*. 4th ed. s.l.:Prentice Hall International.
- Gustavsson, J., 2012. *Cobalt and Nickel Bioavailability for Biogas Formation*, Linköping: Linköping University: Faculty of Arts and Sciences.
- Lees, C. M., Foxon, K. M. & Brouckaert, C. J., 2012. *Dynamic Modelling of Anaerobic Digestion of Fischer-Tropsch Reaction Water*, Durban: University of Kwa-Zulu Natal, Pollution Research Group.
- Lemmer, H. & Nitschke, L., 1994. Vitamin Content of Four Sludge Fractions in the Activated Sludge Wastewater Treatment Process. *Water Research*, 28(3), pp. 737-739.
- Lo'pez-Chuken, U. J., Young, S. D. & Sanchez-Gonzalez, M. N., 2010. The use of Chloro-Complexation to Enhance Cadmium Uptake by Zea mays and Brassica Juncea: Testing a "Free Ion Activity Model" and Implications for Phytoremediation. *International journal of Phytoremediation*, Volume 12, pp. 680-696.
- Mathir, A., 2013. *Analysis of Micronutrient Requirements for the Anaerobic Digestion of Fischer-Tropsch Reaction Water*, Durban: University of Kwa-Zulu Natal.
- Mathir, A., 2013. *Analysis of Micronutrient Requirements for the Anaerobic Digestion of Fischer-Tropsch Reaction Water*, Durban: University of Kwa-Zulu Natal.
- McCarty, P., 1981. *History and Overview of Anaerobic Digestion*, Germany: Second Int'l.Symp.on An Dig., Travemunde.
- Meyer, J. S., 2002. The Utility of the terms "Bioavailability" and "bioavailable fraction" for metals. *Marine Environmental Research*, Volume 53, pp. 417-423.
- Naidu, R. et al., 2008. Bioavailability: Definition, Assessment and implications for Risk Assessment. In: R. Naidu, ed. *Developments in Soil Science*. s.l.:Elsevier, pp. 39-50.
- Newman, M. C. & Jagoe, C. H., 1992. Ligands and Bioavailability of Metals in Aquatic Environments. In: *Bioavailability: Physical, Chemical and Biological Interactions*. Pelleston Workshop, Pellston, Michigan: Lewis Publishers, p. Chapter 1.

- Niyogi, S. & Wood, C. M., 2004. Biotic Ligand Model, a Flexible Tool for Developing Site-Specific Water Quality Guidelines for Metals. *Environmental Science and Technology*, 38(23), pp. 6177- 6192.
- Ntuli, L. & Brouckaert, C. J., 2014. *Micro-nutrient REquirement for Anaerobic Digestion of Concentrated Digestion of Concentrated Industrial Effluents*, Durban, Kwa-Zulu Natal: Pollution Research Group, School of Chemical Engineering, University of Kwa-Zulu Natal.
- Ogunremi, T., Cutler, J., Christensenand, C. & Sparks, C., 2007. *X-ray Absorption Spectroscopic Analysis of Raw and Processed Hog Manure*, Saskatchewan: Institute, College of Agriculture & Bioresources, University of Saskatchewan.
- Oude Elferink, S. J. W. H., 1998. *Sulfate-Reducing Bacteria in Anaerobic Bioreactors*, Netherlands: Landbouwniversiteit Wageningen.
- Pagnanelli, F., Esposito, A., Toro, L. & Veglio, F., 2003. Metal Speciation and pH effect on Pb, Cu, Zn and Cd Biosorption onto *Sphaerotilus natans*: Langmuir-type Empirical Model. *Water Research*, Volume 37, pp. 627-633.
- Parker, D. R., Pedler, J. F., Ahnstrom, Z. A. S. & Resketo, M., 2001. Re-evaluating the Free ion activity model of Trace Element Toxicity Toward Higher Plants: Experimental Evidence with Copper and Zinc. *Environmental Toxicology and Chemistry*, 20(4), pp. 899-906.
- Romera, E. R., Otazua, B. G. & Rossi, F. R., 2007. Optimisation of Anaerobic Processes by Speciation and Bioavailability of Trace Metals. *Toxicology & Environmental Chemistry*, 89(2), pp. 281-293.
- Sag̃, Y. & Kutsal, T., 1996. Fully Competitive Biosorption of Chromium (VI) and iron (III) Ions from Binary Metal Mixtures by *R. arrhizus*: use of the Competitive langmuir Model. *Process Biochemisrty*, 31(6), pp. 573-585.
- Simkiss, K. & Taylor, G., 1995. Transport of Metals Across Membranes. *Metal Speciation and Bioavailability in Aquatic Systems*, Volume 3, pp. 1-44.
- Simkiss, K. & Taylor, M. G., 2000. Trace Element Speciation at Cell Membranes: Aqueous, Solid and Lipid Phase Effects. *Journal of Environmental Monitoring*, Volume 3, pp. 15-21.
- Singleton , I., 1994. Microbial Metabolism of Xenobiotocs: Fundamental and Applied Research. *Journal of Chemical Technology and Biotechnology*, 59(1), pp. 9-23.
- Speece, R. E., 1983. Anaerobic Biotechnology for Industrial Wastewater Treatment. *Environmental Science Technology*, 17(9), pp. 416A-427A.
- Stover, R. C., Sommers, L. E. & Silviera, D. J., 1976. Evaluation of Metals in Wastewater Sludge. *Journal (Water Pollution Federation)*, 48(9), pp. 2165-2175.
- van Hullebusch, E. D., Peerbolte, A., Zandvoort, M. H. & Lens, P. N. L., 2005b. Soprtion of cobalt and nickel on anaerobic granular sludges: isotherms and sequential extraction. *Chemosphere*, Volume 58, pp. 493-505.
- van Hullebusch, E. D., Utomo, S., Zandvoort, M. H. & Lens, P. N. L., 2005a. Comparison of three sequential extraction procedures to describe metal fractionantio in anaerobic granular sludges. *Talanta*, Volume 65, pp. 549-558.



- Van Zyl, P., 2008. *Anaerobic Digestion of Fischer-Tropsch Reaction Water; Submerged Membrane Anaerobic Reactor design, Performance Evaluation and Modeling*, Cape Town: University of Cape Town.
- van Zyl, P. J., 2008. *Anaerobic Digestion of Fischer-Tropsch Reaction Water- Submerged Membrane Anaerobic Reactor Design, Performance Evaluation & Modelling*, Cape Town: University of Cape Town.
- VanBriesen, J. M., Small, M., Weber, C. & Wilson, J., 2010. Modelling Chemical Speciation: Thermodynamics, Kinetics and Uncertainty. In: G. Hanrahan, ed. *Modelling of Pollutants in Complex Environmental System*. s.l.:International Labmate Limited, p. Chapter 4.
- Wadley, S. & Buckley, C. A., 1997. *Chemical Speciation Self-Study Work Manual*, Durban: Pollution Research Group, University of Kwa-Zulu Natl.
- Westergreen, S., Brouckaert, C. J. & Foxon, K. M., 2012. Modelling of Ionic Interactions with wastewater Treatment Biomass. *water Science & Technology*, 65(6), pp. 1014-1020.
- Wilson, C., 2008. *Best Management Practises to Attain Zero Effluent Discharge in South African Industries*, Potschefstroom: North-West University.
- Wood, D. K. & Tchobanoglous, G., 1975;47:1933-45. *Trace Elements in Biological Waste Water Treatment*, s.l.: J Wat Pollut Control Fed.
- Wood, D. K. & Tchobanoglous, G., 1975. Trace Elements in Biological Waste Treatment. *Water Pollution Control Federation*, 47(7), pp. 1933-1945.
- World, U. -. S. f. a. C., 1998. *Frequently Asked Questions for PHREEQC and PhreeqcI*. [Online] Available at: [http://wwwbrr.cr.usgs.gov/projects/GWC\\_coupled/phreeqc/faq.html](http://wwwbrr.cr.usgs.gov/projects/GWC_coupled/phreeqc/faq.html) [Accessed 25 March 2013].
- Worms, I., Simon, D. F., Hassler, C. S. & Wilkinson, K. J., 2006. Bioavailability of Trace Metals to Aquatic Microorganisms: Importance of Chemical, Biological and Physical Processes on Biouptake. *Biochemie*, Volume 88, pp. 1721-1731.
- Zandvoort, M. H., van Hullebusch, E. D., Feroso, F. G. & Lens, P. N. L., 2006. Trace Metals in Anaerobic Granular Sludge Reactors: Bioavailability and Dosing Strategies. *Eng. Life. Sci*, 6(3), pp. 293-301.
- Zhang, Y., Zhang, Z., Suzuki, K. & Maekawa, T., 2003. Uptake and Mass Balance of Trace Metals for Methane Producing Bacteria. *Biomass and Bioenergy*, Volume 25, pp. 427-433.
- Zouboulis, A. I., Matis, K. A. & Hancock, I. C., 1997. Biosorption of Metals from Dilute Aqueous Solutions. *Separation & Purification Reviews*, 26(2), pp. 255-295.

## APPENDICES

### APPENDIX A: Supporting Figures and Tables

**Table 13: Composition of Conventional Basal Medium for methanogens (Zhang, et al., 2003)**

Component	Concentration	Concentration Units
KH <sub>2</sub> PO <sub>4</sub>	3.4	g/l distilled water
K <sub>2</sub> HPO <sub>4</sub>	3.4	g/l distilled water
NH <sub>4</sub> Cl	2.13	g/l distilled water
Na <sub>2</sub> CO <sub>3</sub>	2.54	g/l distilled water
Trace metal solution:	10	ml/l distilled water
MgCl <sub>2</sub> •6H <sub>2</sub> O	41	mg/l trace metal solution
MnCl <sub>2</sub> •4H <sub>2</sub> O	5	mg/l trace metal solution
FeCl <sub>2</sub> •4H <sub>2</sub> O	5	mg/l trace metal solution
NiCl <sub>2</sub> •6H <sub>2</sub> O	1.2	mg/l trace metal solution
ZnSO <sub>4</sub> •7H <sub>2</sub> O	1	mg/l trace metal solution
CoCl <sub>2</sub> •2H <sub>2</sub> O	1	mg/l trace metal solution
CaCl <sub>2</sub> •6H <sub>2</sub> O	4	mg/l trace metal solution
Na <sub>2</sub> SeO <sub>3</sub>	0.8	mg/l trace metal solution
Na <sub>2</sub> MoO <sub>4</sub> •2H <sub>2</sub> O	0.2	mg/l trace metal solution
CuSO <sub>4</sub> •5H <sub>2</sub> O	0.1	mg/l trace metal solution
AlK(SO <sub>4</sub> ) <sub>2</sub>	0.1	mg/l trace metal solution
H <sub>3</sub> BO <sub>3</sub>	0.2	mg/l trace metal solution
NaWO <sub>4</sub> •2H <sub>2</sub> O	0.1	mg/l trace metal solution

### APPENDIX B: Chemical Speciation Mass Balances

Mass balances to carry out speciation modelling are written as follows:

$$M_j = \sum_{i=1}^{N_c} A_{ij} \frac{x_i}{\gamma_i} \dots\dots\dots (B-1)$$

Where:  $M_j$  = total mass of component  $j$ ,  $A_{ij}$  is the stoichiometric coefficient giving the number of moles of component  $j$  in species  $i$ ,  $x_i$  is the activity of the aqueous species  $i$ ,  $\gamma_i$  is the activity coefficient of species  $i$ , and  $N_c$  is the number of components.

$$x_i = \beta_i \prod_{j=1}^{N_c} C_j^{A_{ij}} \dots\dots\dots (B-2)$$

Where  $\beta_i$  is the overall equilibrium formation constant for species  $i$  and  $C_j$  is the concentration of species  $j$ .

## APPENDIX C: Model Development and Design

**Table 14: Initial Conditions for Anaerobic digester (Mathir, 2013)**

<b>Species</b>	<b>Initial Concentrations (mol/l)</b>
Na <sup>+</sup>	3.72E-02
H <sup>+</sup>	1.02E-01
CO <sub>3</sub> <sup>-2</sup>	6.11E-02
NH <sub>4</sub> <sup>+</sup>	2.41E-02
PO <sub>4</sub> <sup>-3</sup>	7.39E-04
Cl <sup>-</sup>	2.72E-04
K <sup>+</sup>	7.39E-04
HS <sup>-</sup>	0.00E+00
Ca <sup>+2</sup>	1.66E-05
Mg <sup>+2</sup>	1.19E-04
Fe <sup>+2</sup>	4.38E-05
Cu <sup>+2</sup>	5.25E-07
Zn <sup>+2</sup>	1.12E-06
Mn <sup>+2</sup>	8.09E-07
MoO <sub>4</sub> <sup>-2</sup>	1.62E-08
Co <sup>+2</sup>	7.54E-08
H <sub>3</sub> BO <sub>3</sub>	1.85E-07
SO <sub>4</sub> <sup>-2</sup>	2.06E-04
Ni <sup>+2</sup>	3.79E-07
I <sup>-</sup>	5.68E-09
Acetate <sup>-</sup>	2.25E-02
Butyrate <sup>-</sup>	5.20E-03
Propionate <sup>-</sup>	9.27E-03
Valerate <sup>-</sup>	2.30E-03

**Table 15: Sasol Nutrient Recipe for Anaerobic Digestion of FTRW (Mathir, 2013)**

<b>Species</b>	<b>Element</b>	<b>Element concentration (mg/l)</b>
FTRW (COD)	COD	18 000
Urea	N	250
KH <sub>2</sub> PO <sub>4</sub>	P	60
	K	130
MgCl <sub>2</sub> .6H <sub>2</sub> O	Mg	13
Na <sub>2</sub> SO <sub>4</sub>	S	23
CaCl <sub>2</sub> .2H <sub>2</sub> O	Ca	3
FeSO <sub>4</sub> .7H <sub>2</sub> O	Fe	11
MnSO <sub>4</sub> .5H <sub>2</sub> O	Mn	0.2
ZnSO <sub>4</sub> .7H <sub>2</sub> O	Zn	0.33
NiCl <sub>2</sub> .6H <sub>2</sub> O	Ni	0.1
CuSO <sub>4</sub> .5H <sub>2</sub> O	Cu	0.15
CoCl <sub>2</sub> .6H <sub>2</sub> O	Co	0.02
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	Mo	0.007
H <sub>2</sub> BO <sub>3</sub>	B	0.009
KI	K	0.001
Yeast Extract		5

**Table 16: Experimental pH results for anaerobic digester (Mathir, 2013)**

<b>Cycle</b>	<b>Experimental pH</b>
-3	6.710
-2	6.695
-1	6.680
0	6.670
1	6.620
2	6.680
3	6.740
4	6.715
5	6.690
6	6.710
7	6.770
8	6.830
9	6.760
10	6.780
11	6.710
12	6.870
13	6.810
14	6.940
15	6.940

## APPENDIX D: Sequential Extraction Supporting Documentation

**Table 17: Adsorption Constants for Ca<sup>2+</sup>, Cu<sup>2+</sup> and Fe<sup>2+</sup> in Sludge**

UKZiNe group	Group (PHREEQC Reprsn.)	Equation	log K
COOH1	Formic acid (Ukzine_f)	$\text{Ca}^{+2} + \text{Ukzine\_f} = \text{CaUkzine\_f}^{+}$	0.994
		$\text{Cu}^{+2} + \text{Ukzine\_f} = \text{CuUkzine\_f}^{+}$	0.222
		$\text{Cu}^{+2} + 2\text{Ukzine\_f} = \text{Cu}(\text{Ukzine\_f})_2$	0.363
		$\text{Cu}^{+2} + 3\text{Ukzine\_f} = \text{Cu}(\text{Ukzine\_f})_3$	0.310
		$\text{Cu}^{+2} + 4\text{Ukzine\_f} = \text{Cu}(\text{Ukzine\_f})_4$	0.290
		$\text{Fe}^{+2} + \text{Ukzine\_f} = \text{FeUkzine\_f}^{+}$	1.260
COOH2	Acetic acid (Ukzine_ac)	Same as Formic acid data	
NH4+	Ammonium (Ukzine_am)	$\text{Ca}^{+2} + \text{Ukzine\_amH}^{+} = \text{CaUkzine\_am}^{+2} + \text{H}^{+}$	-10.973
		$\text{Ca}^{+2} + 2\text{Ukzine\_amH}^{+} = \text{Ca}(\text{Ukzine\_am})_2^{+2} + 2\text{H}^{+}$	-22.546
		$\text{Cu}^{+2} + \text{Ukzine\_amH}^{+} = \text{CuUkzine\_am}^{+2} + \text{H}^{+}$	-9.945
HPO4-2	Phosphate (Ukzine_phos)	$\text{Ca}^{+2} + \text{Ukzine\_phosH}^{-2} = \text{CaUkzine\_phosH}$	2.128
		$\text{Ca}^{+2} + \text{H}^{+} + \text{Ukzine\_phosH}^{-2} = \text{CaHUkzine\_phosH}^{+}$	6.838
		$\text{Fe}^{+2} + \text{H}^{+} + \text{Ukzine\_phosH}^{-2} = \text{FeHUkzine\_phosH}^{+}$	8.908
		$\text{Fe}^{+2} + \text{Ukzine\_phosH}^{-2} = \text{FeUkzine\_phosH}$	3.240

## APPENDIX E: Experimental Results and Initial Conditions

**Table 18: Experimental Results for Washout Experiment (Mathir, 2013)**

Metal Concentration of Effluent (mg/l)						
Cycle	-3	-2	9	10	14	15
Al	0.193	0.188	0.109	0.187	0.166	0.060
Ca	16.276	14.555	6.690	7.551	5.637	4.833
Fe	1.321	1.253	1.176	1.197	0.968	0.335
Mg	14.052	12.200	5.961	5.565	4.043	3.527
Metal Concentration of Sludge at end of cycle (mg/l)						
Cycle	-3	-2	9	10	14	15
Al	6.127	5.581	6.179	6.611	5.834	1.394
Ca	3.388	2.601	2.018	1.962	1.478	0.462
Co	0.186	0.162	0.170	0.183	0.157	0.000
Cr	0.315	0.253	0.307	0.306	0.263	0.000
Cu	0.225	0.173	0.215	0.271	0.181	0.000
Fe	13.859	12.557	13.910	14.418	12.718	2.631
Mg	1.441	1.291	0.874	0.907	0.651	0.163
Mn	1.471	1.316	0.877	0.923	0.652	0.146
Zn	3.936	3.626	3.895	4.154	3.705	0.585

\*Shaded rows indicate experimental results for metals in the current study

## APPENDIX F: Sulphate-Sulphide System

### Appendix F-1: Pilot Plant Reaction and Reactor Conditions

The experimental set-up between the washout experiment and that of the pilot plant were slightly different. The main difference was that the pilot plant was being operated continuously and in the washout experiment, a batch-type of operation was carried out. The reactor conditions for the pilot plant are presented below in Table 16.

**Table 19: Model Reactor and Reaction Conditions for Pilot Plant**

Reactor Conditions		
Volume	litres	30
Supernatant Liquid Flowrate	litres/day	10 to 13
Sludge Flowrate	litres/day	200 to 400
Reactor Type	-	Anaerobic Bioreactor with Membrane Tank
Reactor Operation	-	Continuous
Temperature	°C	35 to 38
Pressure	atm	1
Membrane Area	m <sup>2</sup>	0.047
Reaction Conditions		
Volatile Fatty Acids	-	Acetic (CH <sub>3</sub> COOH) acid.
Reaction type	-	Biological reaction

### Appendix F-2: Sulphate Reducing Bacteria

PHREEQC default representation of the reduction of sulphate (SO<sub>4</sub><sup>2-</sup>):



The forward reaction favours the formation of the following precipitates:

**Table 20: Reactions showing precipitates forming in P-Model and AP-Model**

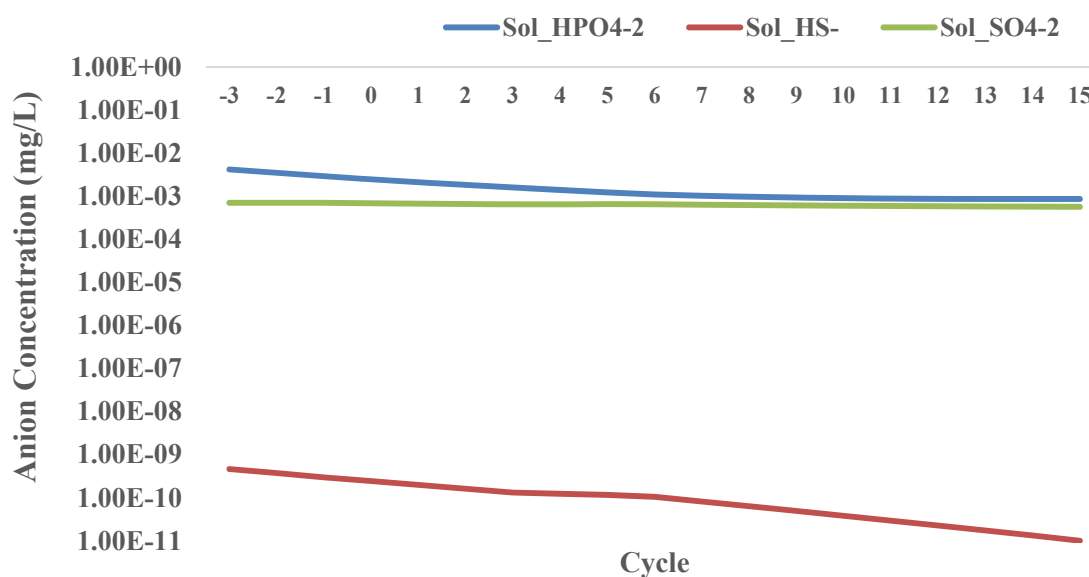
Mineral	Reaction	logK	Δ H (kJ)
CoS	$\text{Co}^{+2} + \text{HS}^- = \text{CoS} + \text{H}^+$	11.07	0.00
NiS	$\text{Ni}^{+2} + \text{HS}^- = \text{NiS} + \text{H}^+$	12.80	0.00
ZnS	$\text{Zn}^{+2} + \text{HS}^- = \text{ZnS} + \text{H}^+$	11.45	-30.00
CuS	$\text{Cu}^{+2} + \text{HS}^- = \text{CuS} + \text{H}^+$	22.30	-97.00
Ca <sub>5</sub> (PO <sub>4</sub> ) <sub>3</sub> OH	$5\text{Ca}^{+2} + 3\text{PO}_4^{-3} + \text{H}_2\text{O} = \text{Ca}_5(\text{PO}_4)_3\text{OH} + \text{H}^+$	44.33	0.00
Fe <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> :8H <sub>2</sub> O	$3\text{Fe}^{+2} + 2\text{PO}_4^{-3} + 8\text{H}_2\text{O} = \text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$	36.00	0.00
FeS <sub>2</sub>	$\text{Fe}^{+2} + 2\text{HS}^- = \text{FeS}_2 + 2\text{H}^+ + 2\text{e}^-$	18.51	-49.84
MnHPO <sub>4</sub>	$\text{Mn}^{+2} + \text{PO}_4^{-3} + \text{H}^+ = \text{MnHPO}_4$	25.40	0.00
MoS <sub>2</sub>	$\text{MoO}_4^{-2} + 6\text{H}^+ + 2\text{HS}^- + 2\text{e}^- = \text{MoS}_2 + 4\text{H}_2\text{O}$	70.26	-389.02

## APPENDIX G: Sequential Extraction Results – With Sulphide in the System

### COMPARISON BETWEEN PRECIPITATES FORMED IN THE P-MODEL AND AP-MODEL AND METAL PARTITIONING

This section highlights the sequential extraction results given the presence of sulphide in the system as the bulk of the study was done with a model including sulphide in the system. This section is aimed at detailing the impact of the conclusions drawn when there is sulphide content in the system; which according to the conclusions drawn from the study, still needs further work for correct representation of the sulphate-sulphide system.

Figure 42 below demonstrates the trend of the anions in the environment inclusive of sulphide; a gradual decrease is seen in the sulphate and sulphide content and this is due to reduction of sulphate and uptake and use of sulphide for the precipitation of minerals in the form of sulphide precipitates. Until cycle 12, the sulphate present in the system is the source of additional sulphide via the reduction reaction, it plateaus thereafter once the reaction has reached its equilibrium concentrations.



**Figure 41:  $\text{HPO}_4^{2-}$ ,  $\text{HS}^-$  and  $\text{SO}_4^{2-}$  concentrations per cycle**

In the first 3 cycles (-3, -2, -1), the precipitates increase with the addition of the nutrient recipe, as the nutrient recipe is increased; dissolved metals increase and further contribute to the saturation conditions of the respective metals. This results in further precipitation given the saturation conditions and sufficient sulphide in the system with which the metals can form minerals.

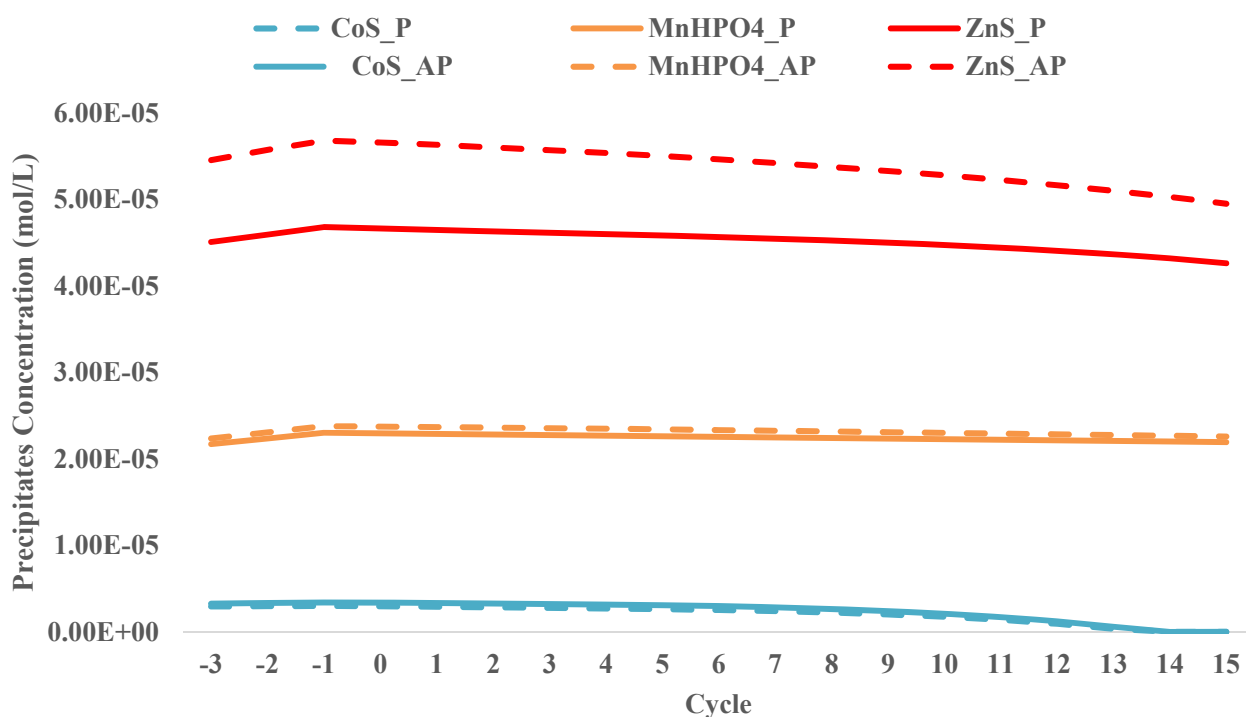
Figures 42 to 52 show the difference in precipitates formation as a result of the inclusion of the adsorbed phase. The biggest difference, occurs at the beginning of the washout experiment, where there are metals already adsorbed onto the surface of the sludge. These adsorbed metals



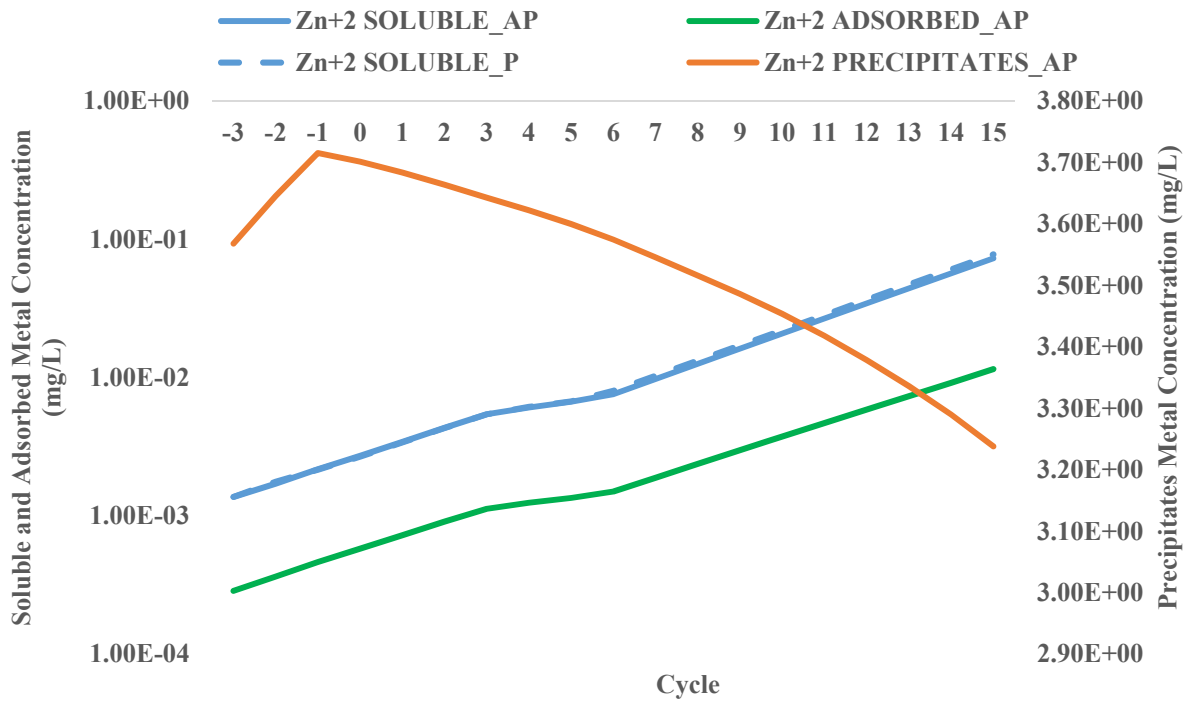
easily detach from the surface into the soluble phase and modify the manner in which the soluble phase reacts to the changes in the material balance in comparison to a precipitate-only model.

The generic concentration profile for precipitates formation is that of an initial increase, followed by a decrease as from cycle 0, which is true for all mineral precipitates formed. The precipitates in the system gradually decrease due to minimal washout via decanting and a considerable extent of displacement of metals into the soluble phase by re-solubilisation.

Figure 42 shows a deviation in the ZnS formed between the P-Model and AP-Model, which is explained by a higher adsorption constant than other metals. On re-solubilisation, an increase in the soluble and adsorbed phase concentrations results, as shown by Figure 43. It should also be noted that the extent to which metals are washed out is very minimal compared to the extent of retention in the system as the amount decanted is merely 1 litre, together with low concentrations. 20% of all metals are decanted, and 80% of the metals remain in the system.

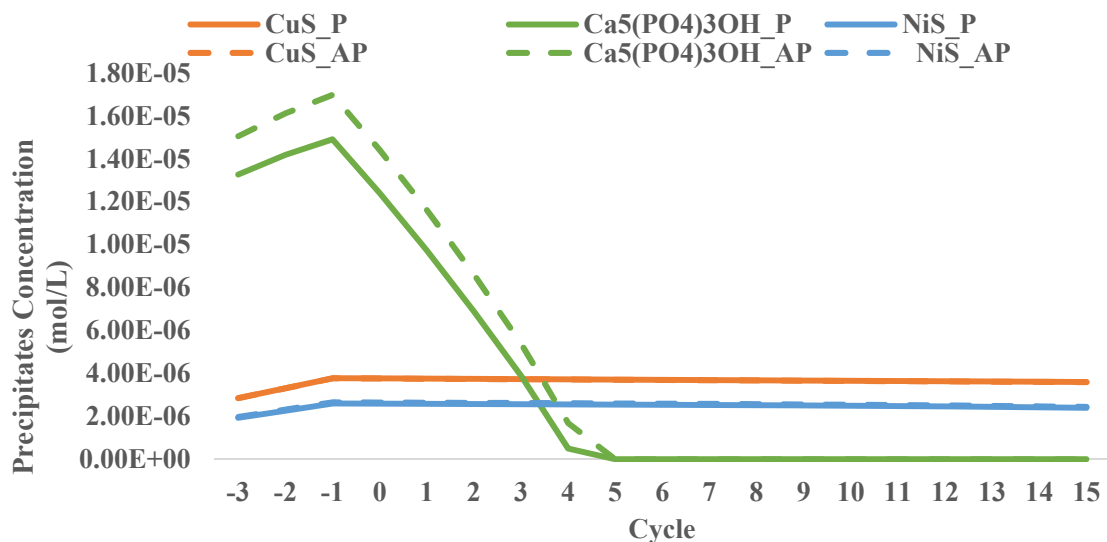


**Figure 42: CoS, MnHPO4 and ZnS precipitates formation for P-Model and AP-Model**

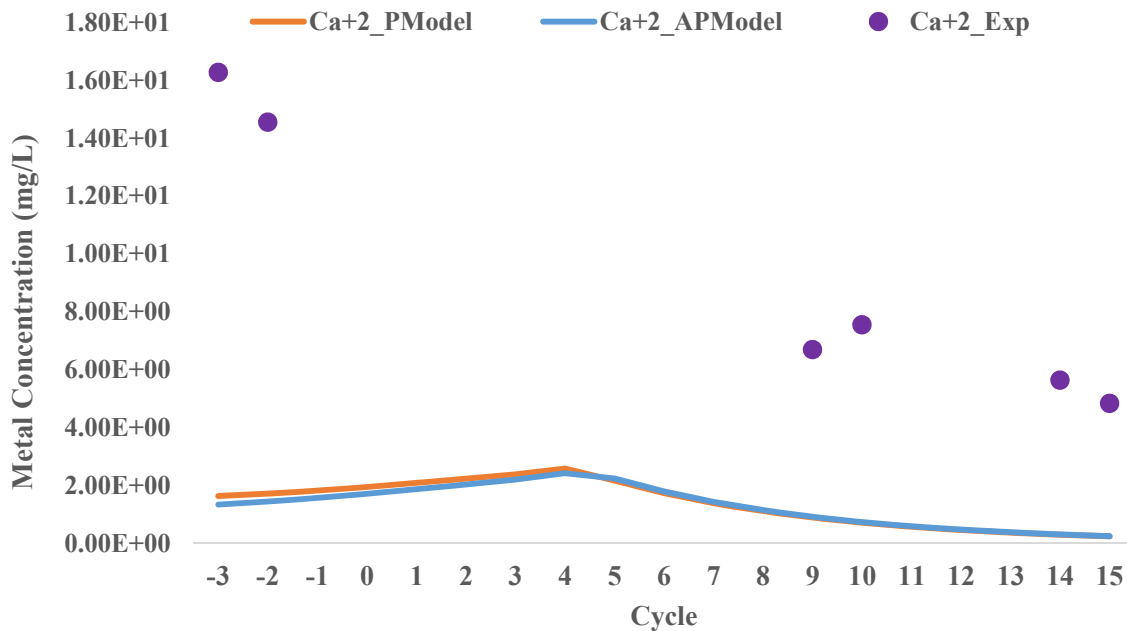


**Figure 43: Concentration Profile of Zn<sup>+2</sup> in the Soluble, Precipitates and Adsorbed Phase for AP-Model**

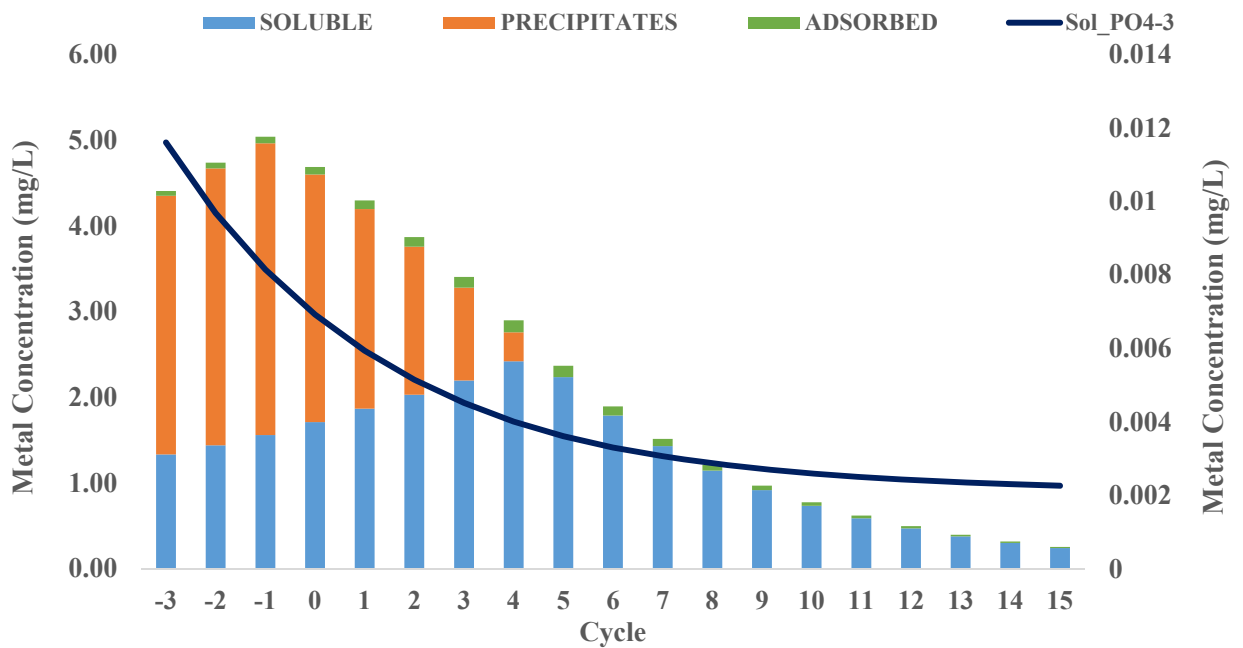
Figure 44 shows the same behaviour of Ca<sup>+2</sup> to that of Zn<sup>+2</sup>, however, for Ca<sup>+2</sup>, the mineral formed fully re-solubilises at the end of cycle 5. Figures 45 and 46 indicate that the soluble and adsorbed phase Ca<sup>+2</sup> concentrations increase from cycle -2 to 5, with the precipitates phase acting as a reservoir from which the Ca<sup>+2</sup> re-solubilises. Once the reservoir has been depleted, in cycle 5, a gradual decrease in the soluble and adsorbed phase is seen as a result of decanting and high concentrations of Ca<sup>+2</sup> in the decanted medium. The experimental soluble phase concentrations are significantly higher than the model, depicting a constant decreasing trend. The misalignment is highly due to misaligned initial conditions for Ca<sup>+2</sup>, as they were iteratively calculated by a change in the precipitates. This points to the need of changing both adsorbed and precipitates initial conditions to get a full set of initial conditions aligning to the experimental.



**Figure 44: CuS, Ca<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>OH and NiS precipitates formation for P-Model and AP-Model**

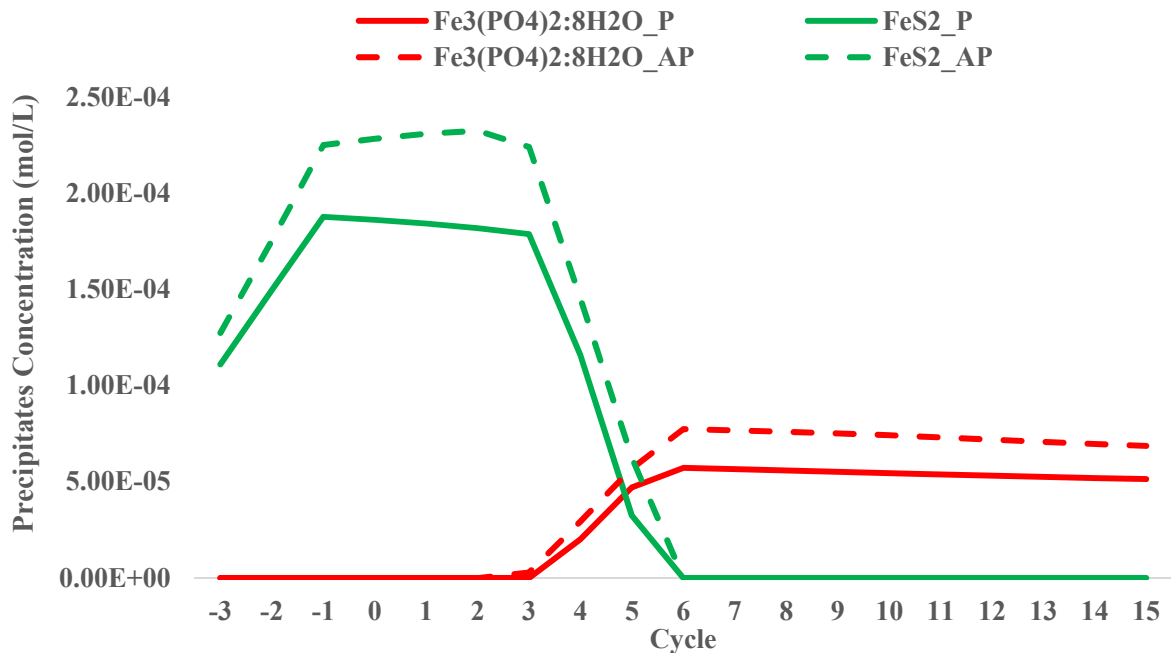


**Figure 45: Soluble Phase Concentration of Ca<sup>2+</sup> for the P-Model and AP-Model**



**Figure 46: Ca<sup>2+</sup> Partitioning in Soluble, Precipitates and Adsorbed Phase for AP-Model Comparing to PO<sub>4</sub><sup>3-</sup> Concentration**

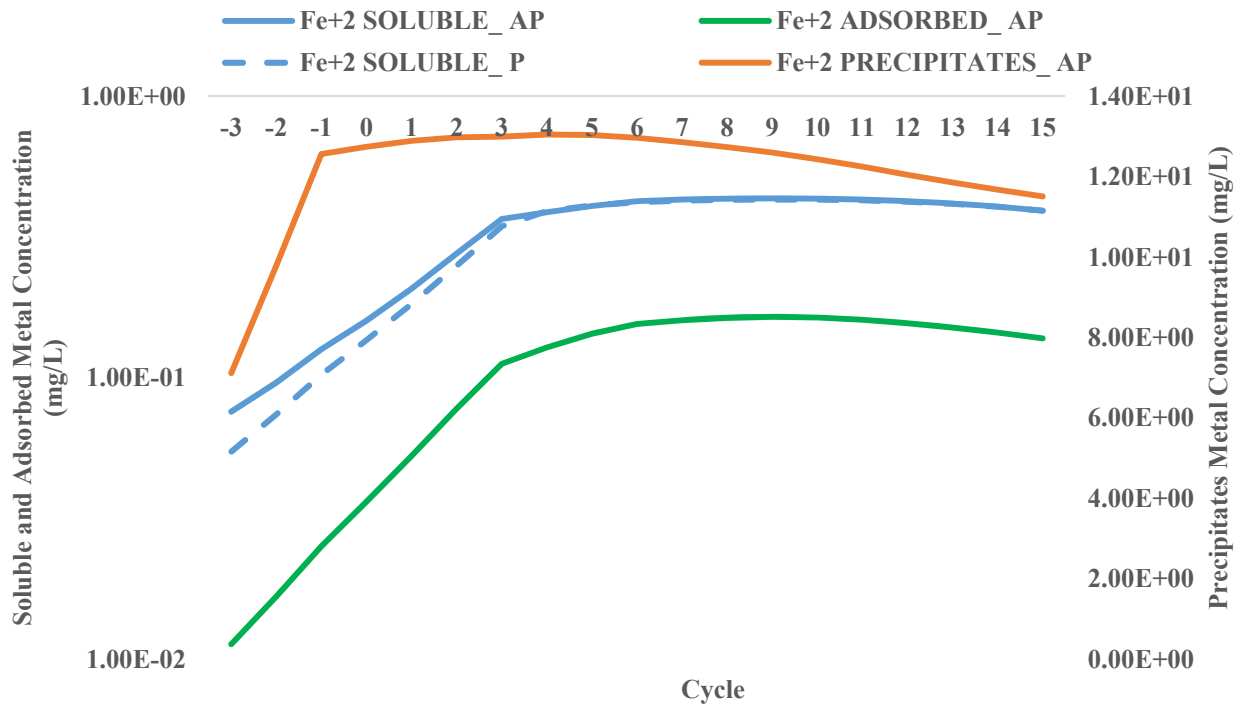
Figure 47 depicts an interesting relationship between Fe<sup>2+</sup> and the respective minerals it forms in the washout experiment. At the beginning of the experiment; the Fe<sup>2+</sup> is inclined to forming FeS<sub>2</sub> precipitate which is explained by favourable saturation conditions for the formation of the FeS<sub>2</sub> mineral and high sulphide concentration in cycle -3. In comparison to the other sulphate precipitates, FeS<sub>2</sub> “sweeps” the initial sulphide in the system, as it forms the most sulphide precipitate. This is due to Fe<sup>2+</sup> having a high concentration in the nutrient recipe than other metals, which is further exaggerated in the actual anaerobic process as a result of the Fe-based catalyst.



**Figure 47: Fe<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>:8H<sub>2</sub>O and FeS<sub>2</sub> precipitates formation for P-Model and AP-Model**

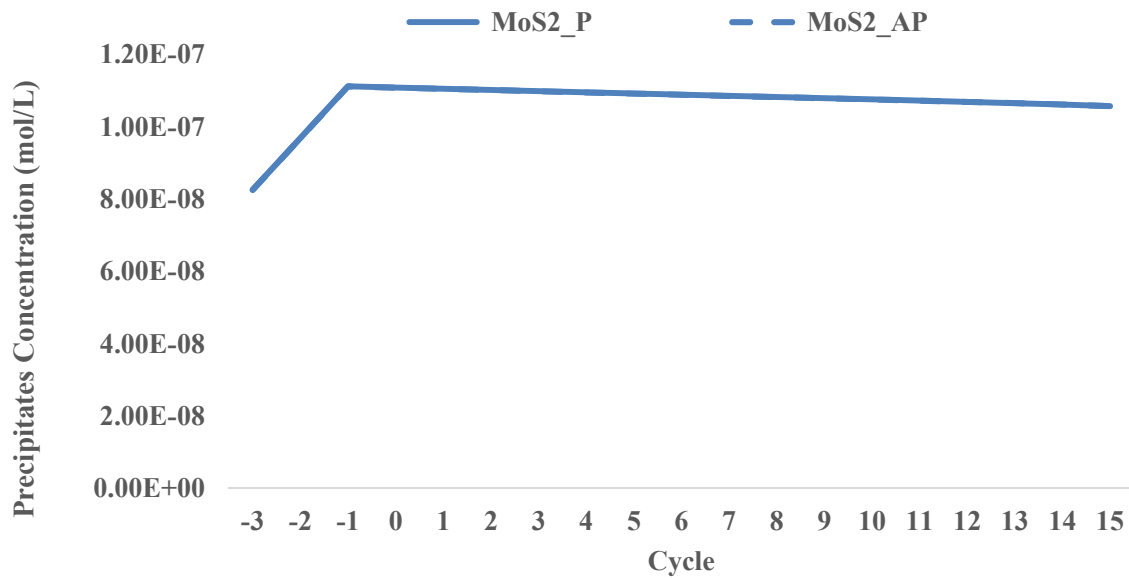
FeS<sub>2</sub> begins by an increase till cycle -1, which is owed to the increase in Fe<sup>+2</sup> with addition of the nutrient recipe, together with conversion of SO<sub>4</sub><sup>-2</sup> to HS<sup>-</sup> (as shown in Figure 40). Fe<sup>+2</sup> gradually re-solubilises from cycle 0 to 3, into the soluble phase, releasing Fe<sup>+2</sup> and HS<sup>-</sup>. HS<sup>-</sup> is mostly washed out of the system via decanting, however, the Fe<sup>+2</sup> remains in the soluble and adsorbed phases till the saturation conditions for the formation of Fe<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>:8H<sub>2</sub>O are favourable.

Between Cycle 3 and 6, the steepest re-solubilisation of Fe<sup>+2</sup> from FeS<sub>2</sub> is seen, which is directly linked to the formation of Fe<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>:8H<sub>2</sub>O accompanied by an increase in soluble and adsorbed phase concentration. From cycles 6 to 15, Fe<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>:8H<sub>2</sub>O re-solubilises and Fe<sup>+2</sup> is adsorbed, it is also gradually washed out of the system.

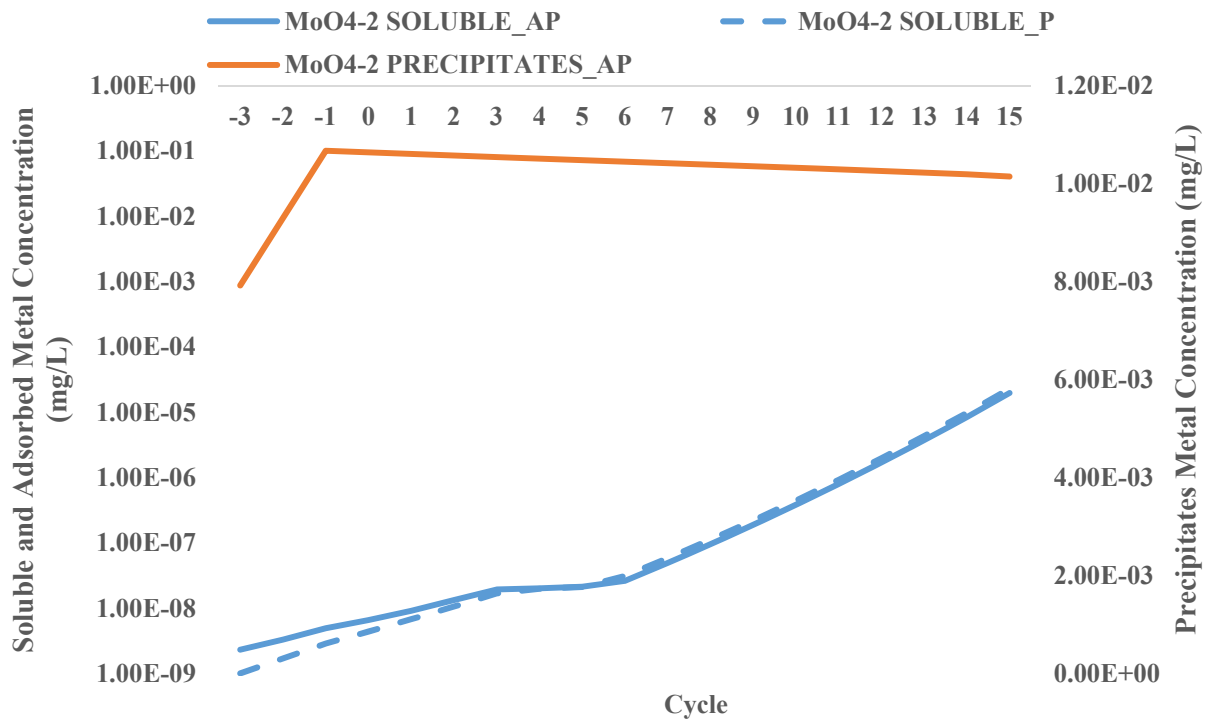


**Figure 48: Concentration Profile of Fe<sup>+2</sup> in the Soluble, Precipitates and Adsorbed Phase for AP-Model**

Figure 49 shows no deviation in MoS<sub>2</sub> formation with the inclusion of an adsorbed phase, Figure 50 shows that MoS<sub>2</sub> is solely in the soluble and precipitates phase and none is adsorbed onto the sludge surface.



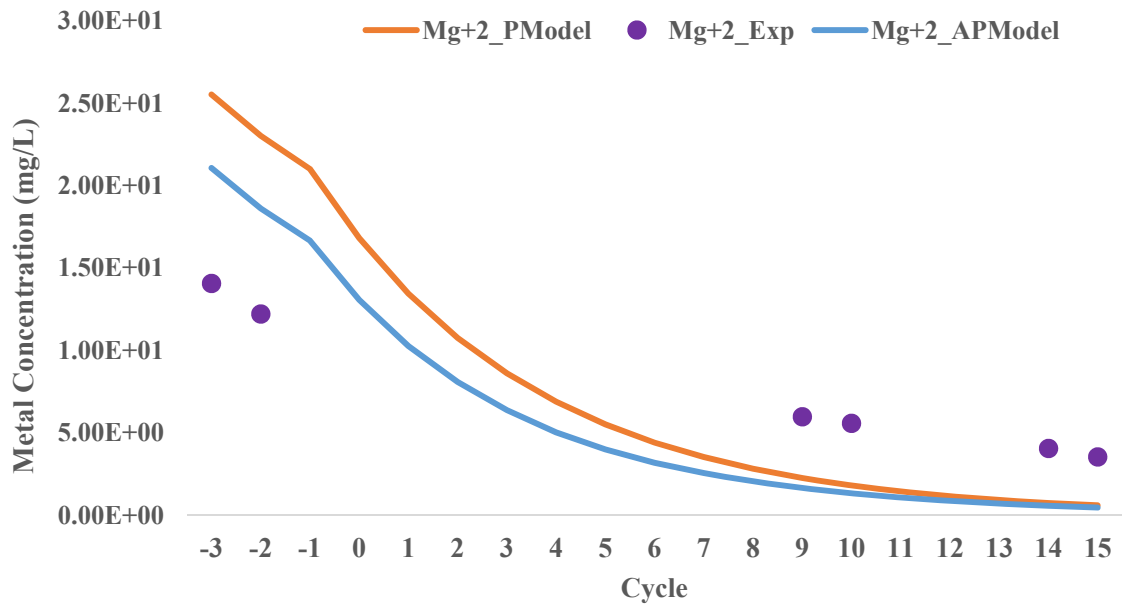
**Figure 49: MoS<sub>2</sub> precipitates formation for P-Model and AP-Model**



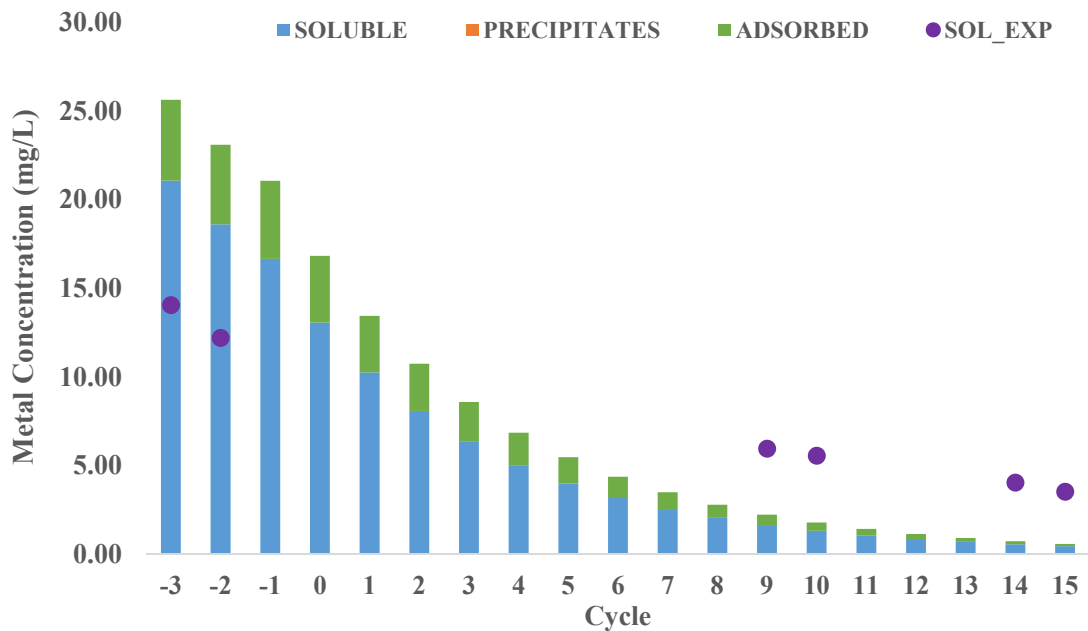
**Figure 50: Concentration Profile of  $\text{MoO}_4^{2-}$  in the Soluble, Precipitates and Adsorbed Phase for AP-Model**

As the washout experiment proceeds, the metals follow the sequence: precipitates  $\rightarrow$  soluble  $\rightarrow$  adsorbed; where the metals are first released into the soluble phase with the re-solubilisation of the precipitates and are thereafter retained in the system as they are adsorbed to the sludge surface. The reverse order is also possible, where metals loosely adsorbed onto the surface detach into the soluble phase and result in precipitation of metals. However, in this case, the former sequence occurs to a greater extent and this is shown in the trends of soluble phase concentration and metal partitioning in the respective phases.

Figure 51 shows a lower soluble phase concentration for  $\text{Mg}^{+2}$  as predicted by the AP-Model given the same initial conditions. Figure 52 indicates that this deviation is due to adsorption of  $\text{Mg}^{+2}$  on the sludge surface. In comparison between the model and experimental data, the model trend follows the same shape and traverses between the experimental data. Looking at cycle 9 to 15 a slight retention of  $\text{Mg}^{+2}$  in the latter part of the washout experiment suggests greater adsorption in latter part of the experiment as opposed to the former.



**Figure 51: Soluble Phase Concentration of Mg<sup>+2</sup> for the P-Model and AP-Model**

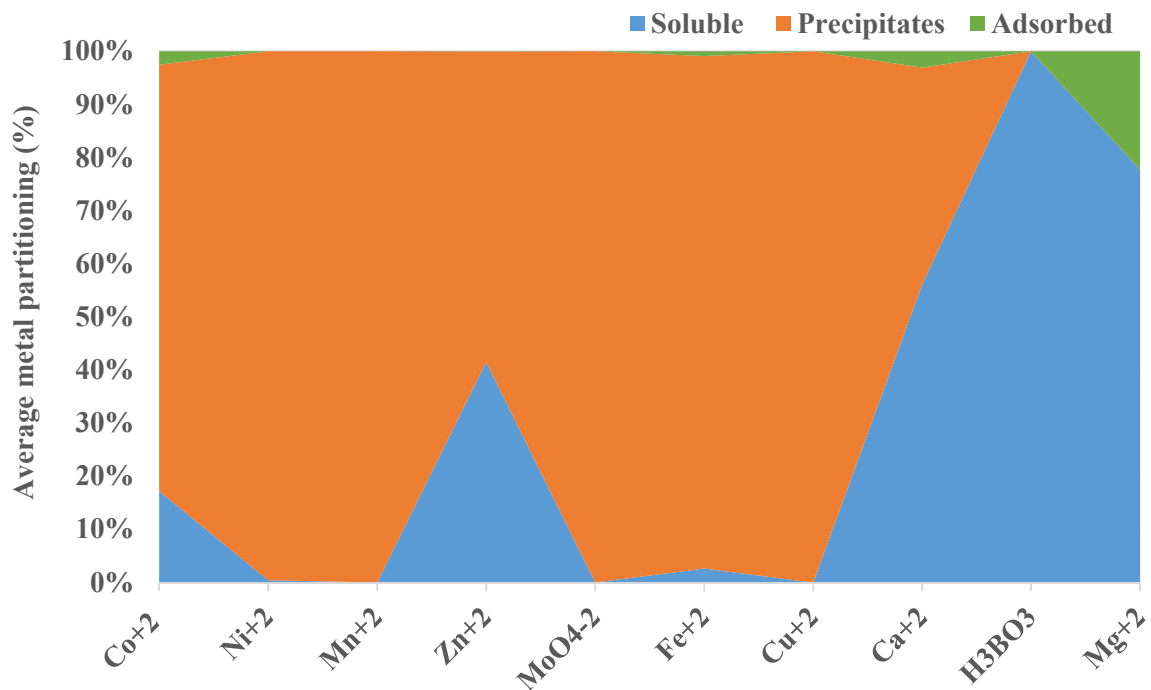


**Figure 52: Mg<sup>+2</sup> Partitioning in Soluble, Precipitates and Adsorbed Phase for AP-Model**

A trend indicating metal retention before a metal concentration decrease is seen for Mn<sup>+2</sup> due to re-solubilisation of metals from the various phosphate minerals, which have been shown to behave as buffers for metals in the system.

Figure 53 shows the average metal partitioning for the washout experiment, giving an indication of the ranking of metal bioavailability. A trend of decreasing bioavailability is as follows: H<sub>3</sub>BO<sub>3</sub> > Mg<sup>+2</sup> > Ca<sup>+2</sup> > Zn<sup>+2</sup> > Co<sup>+2</sup> > Fe<sup>+2</sup> > Ni<sup>+2</sup> > MoO<sub>4</sub><sup>-2</sup>, Cu<sup>+2</sup>, Mn<sup>+2</sup>. According to (van Hullebusch, et al., 2005a), the sequence for bioavailability of metals in anaerobic sludge should be as follows: Ni<sup>+2</sup> > Cu<sup>+2</sup> > Zn<sup>+2</sup> > Mg<sup>+2</sup> > Fe<sup>+2</sup> > Co<sup>+2</sup> > Mn<sup>+2</sup>.

The following consistencies are seen between the model sequence and literature data:  $Mn^{+2}$  is shown to be the least bioavailable metal,  $Zn^{+2}$  is more bioavailable than  $Co^{+2}$ . The following inconsistencies are seen;  $Ni^{+2}$  and  $Cu^{+2}$  are seen to be the most bioavailable by the literature data, which is not reflected by the model, and is due to increased sulphate precipitation of these two metals.



**Figure 53: Average metal partitioning for wash out experiment to indicate ranking of free metal ions**

Throughout the model results, it is evident that a considerable amount of metals are retained in the system as a result of precipitation. With metals being retained in the precipitates phase, they are unavailable for use by the microorganisms. The presence of anions with which metals precipitate, affects the bioavailability of the metal cations. This resulted in more attention being given to understand the representation of the anions with which the cations precipitate, particularly the sulphate-sulphide system as more precipitates form due to the presence of sulphide in the system. This section shows supporting data for further investigation of the sulphate and sulphide in the system as shown in the result section of this paper.



**APPENDIX H: Comparison of P-Model and AP-Model Results to Literature Data**

**Table 21: Comparison of Model Results to Literature Data**

Reference	(Alvarez, et al., 2002)				(Alvarez, et al., 2002)				(Feuntes, et al., 2004)							
Sludge Type and Sequ. Extr. Scheme	Secondary Sludge in BCR Scheme of municipal activated sludge plant				Dewatered and Digested Sludge in BCR Scheme of municipal activated sludge plant				Anaerobic sludge using the BCR scheme							
	%				%				%							
Metal	Exch.	Red.	Oxid.	Res.	Exch.	Red.	Oxid.	Res.	Exch.	Red.	Oxid.	Res.				
Fe <sup>+2</sup>	0.50	12.50	19.00	68.00	0.30	1.00	17.50	82.00	0.27	0.25	9.72	76.88				
Co <sup>+2</sup>	22.00	12.00	43.50	24.00	12.00	3.00	52.00	35.00	-	-	-	-				
Cu <sup>+2</sup>	1.50	1.00	83.00	17.00	0.30	0.30	81.00	18.00	2.97	0.65	63.50	18.10				
Mn <sup>+2</sup>	55.00	29.00	10.00	7.00	44.00	24.50	20.00	13.00	-	-	-	-				
Ni <sup>+2</sup>	23.00	15.00	40.00	22.00	18.00	5.50	44.00	34.00	20.35	5.52	22.07	34.48				
Zn <sup>+2</sup>	12.00	39.00	35.50	9.00	10.00	18.50	56.00	15.00	10.79	8.84	49.48	20.32				
Reference	(van Hullebusch, et al., 2005a)				(Mathir, 2013)				AP Model - Sulphide Present				AP Model - No Sulphide			
Sludge Type and Sequ. Extr. Scheme	Anaerobic Nedalco sludge according to modified Tessier Scheme				Anaerobic sludge according to the Stover Scheme				Anaerobic sludge according to PHREEQC speciation model				Anaerobic sludge according to PHREEQC speciation model			
	%				%				%				%			
Metal	Exch.	Red.	Oxid.	Res.	Exch.	Red.	Oxid.	Res.	Exch.	Red.	Oxid.	Res.	Exch.	Red.	Oxid.	Res.
Mg <sup>+2</sup>	-	-	-	-	52.00	-	32.00	16.00	77.72	0.00	22.28	0.00	77.72	0.00	22.28	0.00
Fe <sup>+2</sup>	3.54	42.53	8.10	45.82	18.61	-	78.67	2.71	2.65	96.44	0.91	0.00	75.94	0.00	24.06	0.00
Co <sup>+2</sup>	10.67	16.00	56.00	17.33	-	-	-	-	17.21	80.26	2.53	0.00	89.23	0.00	10.77	0.00
Cu <sup>+2</sup>	1.89	1.89	69.81	26.42	26.99	-	73.01	0.00	0.00	100.00	0.00	0.00	100.00	0.00	0.00	0.00
Mn <sup>+2</sup>	24.71	45.88	18.82	10.59	-	-	-	-	0.01	99.99	0.00	0.00	0.01	99.99	0.00	0.00
Ni <sup>+2</sup>	7.11	45.69	34.52	12.69	-	-	-	-	0.45	99.54	0.01	0.00	97.77	0.00	2.23	0.00
Zn <sup>+2</sup>	0.95	8.06	80.57	10.43	12.17	-	66.46	21.37	0.50	99.42	0.09	0.00	83.21	0.00	16.79	0.00

