MICRO-NUTRIENT REQUIREMENTS FOR ANAEROBIC DIGESTION OF CONCENTRATED INDUSTRIAL EFFLUENTS

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EXECUTIVE SUMMARY

INTRODUCTION

Sasol has been one of South Africa's largest producers of fuel since the early 1950s. The chemical and physical change undergone by the raw material into fuel occurs in Lurgi dry-ash gasifiers, where fuel is produced from the gasification of coal in the presence of oxygen and steam. This process makes use of Fischer-Tropsch technology. Coal gasification produces a synthesis gas (syngas) composed of a mixture of CO and H_2. 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_1 to C_{20} and water: The reaction water becomes an effluent referred to as Fischer-Tropsch Reaction Water (FTRW).

FTRW has a high organic acid 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 to its reuse in the plant. Aerobic digestion is currently employed by the Sasol Secunda plant, but Sasol has been investigating anaerobic treatment of FTRW, due to its potential advantages including decreased energy requirements and sludge production. Supplementing micronutrients is a major operating cost in anaerobic digestion of FTRW. It is not clear whether the recipe used meets the micronutrient requirements in the most cost effective manner. Sasol is therefore interested in reduction of the micronutrient dosing costs by optimisation of the micronutrient recipe without compromising the biological activity.

OBJECTIVE

The study aimed to develop a theoretical model of the controlling factors of the distribution of metals in anaerobic digester mixed liquor, in order to manage the dosing of micro-nutrients in the most effective manner. The micro-nutrient metals studied were calcium, cobalt, copper, chromium, iron, magnesium, manganese, molybdenum, nickel and Zinc

METHODOLOGY

This was purely a modelling project, with no experimental component.

The model considered the equilibrium distribution of the metals between the soluble phase, precipitates and adsorption onto the biomass. It was expected that the metals dosed to the system would form precipitate with carbonate and phosphate present. For the equilibrium speciation, the software package PHREEQC was used. The equilibrium precipitation model is built into PHREEQC, however the adsorption model had to be added. This was achieved by formulating the biomass as a customised component with reactive sites which could bind the metals. The thermodynamic parameters for the binding reactions were initially estimated from literature, with the intention of using experimental data to calibrate their values.

The experimental data was sourced from a previous investigation by Mathir (2013), supplemented by some measurements made by Sasol on a pilot plant anaerobic digester.

OUTCOMES

In the process of calibrating the model against the available measurements it became evident that any conversion of sulphate to sulphide during anaerobic digestion would have a crucial effect on the results, due to the extremely low solubility of the metal sulphides. The model shows that, because the doses of the micro-nutrients metals are so low, even a very small amount of sulphide will have a major impact on the distribution of the metals. However, it was not possible to predict the conversion of sulphate to sulphide, so experimental measurements were needed to resolve the question. In the absence of such data, it was not possible to prove or disprove the validity of the adsorption model. Overall, the model correctly reflected the experimental result that, apart from calcium and magnesium, none of the metals appeared in the soluble phase to any measureable extent, but it was not possible to predict the distribution between adsorption onto the biomass and precipitates with any certainty.

DISCUSSION AND CONCLUSIONS

The sulphide question was entirely unexpected when the project methodology was being formulated. It had also not been anticipated in the experimental investigations that provided the experimental data for the model, which explains why it was never measured.

No sulphide was fed to the system, but the possibility existed that sulphate that was present might be partially converted to sulphide by biological action. It was the model itself that drew attention to the issue. The sulphate to sulphide conversion is a redox reaction, and by default

PHREEQC assumes redox equilibrium. Accordingly it predicted that some of the sulphate present in the system would be converted to sulphide. However, the equilibrium model cannot be expected to provide a reliable prediction of the conversion to sulphide, because such redox reactions are very unlikely to approach equilibrium under the conditions prevailing in a methanogenic anaerobic digester. The conversion required the action of sulphidogenic micro-organisms, and the conditions were not clearly favourable for their survival. So measurement was the only means of establishing how much sulphide was present, if any at all.

The uncertainties that remain around sulphide not only prevented the successful calibration of the model, but also constitute a major gap in understanding the dynamics of the micronutrient metals in the system, which was the ultimate goal of the project. The fact that soluble metal concentrations were not detected in most of the samples provided an indirect indication that sulphide was indeed present, and that it may have constituted a strong limitation on the bioavailability of the dosed metals. This suggests that the identification of the sulphide problem may prove to be the most important outcome of the investigation.

It even seems possible, from the model results, that minimising the precipitation of micro-nutrient metal sulphides (perhaps by dosing excess iron) might turn out be the most important factor in optimising the effectiveness of micro-nutrient metal dosing. However, without any sulphide measurements to guide the model these issues could not be resolved with any certainty. The model results that have been presented are just scenarios based on assumptions, in order to give an indication the range of possibilities.

RECOMMENDATIONS

Modelling has exposed, but not resolved, a number of shortcomings in the experimental programmes undertaken so far, and the chief recommendation is to undertake a new experimental programme that addresses these shortcomings. Since Mathir's (2013) investigation, Sasol have developed experimental facilities that should be ideal for such an investigation, and could not conceivably be matched by the university. These are the laboratory-scale and large-scale pilot plants.

The large-scale pilot plant is fed with real factory effluent, and so is exposed to all the unexpected components that might affect the micro-nutrients. It needs a conservative, stable operating regime, but can provide large samples of material for intensive and extensive chemical analyses. Thus, it would be possible to undertake crystallographic analysis to confirm the presence of specific precipitates under normal operating conditions. It can also be used to provide data on the key issue of conversion of sulphate to sulphide under realistic operating conditions.

The laboratory scale pilot plant can be used for complementary experiments, particularly those which involve the risk of reaction failure, such as a nutrient wash-out experiment. This is because waste sludge from the large reactor can be used to restore it to full operation almost immediately. It set up for continuous operation, ands comprehensively instrumented for monitoring the anaerobic digestion performance.

The experimental programme that is envisaged is a series of nutrient washout experiments performed on the lab-scale reactor, each starting with sludge taken from the large-scale reactor during stable operation. The laboratory reactor can be operated under that same conditions as the large reactor, except for the micro-nutrient dosing. Once the anaerobic digestion performance is clearly impaired, the experiment can be stopped, and a new one initiated with sludge from the large reactor.

A series of such experiments in which different micro-nutrient components are removed from the recipe, should soon yield information which can be used to identify which ones are most critical, and eventually to optimise the formulation.

The modelling has suggested that the levels of sulphide in the system has a strong influence on the bioavailability of the micro-nutrient metals. Experiments should be designed to test this hypothesis, because if it is correct, controlling the level of sulphide in the system may prove to be a key strategy for increasing the effectiveness of micro-nutrient dosing.

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GLOSSARY

Adsorption	The accumulation of molecules of a gas or liquid to form a thin film on the surface of a solid.
Aerobic Digestion	The process carrying out the mineralisation of the complex organic matter in wastewater to final products of CH_4, CO_2, H_2 and H_2O *in the presence of oxygen*.
Anaerobic Digestion	The process carrying out the mineralisation of the complex organic matter in wastewater to final products of CH_4, CO_2, H_2 and H_2O *in the absence of oxygen.*
Bioavailability	The extent to which metals in sludge chemically avail themselves for biological uptake by the microorganisms to bring about a biological response.
Biological Response	A response to the catalytic effect of enzymes produced by micro-organisms bringing about the degradation of organic content to form CH_4, CO_2, H_2 and H_2O.
Biomass	The total measure (quantity or weight) of organisms in a given area or volume, sludge in this case.
Complexation	A mechanism by which metal ions join onto the surface of organic content, resulting in the formation of a metal-surface complex.
Metal Toxicity	The boundary condition for metal addition to activated sludge corresponding to an excess of metals.
Methanogens	Methanogenic bacteria responsible for conversion of acetate and hydrogen into methane in anaerobic digestion.
Microorganism	Minute living organisms that feed on organic material in anaerobic digestion, bringing forth decomposition into various products.
Micronutrients	A mixture (recipe) of trace metals supplemented to sludge, including : Mn, Zn, Co, Mo, Ni, Cu, Va, B, Fe, I, Se, Cr and W.
pH	A measure of the molar concentration of hydrogen ions in the solution, giving a measurement of the acidity or basicity of the solution.
Precipitation	The process of the formation of solids from a solution by a reaction between metal ions and anions to form minerals.
Sequential Extraction	An experimental technique in which metal ions are extracted from the different phases in which they exist in a system (sludge) by a solvent.

Sludge	A semi-solid slurry, a mixture of solid and liquid. In this study, the liquid is FTRW and the solid is biomass.
Speciation	The distribution of an element between the chemical species it may exist in, in a system.
UKZiNe	Ionic representation of anaerobic sludge surface consisting of two carboxylic acid groups, a phosphate and an ammonium group (pr. You-ka-zeen)

LIST OF ACRONYMS AND ABBREVIATIONS

Acronym or Abbreviation	Explanation
ADMLSS	Anaerobic Digester Mixed Liquor Suspended Solids
Aij	Stoichiometric coefficient giving the number of moles of component j in species i.
Al	Aluminium
AP-Model	Adsorbed and Precipitates Phase Controlled Speciation Model
AVS	Acid Volatile Sulphide
AVS-Me	Semi-continuously extracted metals
B	Boron
BCR	The commission of the European communities Bureau of Reference
βi	Overall equilibrium formation constant for species i.
BFMpH	Best Fit Model pH
BLM	Biotic Ligand Model
BR	Biological response
BRT	Biological receptor theory
C	Compost
Cd	Cadmium
CH_4	Methane gas
Cj	Concentration of component j
C_nH_{2n}	Alkene hydrocarbon functional group
CO	Carbon Monoxide
Co	Cobalt
CO_2	Carbon Dioxide
CO_3^{-2}	Carbonate ion
COD	Chemical Oxygen Demand

Cr	Chromium
Cu	Copper
D+DS	Dewatered and digested sludge
DOM	Dissolved Organic Matter
ECP	The human eosinophil cationic protein (ECP), also known as RNase
EDTA	ethylenediaminetetraacetic acid
Fe	Iron
FIAM	Free Ion Activity Model
FTRW	Fischer-Tropsch Reaction Water
GSIM	Gill Surface Interaction Model
H_2	Hydrogen gas
H_2O	Chemical formula of water
HNO_3	Nitric acid
I	Iodine
K	Potassium
KF	Potassium fluoride
KNO_3	Potassium nitrate
LC_{50}	Concentrations of the biotic ligand bringing about a mortality of 50%
M or M^z	Free metal
$MgCl_2$	Magnesium Chloride
ML	Metal-Ligand complex
$M\text{-}A_{cell}/M\text{-}R_{cell}$	Adsorbed metal
Mj	Total mass of component
Mn	Manganese
Mo	Molybdenum
mol/litre	Units of concentration
MPa	Pressure units: Megapascals

MpH	Model pH
N	Nitrogen
$Na_4P_2O_7$	Sodium diphosphate
N_C	Number of components
Ni	Nickel
NOM	Natural Organic Matter
OLR	Organic Loading Rate
OM	Organic Matter
P	Phosphorus
P-Model	Precipitates Phase Controlled Speciation Model
Pb	Lead
PHREEQC	PHREEQC stands for pH (PH), redox (RE), equilibrium (EQ) and programme written in C (C)
PS	Primary Sludge
PO_4^{3-}	Phosphate ion
Qe/Qmax	Uptake and maximum uptake of metal ion by biomass
R&D	Research and Development
S	Sulphur
S^{2-}	Sulphide ion
SBR	Sequencing Batch Reactor
SCFA	Short Chain Fatty Acid
Se	Selenium
SMP	Soluble Microbial Products
SS	Secondary Sludge
Ti	Titanium
TSS	Total Suspended Solids
UASB	Up-flow Anaerobic Sludge Bed
Va	Vanadium

VS	Volatile Solids
W	Tungsten
WEST	An open source waste water treatment modelling software.
X-cell	Free surface site on cell membrane
$\boldsymbol{x_i}$	Activity of the aqueous species i
ZED	Zero Effluent Discharge
Zn	Zinc
$\boldsymbol{\gamma_i}$	Activity coefficient of species i

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1 INTRODUCTION

1.1 CONTEXT OF THE STUDY

Sasol is one of South Africa's largest producers of fuel since the early 1950's (Dry, 2002). The chemical and physical change undergone by the raw material into fuel occurs 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_2. 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_1 to C_{20} and water: reaction water is produced. This reaction proceeds as follows (van Zyl, 2008):

$$\mathrm{nCO + 2nH_2 \rightarrow C_nH_{2n} + nH_2O + CH_4} \qquad \text{......(1-1)}$$

As can be seen in reaction 1-1 above, water is chemically produced as a by-product in the gasifiers, and is referred to as Fischer-Tropsch Reaction Water (FTRW). The FTRW is studied to be an aqueous solution deficient in both macronutrients (N, P, etc.) and micronutrients (Van Zyl, 2008) whose solutes principally consist of short chain fatty acids (SCFAs) and alcohols. It 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 carried out 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 to 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 been extensively looking into anaerobic treatment of FTRW, due to its potential advantages including decreased oxygen demand costs, thereby decreasing energy requirements due to 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.

Anaerobic digestion's major concern is its control, as anaerobic systems have difficulty in responding to process fluctuations and consequently operate 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_2 and CH_4 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, they are essential for growth, enzymatic processes and metabolic functions (Fermoso, et al., 2009). 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 on 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 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 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 modelled 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

Supplementing micronutrients results in a major operating cost in anaerobic digestion of FTRW and requires that the micronutrient recipe used 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 reduction of the micronutrient dosing costs 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 is to develop a theoretical model to depict the controlling factors of 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. The model is to be constructed to direct experiments designed to bring about modifications to the current dosing strategy such that the micronutrient supplementation operating cost is lowered without compromising metal activity in the system.

1.4 SPECIFIC OBJECTIVES

- To develop an integrated ionic Speciation model that can describe the fate of metals dosed an anaerobic digester treating FTRW. The model should account for both precipitation of the micro-nutrient metals and adsorption onto biomass.
- To calibrate the model against experimental data of Mathir (2013) and additional data measured on the Sasol pilot plant and laboratory digesters.
- To use the calibrated model to explore strategies for maximizing the efficiency of micro-nutrient dosing.

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 provided certain inputs.

1.6 LIMITS OF THE STUDY

This was a pure modelling study: experimental data used for validation was performed by parties outside the contract. This meant that experimental data identified as necessary for calibrating and validating the model could not always be obtained.

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 brought to attention 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 with its active site remains 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 mineralisation of the complex organic matter in the water to final products of CH_4, CO_2, H_2 and H_2O. 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 microorganisms that are genetically capable of processing the compound; it is desirable that the microorganisms be acclimated[1] to the compound (Fogler, 2009). The complex organics are thereafter 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).

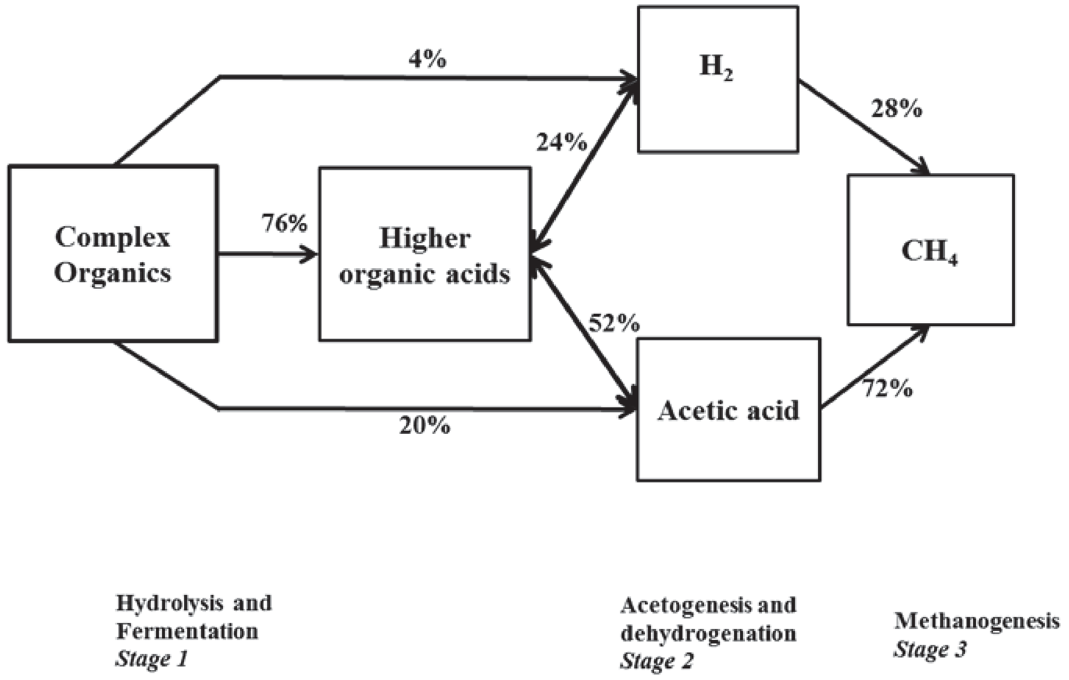

Figure 1: The Three Stages of Methane Fermentation [a]

[a]: 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)

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[1] **Acclimation** is the process in which a population of organisms 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.

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 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 stock, 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 showed 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 greatly determines the efficiency of the degradation process in the treatment of effluent wastewater (Burgess, et al., 1999).

It is paramount 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 serve a purpose of stimulating 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 conducted (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 2.1.

The methanogens which are responsible for converting acetate and hydrogen to methane are in a separate class as compared to the other microorganisms; the levels of nitrogen and phosphorus that are considered adequate for them are different from other micro-organisms (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 [b] (Burgess, et al., 1999)

Micronutrient	Requiring organisms	Role
Iron (Fe^{2+}, Fe^{3+})	Aerobic bacteria	Growth factor
	Aspergillusniger	Growth factor
	Chlorella pyrenoidosa	Adsorbed in quantities directly proportional to the concentration available.
Fe^{3+}	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. Stimulates cell growth. Activity of carbonic anhydrase and carboxypeptidase A. Toxic at low concentrations (1 mg/L), especially to protozoa. Toxic at low concentrations (1 mg/L), especially to protozoa.
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.
	Chlorella pyrenoidosa	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	Cyanobacteria and Chlorella	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.
	Thiothrix and Zoogloea	Increase growth rates and improve flocculation.
	All bacteria	Requirements and effects vary. Interacts with other metals.

[b]: Refer to (Burgess, et al., 1999) for references used in construction of the table.

2.1.4 MICRONUTRIENT DOSING

2.1.4.1 METAL BIOAVAILABILTY

“Bioavailability” is a term with 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 a lot of sectors and therefore highly depends on the environmental discipline its being used in 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 in which they exist by a solvent, after extraction, the total metals concentration and the concentration of the metals in the soluble phase is analytically determined. Analytical methods have difficulty in expressing the metals bound in other phases and are therefore insufficient to be used 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.

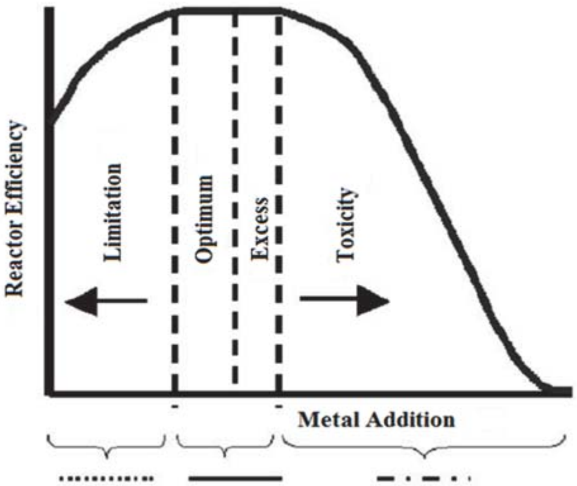

Figure 2: Boundary conditions for metal addition to keep UASB reactor efficiency optimal (Fermoso, et al., 2009)

The relationship between metal addition and reactor efficiency is depicted in Figure 2. 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).

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)

Metal	Organism	Toxic Concentration	Conditions
Co	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	
Ni	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_{50}	Granules were sample from four UASB reactors at COD loading rate of 10 g COD L^{-1} d^{-1} for over six months at 37°C.
Zn	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, consequently decreasing the 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 greatly related 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 catered for 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 harvested by 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. Fermoso (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 chemical 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 processes bring about a drastic decrease 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 (Fermoso, 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 compete for 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 (Fermoso, et al., 2009).

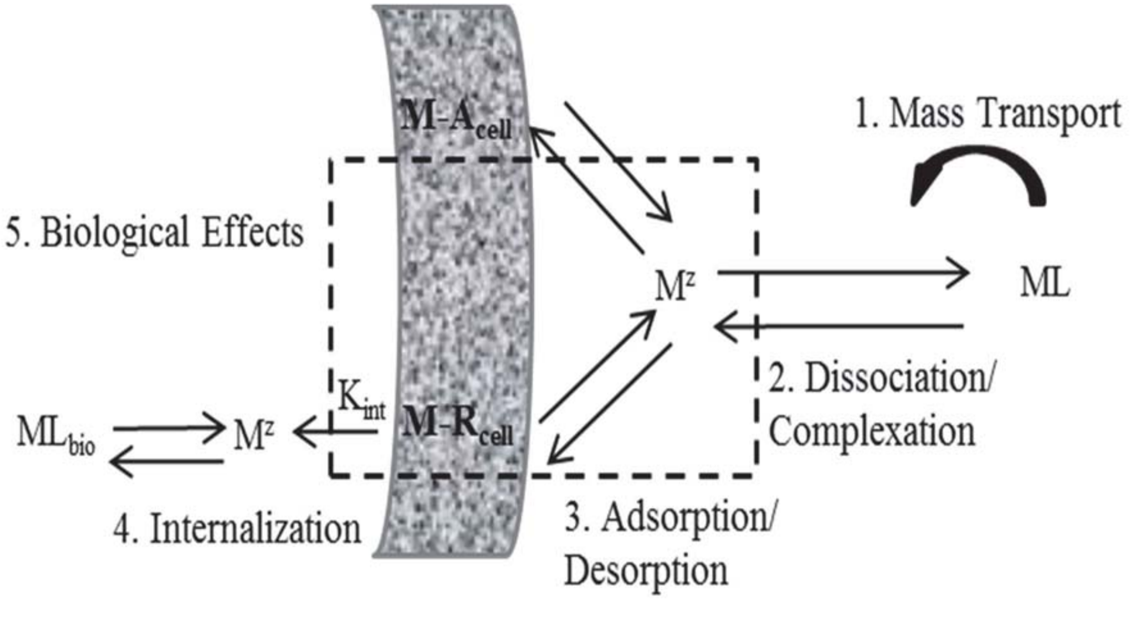

Figure 3: Conceptual Framework of the Free Ion Activity Model and Biotic Ligand Model. M^z: free metal ion, L: ligand, ML: hydrophilic complex, ML_{bio}: metal-intracellular ligand complex, K_{int}: internalisation rate constant, M-A_{cell}: non-specific metal-surface adsorption complex, M-R_{cell}: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

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 (also termed specificity) of reagents may be explained as the ability of the reagent to extract only metals in the fractions it was intended to extract; non-selectivity (non-specificity) would therefore describe 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 $MgCl_2$ and EDTA as extractants, the following redistribution of metals was witnessed: re-adsorption of Pb during the $MgCl_2$ 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) 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 proposed by Filgueiras is shown in Table 3.

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

Fraction	Description
Water Soluble (F_1)	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_2)	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.
Acid Soluble (F_3)	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_4)	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_5)	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_6)	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

According to Álvarez, et al. (2002), the main method used in Europe to dispose 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, in sludge sampled at each treatment step from a total of five municipal activated sludge plants.

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 reference to Filgueiras (2002)

Step	Fraction	With reference to Filgueiras (2002)
Step 1	**Exchangeable** fraction and fractions associated with carbonated phases	F_1, F_2, F_3
Step 2	**Reducible** fraction or fraction associated with Fe and Mn oxides	F_4
Step 3	**Oxidisable** fraction or bound to organic matter	F_5
Step 4	**Residual** fraction	F_6

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, 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 highest organic matter 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. An increasing trend from the SS to the D + DS of metals in the oxidisable and residual fractions can be seen, so 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).

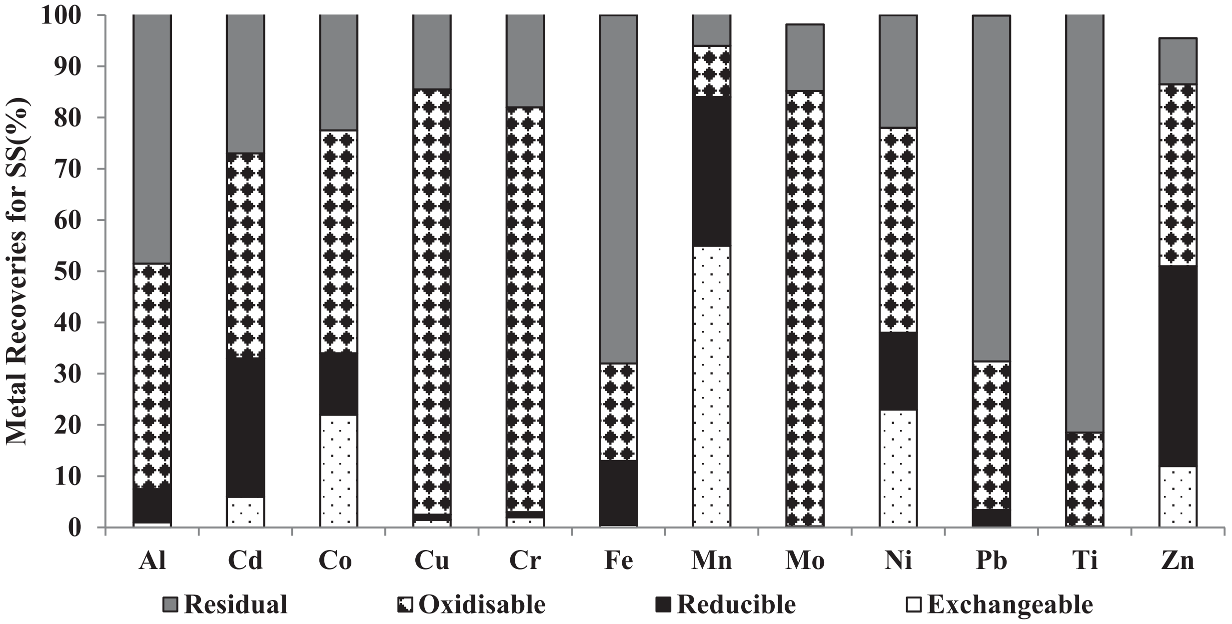

Figure 4: Metal partitioning of Secondary Sludge in BCR Scheme (Alvarez, et al., 2002). Data plotted from Table 2 and Table 3 of Alvarez, et al. (2002)

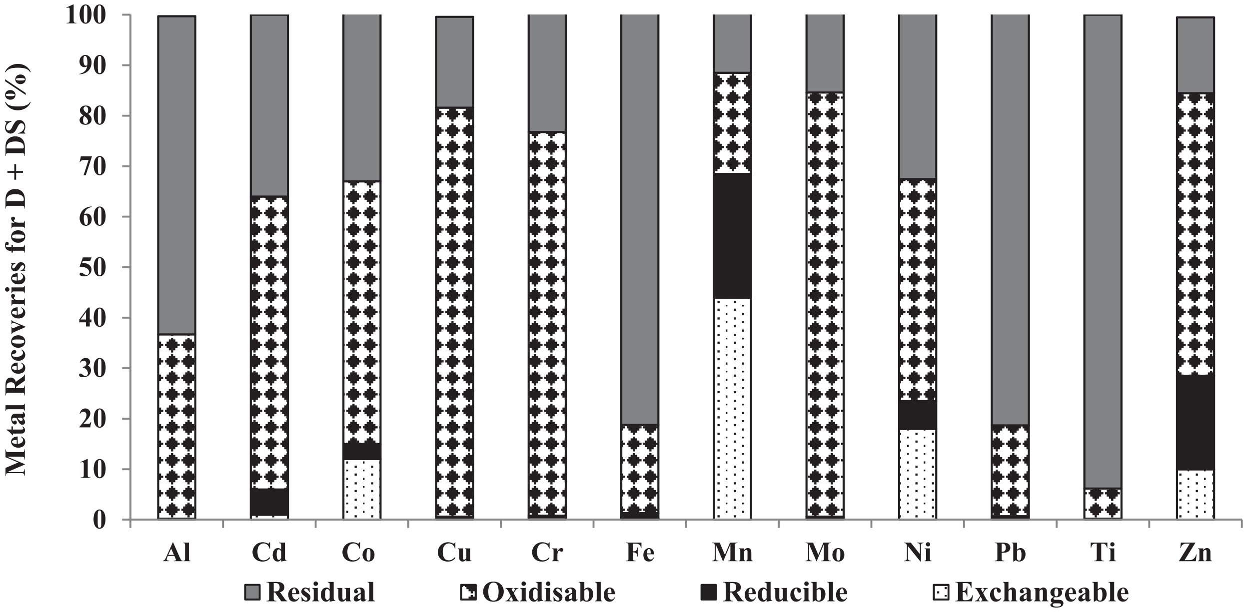

Figure 5: Metal partitioning of Dewatered and Digested Sludge in BCR (Alvarez, et al., 2002). Data plotted from Table 2 and Table 3 of Alvarez, et al. (2002).

Álvarez, et al. (2002) suggested that due to the high water content 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 metals partitioning themselves mostly 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), they were concluded to be the most bioavailable and mobile of the metals studied.

2.2.4 SIMPLE AND SEQUENTIAL EXTRACTION OF HEAVY METALS FROM DIFFERENT SEWAGE SLUDGES

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 using simple extraction (water and DTPA) and the BCR scheme. The BCR extraction scheme included four steps as shown in Table 3; due to the nature of the extractant used for the simple extraction, the results shown will be limited to those obtained by the BCR scheme as shown in Figure 6.

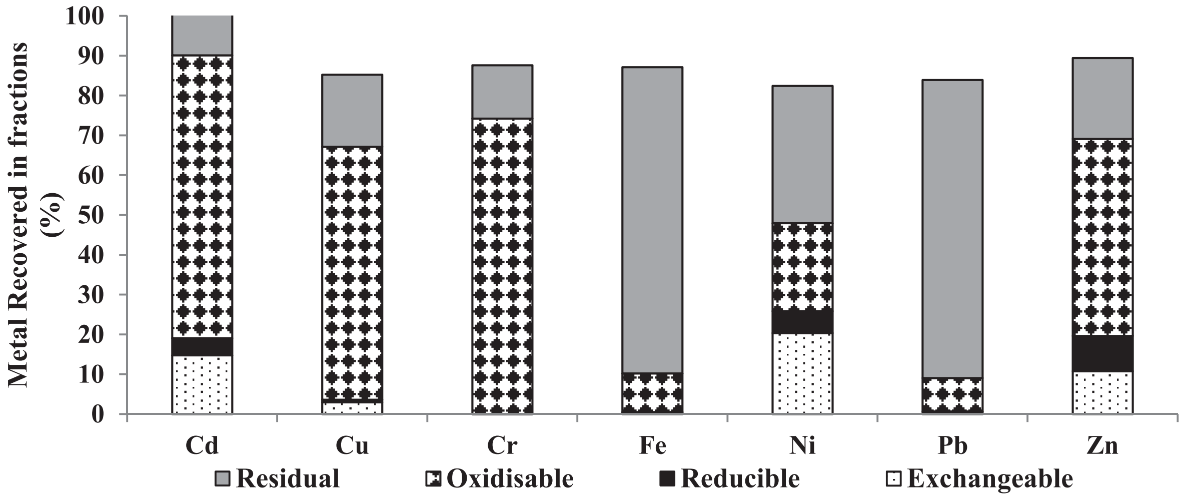

Figure 6: Percent recoveries of total metals in the sequential extraction fractions for anaerobic sludge using the BCR scheme (Feuntes, et al., 2004). Data plotted from Figure 1 and Table 3 of Feuntes, et al. (2004).

Figure 6 shows that most metals fraction themselves 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 DETERMINE METAL FRACTIONATION IN ANAEROBIC GRANULAR SLUDGES

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, et al. (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)

Nedalco sludge							Eerbeek sludge					
	Co	Ni	Cu	Zn	Mn	Fe	Co	Ni	Cu	Zn	Mn	Fe
Tessier	142	108	91	165	114	104	102	109	105	107	87	118
BCR	100	94	93	90	95	97	98	103	97	111	101	119
Stover	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 high 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 don't 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_1, F_2
Carbonates	F_3
OM (Organic Matter) -Sulphides	F_5
Residual	F_6

The results to be shown are limited to the Nedalco sludge as it has a higher organic matter which is analogous to anaerobic sludge to treat industrial wastewater with a high organic load, furthermore, the pH is 7.2 (± 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.

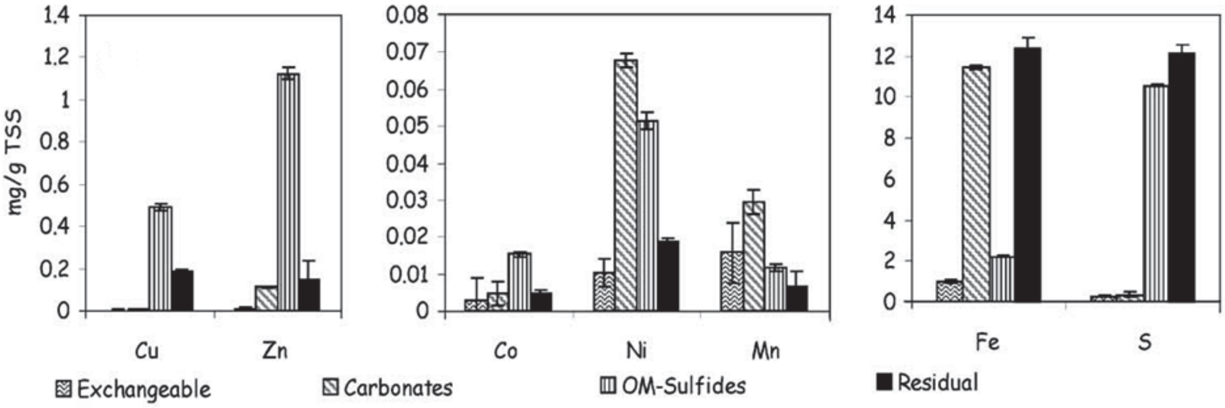

Figure 7: Metal partitioning profiles in Nedalco sludge according to the modified Tessier Sequential extraction scheme (van Hullebusch, et al., 2005a)

As confirmed in the previous study by Alvarez, et al. (2002) together with Feuntes, et al. (2003), all the metals to some degree partition themselves in the oxidisable fraction, in this case, the OM-sulphides, where the metals are bound to organic matter (OM) by bioaccumulation and complexation and may be bound as sulphides.

Cu, due its chemistry, was found to be predominantly bound as sulphides; these sulphides do not dissolve from the solid phases in the anaerobic digester sludge. A total of 53% of Nickel 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 $FeCO_3$.

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-Sulphides 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-Sulphides) 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

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 sulphur 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 sulphide (AVS) extraction and semicontinuously extracted metals (AVS-Me).

The work carried out showed that the bioavailability of Co and Ni was greatly affected by the presence of sulphides. In the case where the total sulphide level, in the form of H_2S, HS^- and

S^{2-}, is greater than the total concentration of Co, Ni, Cu, Fe and Zn, the metals are most likely to be precipitated in sulphide form. The effect of the sulphide 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 sulphate is part of the feed or is part of the micronutrient recipe, its reduction brings about existence of sulphur in the system, further contributing to trace metal precipitation as sulphides. 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 found to have partitioned itself in the organic matter/sulphides and in the acid volatile sulphides (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 sulphides was due to the complexation of Co with organic ligands or sulphides 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_{12} 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/sulphides 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-sulphides 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-sulphide precipitates further influence the bioavailability of Ni.

2.2.7 CONCLUSION OF 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 and from the case studies reviewed was found to successfully identify the distribution of metals (trace and main) in soils and sludge.

Sequential extraction makes use of experimental means to find the distribution of metals in the various forms in which they exist in sludge, it also provides data to be used in conjunction with speciation modelling. 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, in that once a metal is bound in the reducible, oxidisable or residual fraction; 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 microorganisms, given the prevailing sludge conditions (temperature, pH, redox potential) in an anaerobic digester. The fractions considered bioavailable henceforth are 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 sulphides 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 modelling 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 where 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, where they mostly lie in the oxidisable fraction, 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, biosorption 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 biosorption 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). Biosorption 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 biosorption 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 the existence of competition amongst metal for binding sites onto which they wish to adsorb themselves (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 (1996) 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.

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 decreasing 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.

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).

2.3.2.1 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 itself 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 2-1:

   $M + X\text{-cell} \overset{k_1}{\leftrightarrow} MX\text{-cell}$ ……. (2-1) (Brown & Markich, 2000)

   Where the activity of the surface complex is given by:

   $\{MX\text{-cell}\}\text{-}K_1\{X\text{-cell}\}[M]$ …… (2-2) (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-cell} and the BR is derived as follows:

   $BR = kK_1\{X\text{-cell}\}[M]$ …… (2-3) (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-cell} is effectively constant and the surface complex activity {MX-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 doesn't 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).

2.3.2.2 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 $\{X\text{-cell}\}_T$ 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:

$\{X\text{-cell}\}_T = \{MX\text{-cell}\} + \{X\text{-cell}\}$ ……. (2-4)

The BR is then given by the following general equation:

$\mathrm{BR} = \frac{\mathrm{BR_{max}}\{\mathrm{X-cell}\}_\mathrm{T}^\mathrm{n}[\mathrm{M}]^\mathrm{n}}{\mathrm{K_E^n}(\mathrm{K_{-1}}+[\mathrm{M}])^\mathrm{n}+\{\mathrm{X-cell}\}_\mathrm{T}^\mathrm{n}[\mathrm{M}]^\mathrm{n}}$ ……. (2-5)

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 2-5 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:

$\mathrm{BR} = \mathrm{a} + \mathrm{bK_1}\{\mathrm{X} - \mathrm{cell}\}_\mathrm{T}[\mathrm{M}]$ ……… (2-6)

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

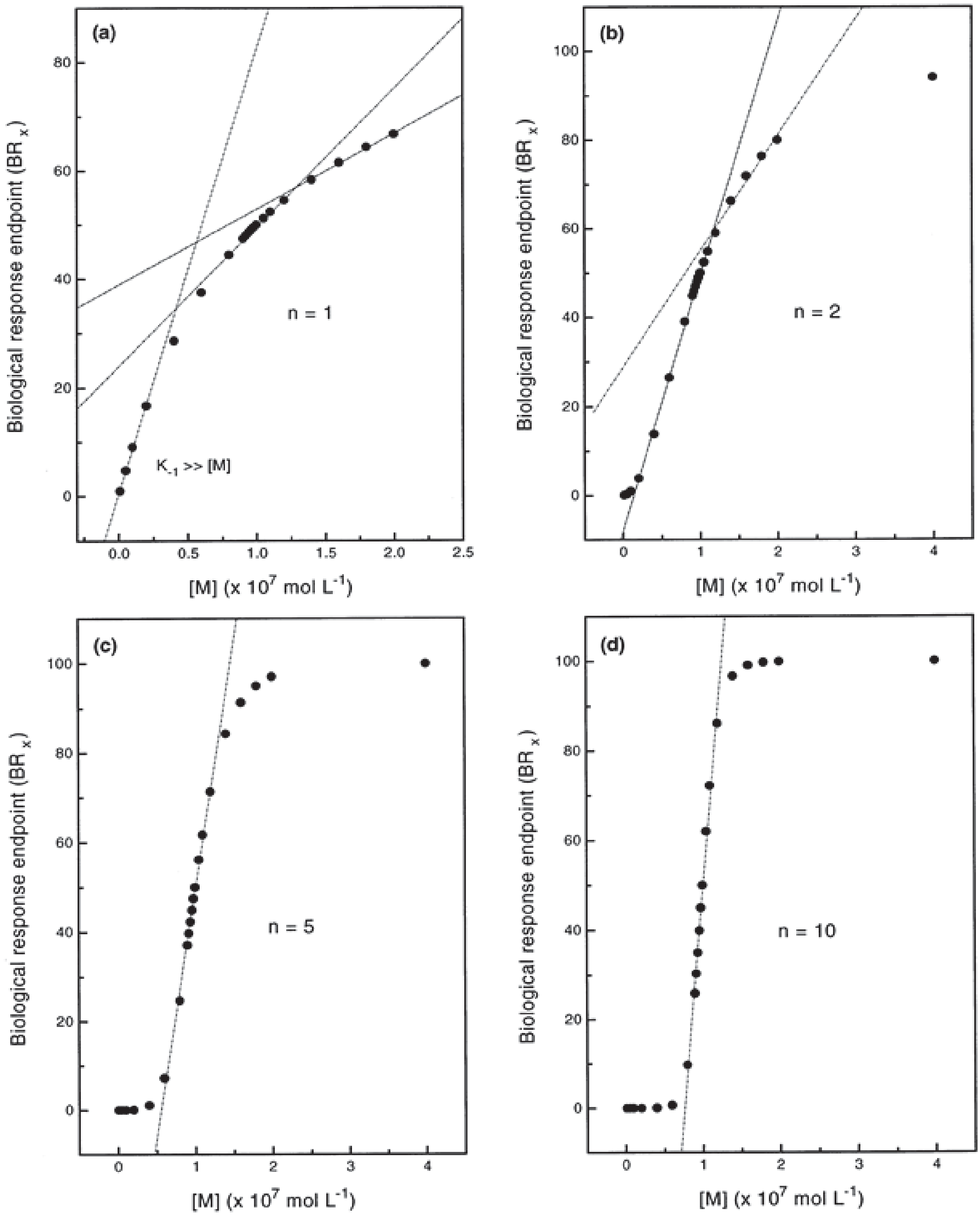

Figure 8: Concentration-response curves for different slope (n) values (Brown & Markich, 2000)

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.

2.3.2.3 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

Soils contaminated by toxic metals are successfully remediated by a well-known cost effective method termed phytoextraction, 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 phytoextraction 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 (Cl^-) to enhance Cd solubility was investigated as Cl^- is known to readily form moderate complexes with Cd^{2+}. One of the mechanisms proposed, by which Cl^- enhances Cd uptake by plants is an increase in the activity of free 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 aren't 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 (M^{2+}) or metal complexes (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:

$$\mathrm{Cd_{shoots}} = \frac{\mathrm{K_{transfer\ 1}R_{tot(1)}K_{Cd}(Cd^{2+})}}{\mathrm{1+K_{Cd}(Cd^{2+})+K_{Zn}(Zn^{2+})+K_{H1}(H^{+})}} + \frac{\mathrm{K_{transfer\ 2}R_{tot(2)}K_{CdCl}(CdCl^{+})}}{\mathrm{1+K_{CdCl}(CdCl^{+})+K_{H2}(H^{+})}}$$

…. (2-7) (Lo´pez-Chuken, et al., 2010)

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

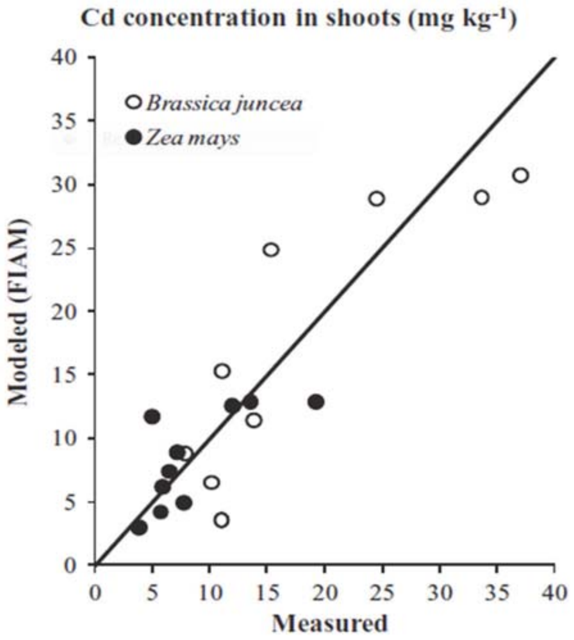

Figure 9: Cd uptake (mg kg^{-1}) by *Brassica juncea* and *Zea may* shoots modelled by the FIAM assuming a common absorption site for Cd^{2+} and CdCl^{+} ions (Lo´pez-Chuken, et al., 2010)

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.

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 makes its way to an organic ligand (biotic ligand) by transportation by a metal transporter; it binds itself to an active site on the organic ligand. The BLM extends into the incorporation of the competition between the metal cation and naturally occurring cations: Ca^{2+}, Na^{+}, Mg^{2+}, and 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.

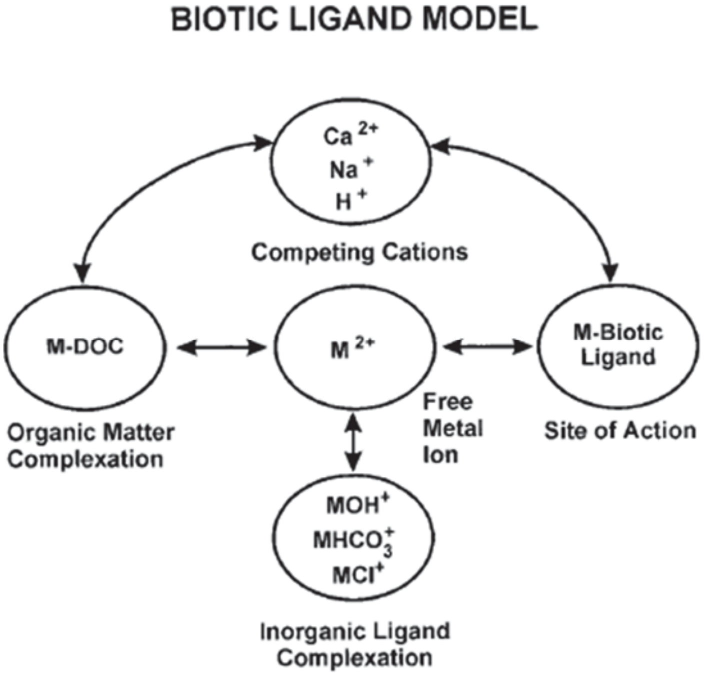

Figure 10: Schematic diagram of the biotic ligand model (Di Toro, et al., 2001)

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 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 competition for binding of the free metal ions on the BL (Bell, et al., 2002), (Niyogi & Wood, 2004).

5. There exists competition between the free metal ions and the naturally occurring hardness cations (Ca^{2+}, Na^{+}, Mg^{2+}, H^{+}) for 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:

$$\mathrm{M} + \mathrm{L} \overset{\mathrm{K}}{\leftrightarrow} \mathrm{ML}$$ ……. (2-8) (Bell, et al., 2002)

Where ML is the metal bound by the ligand.

The equilibrium constant is given by: $\mathrm{K} = \frac{[\mathrm{ML}]}{[\mathrm{M}][\mathrm{L}]}$ ……. (2-9) (Bell, et al., 2002)

The concentrations of ML and L are expressed in terms of the total ligand concentration (L_t) by equations 2-10a and 2-10b0.

$[\mathrm{ML}] = \mathrm{L_t}\frac{[\mathrm{M}]}{[\mathrm{M}]+\mathrm{K}^{-1}}$ …… (2-10a) $[\mathrm{L}] = \mathrm{L_t}\frac{\mathrm{K}^{-1}}{[\mathrm{M}]+\mathrm{K}^{-1}}$ …… (2-10b) (Bell, et al., 2002)

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

$$C_f = \frac{[M]}{[M]+ K^{-1}}$$ ……. (2-11) (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 don't 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

Of the applications of the BLM, computation of LC_{50} 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_{50} 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.

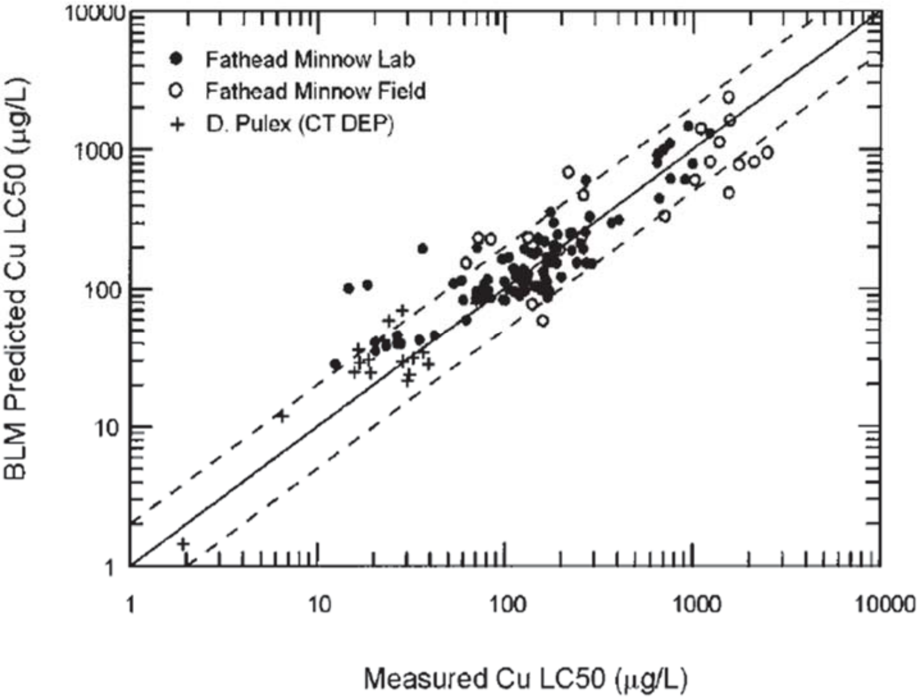

Figure 11: Comparison between measured and BLM predicted LC50s for Cu for *fathead minnow, rainbow trout* and *daphnia magna* (Di Toro, et al., 2001)

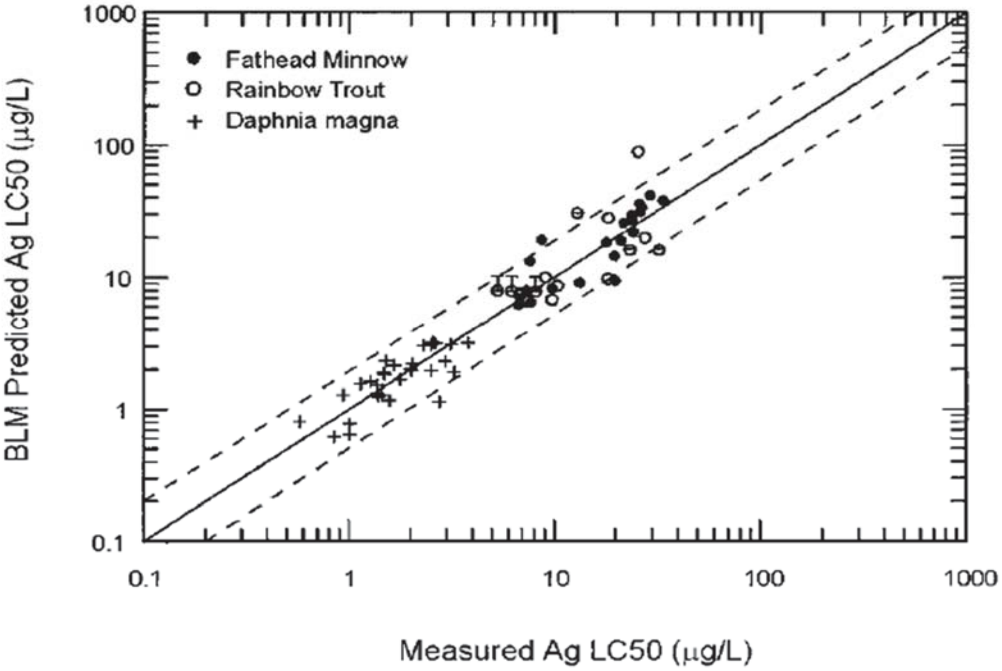

Figure 12: Comparison between measured and BLM predicted LC50s for Ag for *fathead minnow, rainbow trout* and *daphnia magna* (Di Toro, et al., 2001)

Figure 11 and 12 shows the comparison between experimentally obtained and BLM obtained LC_{50}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_{50}s for the aquatic organisms studied. The BLM was shown to be predictive within a factor of two for LC_{50}s covering the range over two orders of magnitude; deviations were brought about by water chemistry variations.

2.3.4 ADSORPTION ISOTHERMS

Another approach is making 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 modelling 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:

Langmuir Isotherm Model: $Q_e = \frac{Q_{max} b C_e}{1+bC_e}$ …… (2-12) (van Hullebusch, et al., 2005a)

Freundlich Isotherm Model: $Q_e = kC_e^{1/n}$ ….. (2-13) (van Hullebusch, et al., 2005a)

Where, Qe: the uptake of the metal ion by biomass (mg g^{-1} TSS), Q_{max}: maximum uptake of the metal ion by the biomass (mg g^{-1} TSS), Ce: final metal concentration in the solution (mg l^{-1}), b: Langmuir adsorption equilibrium constant, k: constant relating to the capacity of adsorption, n: constant relating to the intensity of adsorption.

By inclusion of the Q_{max} in the Langmuir model, it implies that the model acknowledges the possibility of the saturation of the adsorptive surface. Qe and Ce are experimentally determined, regression of equation 12 and 13 using experimental data allows for the determination of the constants Ce and n for the Freundlich isotherm and the Q_{max} and b for 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

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 upflow anaerobic sludge bed (UASB) reactors, the partitioning of the metals in four fractions (exchangeable, carbonates, OM/sulphides 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.

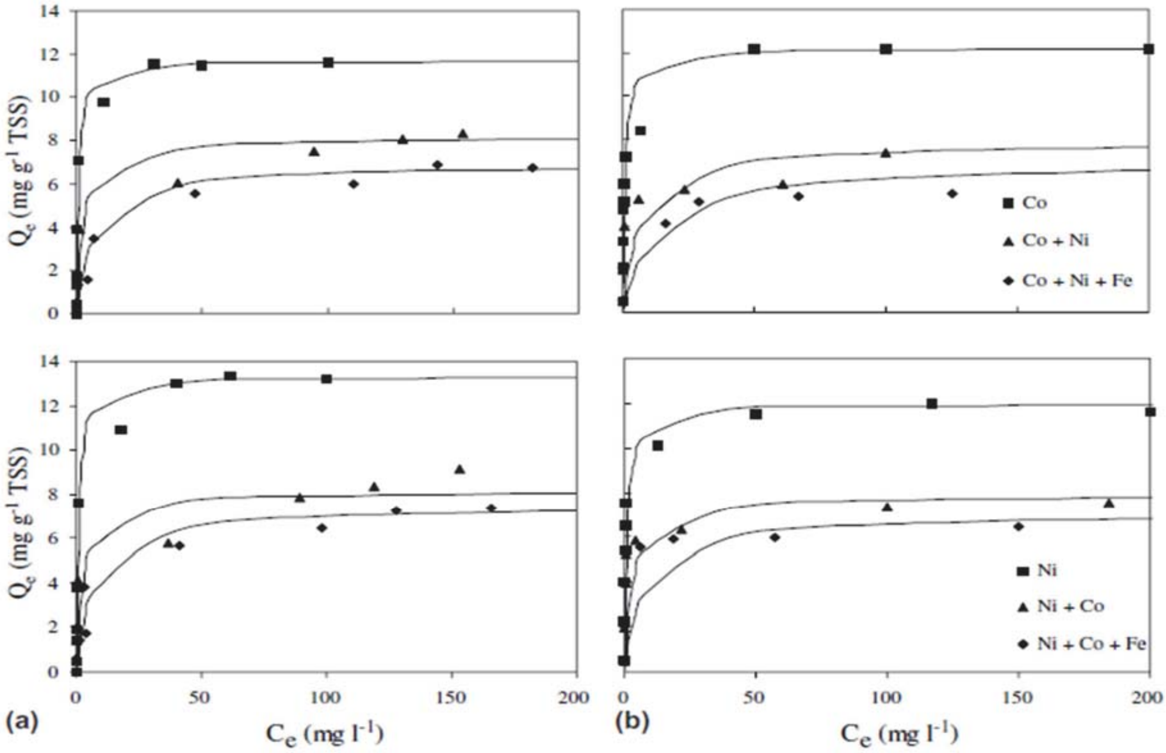

Figure 13: Nickel and cobalt sorption onto Nedalco (a) and Eerbeek (b) anaerobic granular sludges (monometal and competitive (van Hullebusch, et al., 2005b)).

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 (Ce) and the sequential extraction experimental data shows the modelling capability of the Langmuir isotherm when coupled with sequential extraction.

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 modelling of the experimentally obtained data by making use of a computer simulation package MINTEQ to carry out the speciation modelling. The study first involved the analysis of the micronutrient requirements for anaerobic digestion of Fischer-

Tropsch Reaction Water followed by chemical speciation modelling 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 modelling 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). 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 fractions they exist in and to quantify any losses from the various sequential extraction schemes performed, all this was to done in order to understand limitations to be considered in experiment B.

Experiment B was performed where FTRW was being treated in the reactors, forming the bulk of the study. In this part of the study, it was investigated whether the precipitation controlled model was able to predict the concentration of the free metal or not, to find any correlation between the model-predicted and the experimental data. 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

Fraction	With respect to Filgueiras (2002)
Soluble	F_1
Exchangeable	F_2
Adsorbed	F_5
Organically Bound	F_5
Carbonate Precipitate	F_3
Sulphide Precipitate	F_5
Residual	F_6

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

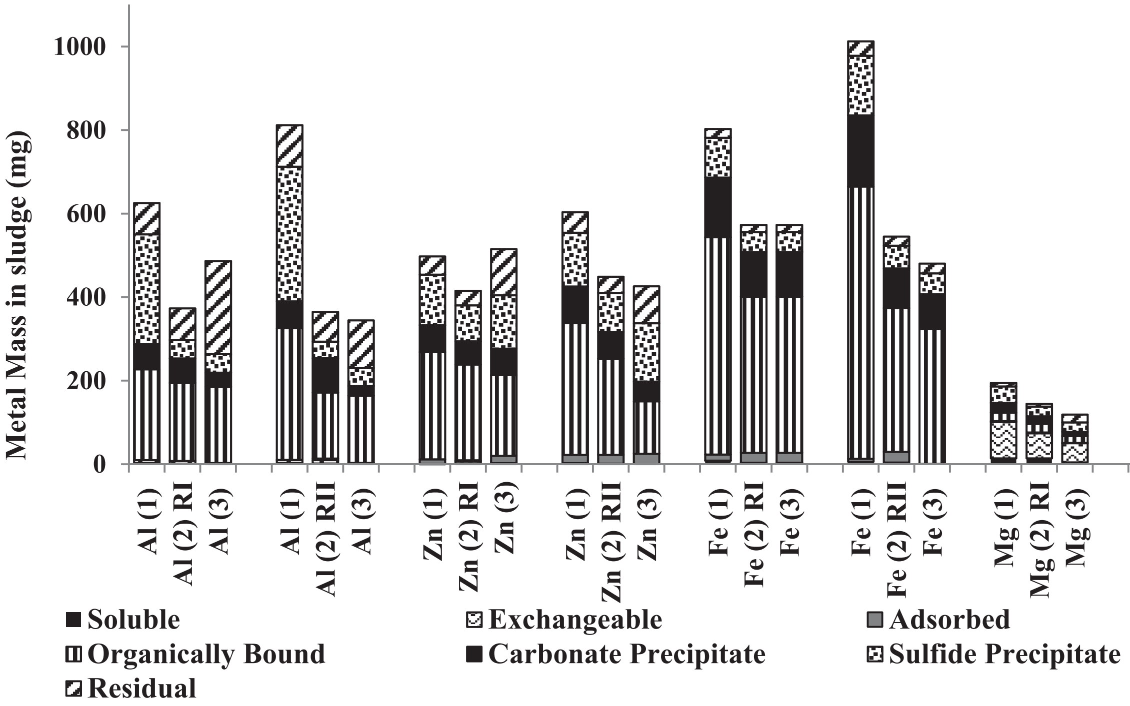

Figure 14: Experimental Metals Speciation Results for Sequential Batch Reactor for Experiment A (Mathir, 2013)

As shown in Figure 14, the dosed metals were distributed between the different phases. 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 brought to surface 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 errors of high extent (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 $Ca_5(PO_4)_3OH$, wash out of the experiment, with the remainder decreasing steadily from cycle to cycle.

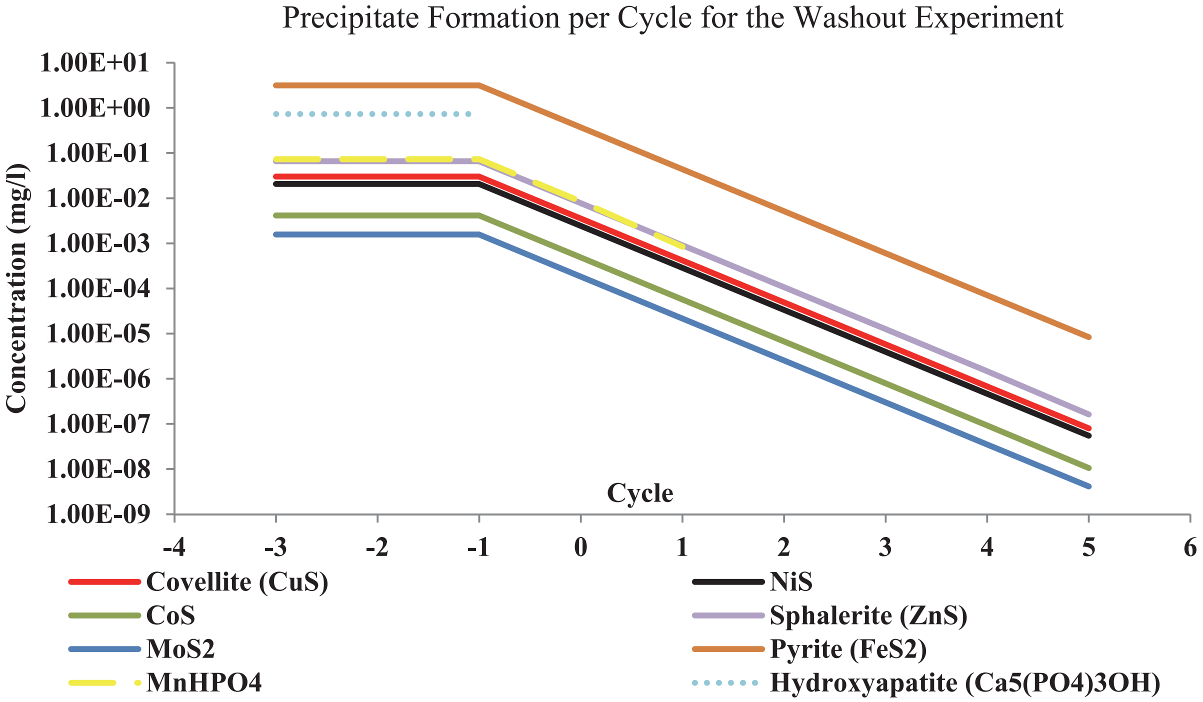

Figure 15: Concentration of precipitated minerals as predicted by model for each successive cycle in Experiment B (Mathir, 2013)

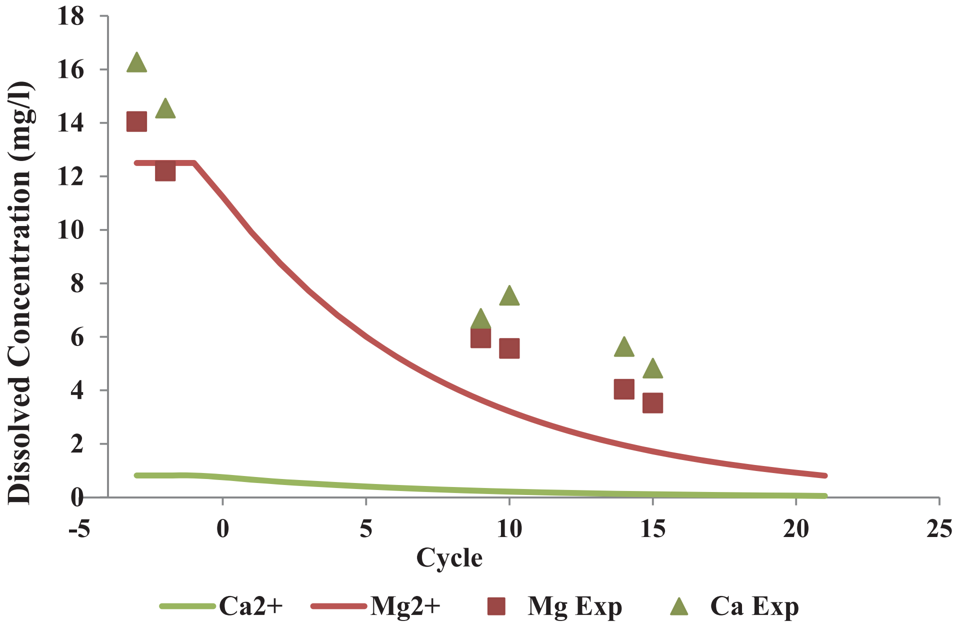

Figure 16: Concentration profile of Ca^{+2} and Mg^{+2} in the dissolved phase (mg/l) including comparisons to experimental data in Experiment B (Mathir, 2013)

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^{+2}, the same trend can be seen between the two sources of data, making them comparable.

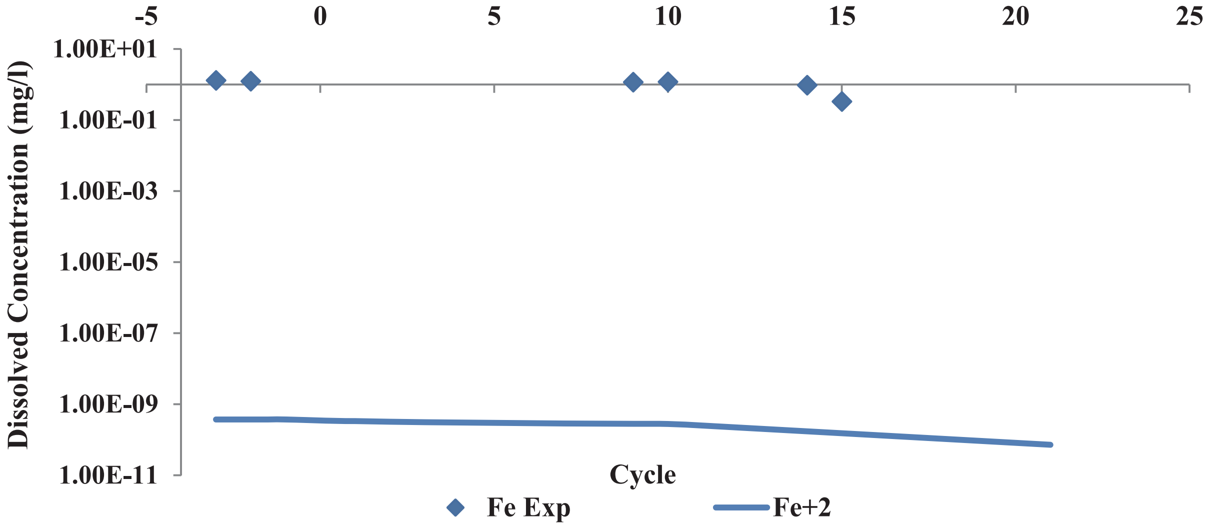

Figure 17: Concentration profile of Fe^{+2} in the dissolved phase (mg/l) including comparisons to experimental data in Experiment B (Mathir, 2013)

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 dosed. 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.

2.3.6 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 microorganisms existing in the anaerobic sludge used to anaerobically treat waste water is insufficient, 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 metals dosed are in a form that the organisms can readily take up, introducing the concept of bioavailability. Bioavailability may be defined as the extent to which metals in sludge chemically avail themselves 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 chemically available for the microorganisms to stimulate biological response or not.

Sequential extraction techniques were used to investigate the manner in which metals partition themselves in the various species and fractions in which they exist. From the extraction results, the metals partitioning themselves 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 into consideration aquatic effects 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.

Making use of the speciation models that have been validated by making use of sequential extraction results, 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 considerable amount of the total metal content partitions itself to a phase 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.

2.3.7 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 METHODOLOGY

3.1 APPROACH

The PHREEQC aquatic chemistry modelling software provides a comprehensive framework for modelling ionic speciation and precipitation reactions. Its basic functionality is to compute the equilibrium state of an aqueous system, and for this it requires little more than a specification of the quantities of chemical components making up the system. Its component database holds the data required for equilibrium speciation of most components likely to be encountered in such systems.

For the purpose of modelling the fate of micro-nutrient metals in an anaerobic digester, this study aimed to extend this basic functionality by adding terms to represent adsorption of micronutrient metals to the anaerobic biomass. The hope was that the adsorption would improve the predictions of the Mathir (2013) model which include on precipitation and liquid phase speciation.

PHREEQC provides several models for representing surface adsorption phenomena, however, unlike the basic speciation, no thermodynamic parameter values are provided, since adsorbing surface characteristics vary greatly and are difficult to predict. The central task was therefore to formulate a model structure, and then calibrate the model parameter values using experimental data from Mathir (2013), supplemented by measurements on the Sasol pilot-plant reactors. The model represents the biomass surface as containing metal binding ligand sites which complex the metal ions. The model equations are essentially the same as used in the BLM model (Equations 2-8 and 2-9, Section 2.3.1) The essential parameters of the adsorption model are the densities of the binding sites and the equilibrium constants for the metal complexes with the surface ligands. The micro-nutrient metals included in the study were those that appear in the recipe dosed to the Sasol reactors, namely B, Co, Fe, Ni, Zn, Mn, Ca and Mg.

According to given inlet conditions (influent FTRW COD, nutrient recipe, temperature, etc.), the model was set up to predict process variables 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 used:

- 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).

The investigation was structured to test two basic hypothesis by comparing the model predictions to the available experimental data:

Hypothesis 1: The distribution of micro-nutrient metals can be adequately described in terms of aqueous phase speciation and mineral precipitation only.

Hypothesis 2: The distribution of micro-nutrient metals can be adequately described in terms of aqueous phase speciation, mineral precipitation and adsorption of the anaerobic biomass.

3.2 MODEL DEVELOPMENT AND DESIGN

For comparison, a precipitation-only equilibrium model was constructed, as well as the model including both precipitation and adsorption.

There is a network of processes that occur within an anaerobic sludge reactor, as shown in Figure 1, the volatile fatty acids go through various stages till they are converted into methane (CH_4). Taking the processes that occur as a result of the presence of micronutrients further complicates the attempt to model all the processes occurring simultaneously in the reactor. The overall model was therefore split into two sub-models.

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 carries out 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
Biological Conversion	Experimental pH.	Model pH (1)
	Initial reactor conditions (mol/litre)	Total biogas production (litres)
	Temperature (°C), Pressure (atm), Reactor Volume (litres)	Reactor composition after biological conversion
Speciation	Nutrient concentration as per Sasol recipe (mol/litre)	Model pH (2)
	Remaining Acetate$^-$, Propionate$^-$ and CO_3^{-2} concentrations (mol/litre)	Precipitated and adsorbed metal per cycle
	Model pH (1)	Free metal ion concentration (mg/litre) per cycle

3.3 MODELLING OF WASHOUT EXPERIMENT BY Mathir (2013)

3.3.1 EXPERIMENTAL DATA

Construction and execution of experimental work was unnecessary, the data used in the study is that which 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 $NaHCO_3$ 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 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.

3.3.2 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 ($CH_4(g)$, $CO_2(g)$ and $H_2O(g)$). 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 as a result of the biological conversion taking place.

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[2] (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_4, $H_2O_{(g)}$, CO_2 and biomass) as suggested by Figure 18.

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[2] MATLAB is a computing package widely used in engineering.

3.3.3 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[3] (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 $NaHCO_3$ 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.

3.3.4 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.

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[3] WEST is a water treatment modelling software suite.

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

Reactor Conditions		
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
Reaction Conditions		
Volatile Fatty Acids	-	Acetic (CH_3COOH) and Propionic (CH_3CH_2COOH) acid.
Reaction type	-	Stoichiometric with extent of reaction

3.4 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.

3.4.1.1 PHREEQC

The speciation model was constructed using PHREEQC, a simulation package used to simulate various reactions and processes in natural waters or laboratory experiments. It is 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^{+2}, instead of its ferric state; Fe^{+3} (Ogunremi, et al., 2007). Due to PHREEQC having a default coding of the relationship between Fe^{+2} and Fe^{+3}, where equilibrium is ensured between the two valence states, disequilibrium between the two states was introduced.

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_3^-) and ammonium (NH_4^+) in an aquifer was necessary, due to equilibrium between the valence states occurring to a negligible extent (World, 1998).

3.4.1.2 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 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 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 11. 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)

Mineralogical name	Chemical Formula	Comments on occurrence of formation
Alum K	$KAl(SO_4)_2.12H_2O$	When hot solutions of equimolecular quantities of aluminium sulphate + K-sulphate are mixed, and the solution cooled, octahedral crystals of a double sulphate of aluminium and potassium separate.
Anhydrite	$CaSO_4$	Precipitate under conditions of very high super-saturation (high $Ca^{+2}/SO4^{-2}$). It forms at temperatures > 40°C.
Aragonite	$CaCO_3$	Unstable relative to calcite at atmospheric pressure and normal temperatures, only likely to form below 100°C or when foreign elements are present in solid solution.
Barite	$BaSO_4$	Frequently found associated with fluorite, calcite, dolomite and quartz. Produced by slow inter-diffusion of dilute solutions of barium chloride and sulphates.
Brucite	$Mg(OH)_2$	Found associated with minerals such as calcite, aragonite, hydromagnesite and artinite.
Covellite	CuS	Double displacement ionic reaction carried out when passing hydrogen sulphide gas through a copper sulphate solution forming a copper sulphide precipitate.
Dolomite	$CaMg(CO_3)_2$	Precipitated from a solution of $MgCl_2$, $CaCl_2$ and area highly elevated pressure (higher than 2/3 atm.) at 228°C.
Epsomite	$MgSO_4.7H_2O$	$MgSO_4$ occurs as kieserite, $MgSO_4.H_2O$.
Fluorite	CaF_2	Most fluorite is 99% CaF_2, with small amounts of Si, Al and Mg due to impurities and inclusions.
Gibbsite	$Al(OH)_3$	It is obtained as a colloidal precipitate when ammonia or an alkaline carbonate is added to a solution of an aluminium salt.
Goslarite	$ZnSO_4.7H_2O$	By acting upon zinc or zinc oxide with dilute sulphuric acid. The concentrated solution deposits transparent

		crystals of the composition $ZnSO_4.7H_2O$.
Gypsum	$CaSO_4.2H_2O$	Prepared by mixing solutions of sulphates with solutions of calcium salts. Some form by hydration of anhydrite.
Halite	NaCl	Formed by evaporation of a highly saturated saline solution.
Hematite	Fe_2O_3	Prepared by decomposing ferric chloride by steam at high temperature.
Hydroxyapatite	$Ca_5(PO_4)_3OH$	Prepared by precipitation from solutions of calcium salts with the addition of ammoniacal phosphate solutions.
Lime	CaO	Obtained by igniting calcium carbonate or calcium oxalate at about 800°C.
CoS	CoS	Double displacement ionic reaction carried out when passing hydrogen sulphide gas through a cobalt carbonate solution forming cobalt sulphide precipitate.
Manganosic Oxide	$MnSO_4$	Manganosic oxide reacts with acid with the formation of a soluble manganous salt and insoluble hydrated manganese dioxide.
Manganous Chloride	$MnCl_24H_2O$	Manganous chloride is prepared by dissolving the oxide or carbonate in hydrochloric acid and evaporating the solution, the heating being continued long enough to drive off all free chlorine.
$MnHPO_4$	$MnHPO_4$	Produced by adding know concentrations of Mn^{+2} to a saturated solution of monopotassium phosphate (MKP) with reaction temperatures ranging between 20 and 60°C.
MoS_2	MoS_2	Reaction of MoO_3 and S at a temperature of 300°C in an argon atmosphere.
Nickel Sulphide	NiS	Produced by treating Nickel (II) salts with hydrogen sulphide (H_2S).

Periclase	MgO	It is made by heating $MgCO_3$ if the hydroxide obtained from sea-H_2O.
Portlandite	$Ca(OH)_2$	Formed on addition of water to CaO. Another way is to heat aqueous solutions of calcium salts with alkalis.
Rhodochrosite	$MnCO_3$	Made by adding sodium carbonate solution to a solution of manganous salt.
Siderite	$FeCO_3$	By heating $(NH_4)_2CO_3$ with $FeCl_2$.
Sphalerite	ZnS	Sphalerite is the main form of zinc found in nature.
Vivianite	$Fe_3(PO_4)_2:8H_2O$	It is found with iron, copper and tin ones. It may occur in clay, and especially in bog iron-ore.
$ZnCl_2$	$ZnCl_2$	It is obtained by passing chlorine or hydrogen chloride over heated zinc.
Zn(OH)	$Zn(OH)_2(A)$	It is precipitated when an equivalent quantity of an alkaline hydroxide is added to solution of a zinc salt.
Zincite	ZnO	It is formed by heating zinc in air and passing the fumes into condensing chambers where the powdered oxide collects.

3.4.1.3 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 (2013) 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") is essential.

3.4.1.3.1 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 biosorption 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.

Building up on the study conducted by Artola and co-workers (1997), a study conducted by Westergreen et al. (2012) first dealt with determination of the effect of biomass on the buffer capacity of a solution comprised of mixed ions by making use of glycine equivalence. In this study, a model was constructed where the biomass was represented by glycine, and the hydrogen affinity as a function of pH was modelled. The model was compared to titrations of strong acid against a solution containing anaerobic sludge, but the model and experimental data were found to be significantly different.

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).

In the experimental work, solutions made consisted of mixed salts and prepared solids from municipal anaerobic digester sludge. Mixed salts considered 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. Basis the inclusion of the above stated components in the solution composition, which are analogous to components in the nutrient recipe, in the model to predict pH and conductivity with use of the UKZiNe formulation; the UKZiNe formulation was incorporated in the current study. Details of the UKZiNe molecule are as follows:

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

Group	Glycine mmol/g VS	pKa (at 25°C)	UKZiNe mmol/g VS	pKa (at 25°C)
COOH1	13.146	2.350	0.170	4.350
COOH2	-	-	0.170	5.650
NH_4^+	13.146	9.778	0.076	9.244
HPO_4^{2-}	-	-	0.021	7.198

The UKZiNe ionic representation of biomass is incorporated into PHREEQC by making use of its characterisation as shown in Table 12. It provides an ionic acceptor for the positively charged metal cations, thereby allowing the formation of complexes between the biomass (organic content) and the metal ions. The complete model is constructed by using both Matlab and PHREEQC, the model algorithm is shown by Figure 18 below. Figure 19 and 20 depict how Hypothesis 1 and 2 will be tested following the successful construction of the speciation models in PHREEQC.

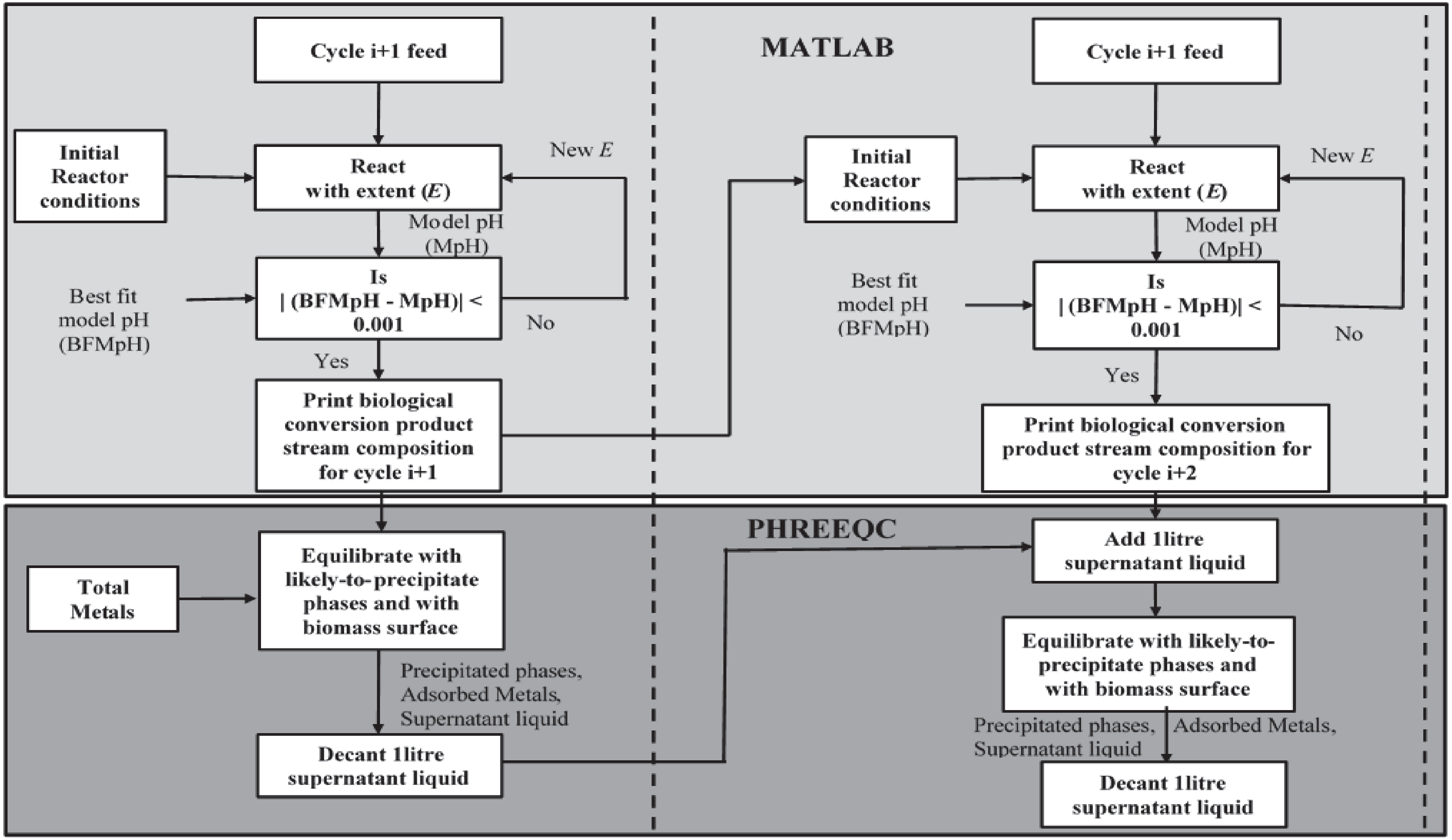

Figure 18: Overall Speciation Model Algorithm

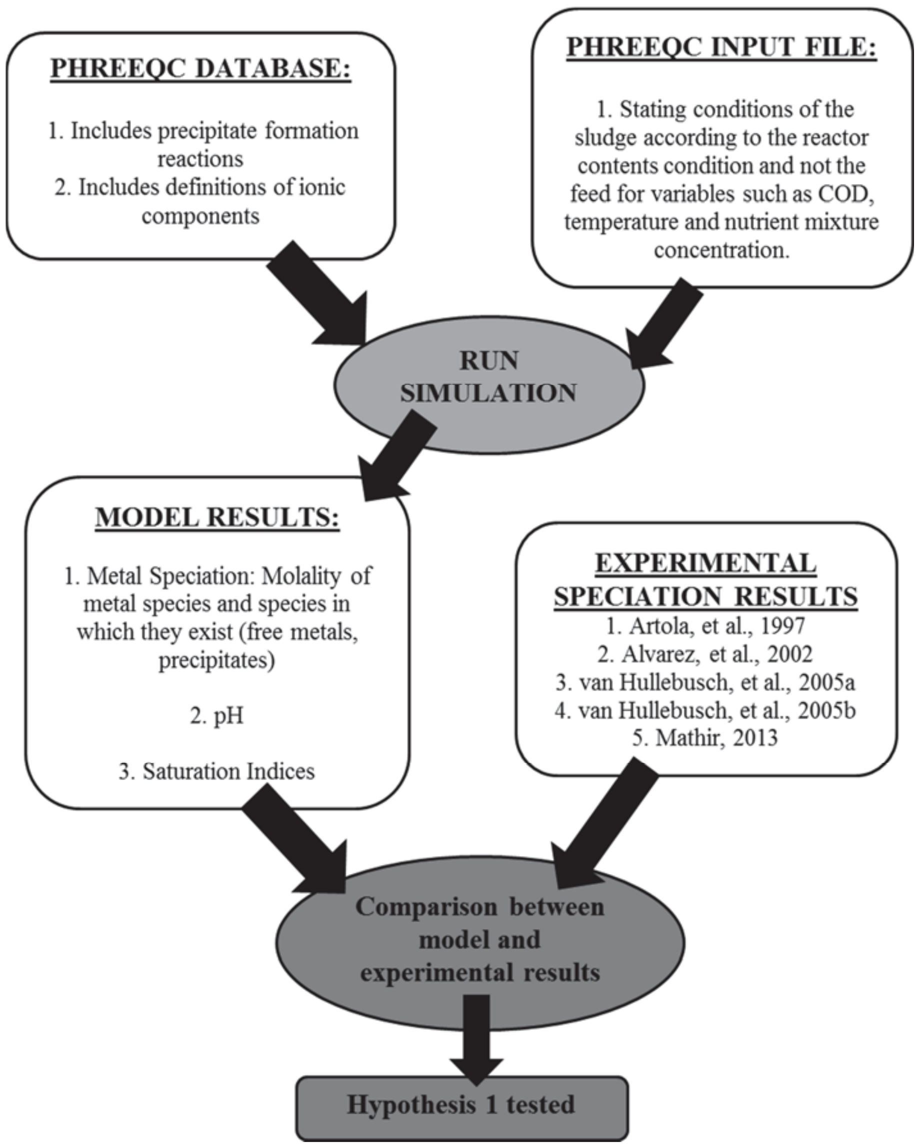

Figure 19: Model construction and Hypothesis 1 testing algorithm

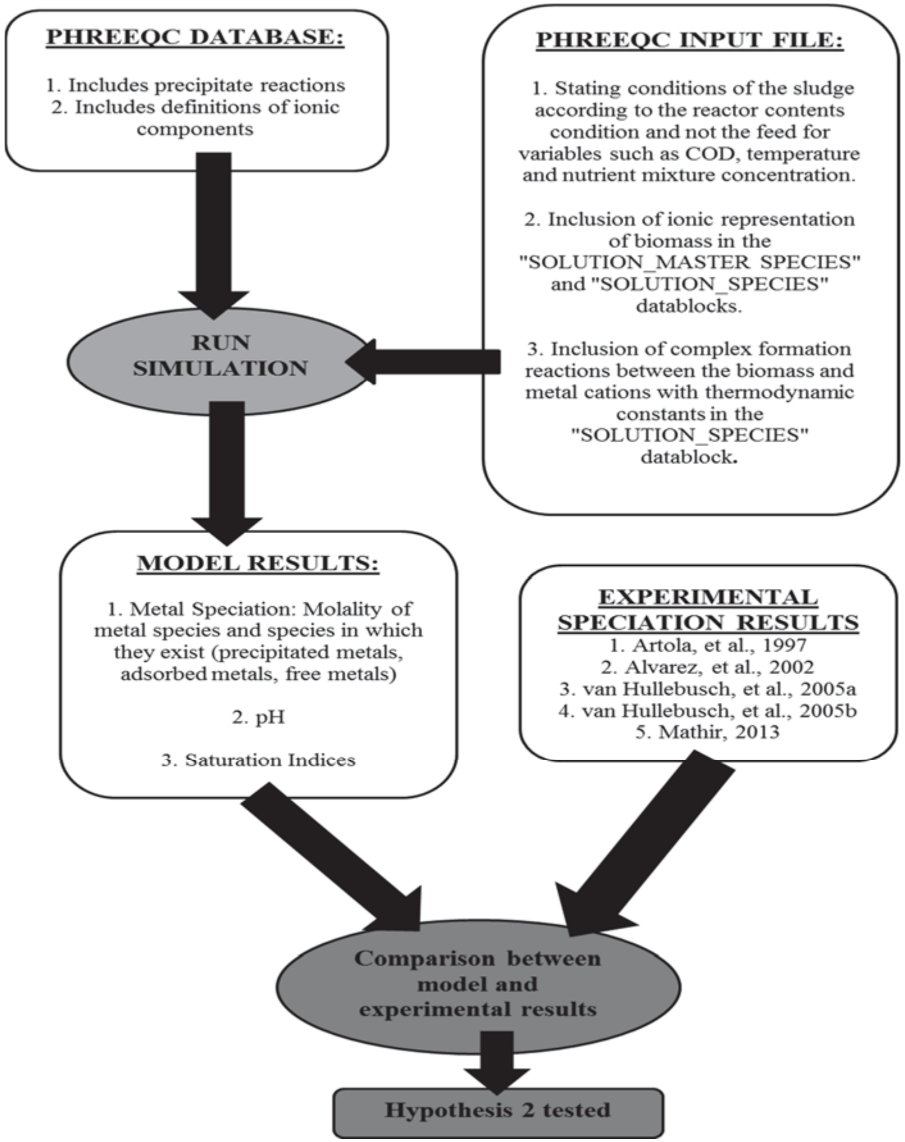

Figure 20: Model construction and Hypothesis 2 testing algorithm

4 RESULTS

This investigation involved modelling only, with no experimental component. It relied on data that had been measured elsewhere. The main source of data was the MSc Eng thesis of Aarefah Mathir. A limited amount of additional data was obtained from the Sasol pilot plant reactor.

The key result was that the modelling revealed the critical importance of sulphide in the system, because of the extreme insolubility of the sulphides of several of the micro-nutrient metals. The possibility existed that sulphate present in the system might be reduce to sulphide during the anaerobic digestion, but this was not certain, because it depended on whether a population of sulphidogenic micro-organisms could establish itself in competition with the dominant methanogens. Unfortunately, no measurements of sulphides were made either during Mathir's experiments, or on the Sasol pilot-plant. The modelling showed that even a low conversion of sulphate to sulphide could dramatically influence the distribution of the metals, because of the low concentrations of metals and the extreme insolubility of their sulphides.

4.1 SULPHATE-SULPHIDE SYSTEM

4.1.1 Sensitivity of the system to sulphide

From studying the nature of the system in question, the following facts suggest minimal existence of sulphide in the system:

- A low concentrations of SO_4^{-2} is dosed into the system, and in order for sulphide to result, sulphate reducing bacteria should thrive.
- Sulphate reducing bacteria compete with the methanogens for acetate. Methanogens only require sufficient acetate concentration to grow, whereas sulphate reducing bacteria that requires sufficient acetate plus sulphate. If there is any conversion to sulphide, it is expected to be low.

- However, the metals concentrations are also very low, and their sulphides are extremely insoluble, so very low levels of sulphide can have a marked influence on the distribution of the metals among the phases.

The behaviour of the sulphate-sulphide system was investigated under the conditions prevailing in the Sasol pilot plant. The effect on the soluble, precipitates and adsorbed phase of various concentrations of sulphide was studied, refer to Appendix F for experimental details.

Figures 20, 21 and 22 show the relationship between soluble phase concentration, precipitate formations and adsorption of Fe, Ni, Co and Zn at various sulphide concentrations. The concentration of sulphide has a great impact on the formation of Ni, Co and Zn precipitates (Figure 21), as they only start forming once a certain sulphide concentration has been reached. For concentrations prior to the precipitate-forming concentration, the Fe forms sulphide precipitate, sweeping out the sulphide until the Fe is fully used up in the system.

Figure 22 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. A shift from the adsorbed to the soluble phase is also seen, further aiding to reaching of the saturation conditions, bringing about precipitation.

Due to the formation of the precipitates and adsorption of the metals affecting the metal concentration in the soluble phase, as shown by Figure 30, the concentration of the sulphide in the system therefore impacts the soluble phase concentration of the metals. The study carried out of the behaviour of the pilot plant system at different sulphide concentrations clearly points to the dependence of the bioavailability of the metals to the concentration of sulphide in the system.

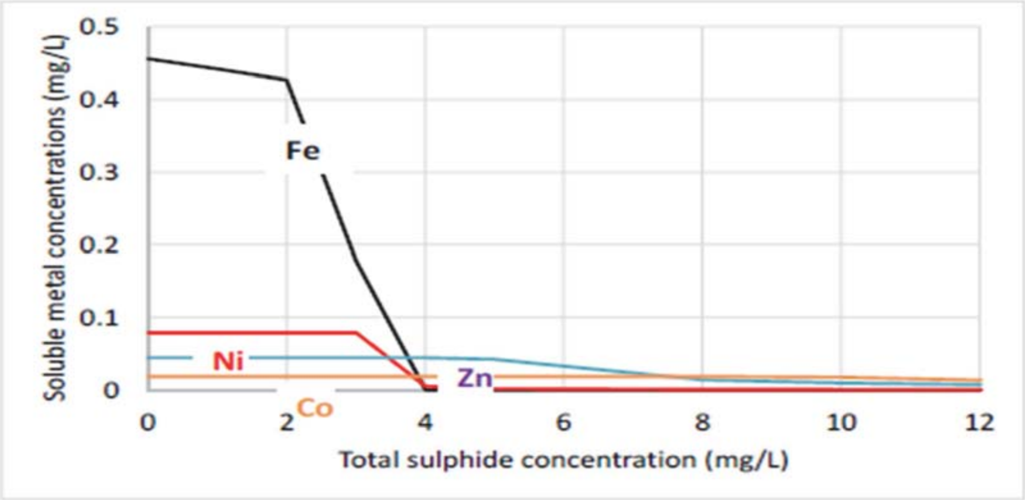

Figure 21: Model predictions of soluble metal concentrations for the pilot plant

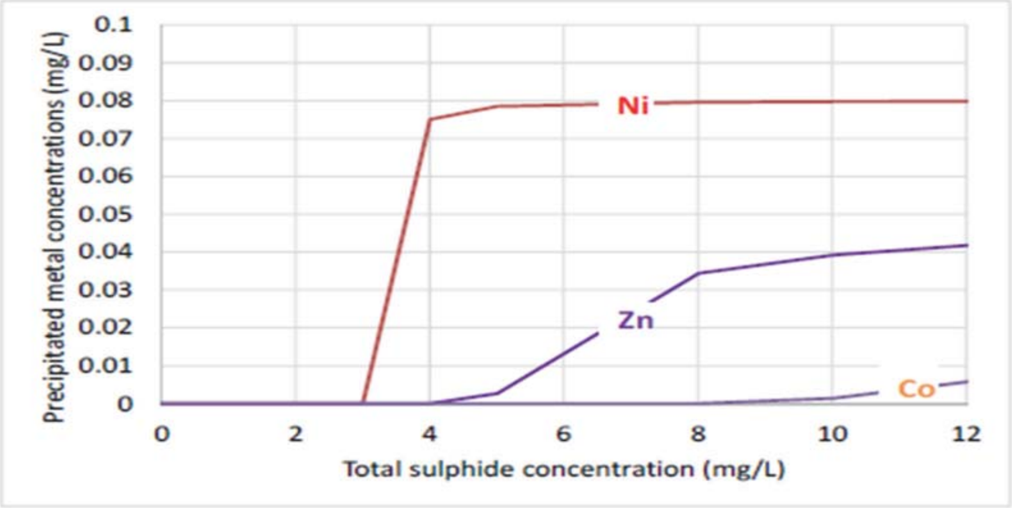

Figure 22 : Model predictions of precipitated metal concentrations for the pilot plant

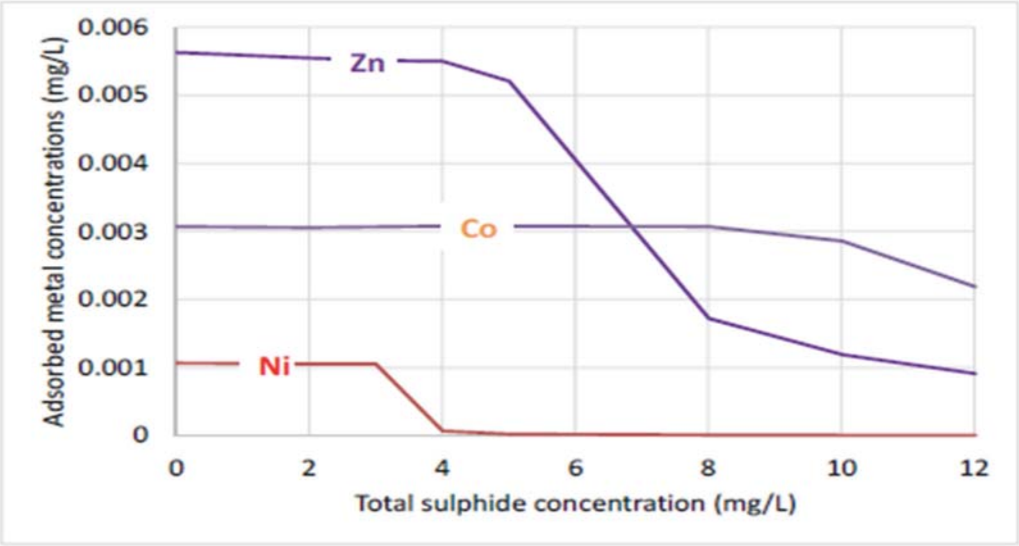

Figure 23: Model predictions of adsorbed metal concentrations for the pilot plant

4.1.2 The effect of sulphide on metal washout

Due to the sulphide concentration having an impact on the distribution of metals in the system, the effect of the sulphide concentration on the washout experiment was tested. At one extreme, the sulphide concentration is generated by the reduction of the sulphate dosed with the micronutrient recipe. By default PHREEQC models complete chemical equilibrium of the material specified to be present in the system, including redox equilibrium. Complete redox equilibrium is seldom attained in industrial systems, such as the one under study. In particular, the sulphate/sulphide reaction needs to be biologically catalysed. Furthermore, the acetate/methane equilibrium was not represented in the model, for lack of measured data, and indeed was probably not at equilibrium. Thus, the relevance of the equilibrium simulation is very uncertain. Nevertheless, it was included in the following series of figures as one scenario. Two other scenarios were also prepared for comparison: one with no conversion of sulphate to sulphide, and one with 20% conversion. Note that the simulated equilibrium conversions are much less than 20%. In the extreme of no sulphide, the only precipitates that form are phosphates and carbonates. Figures 23 and 24 below show the behaviour of the **soluble phase** concentration for these two extremes as well as a trend where the conversion of sulphate to sulphide is restricted to 20%.

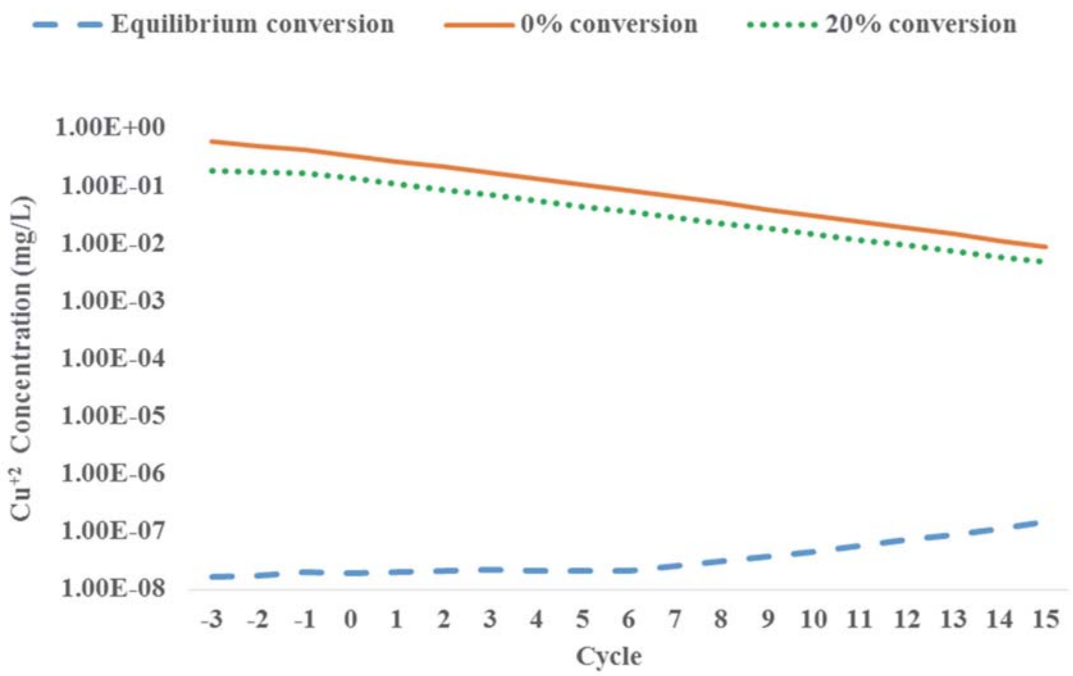

Figure 24: Soluble Cu^{+2} for the AP-Model at different conversions of SO_4^{-2} to HS^-

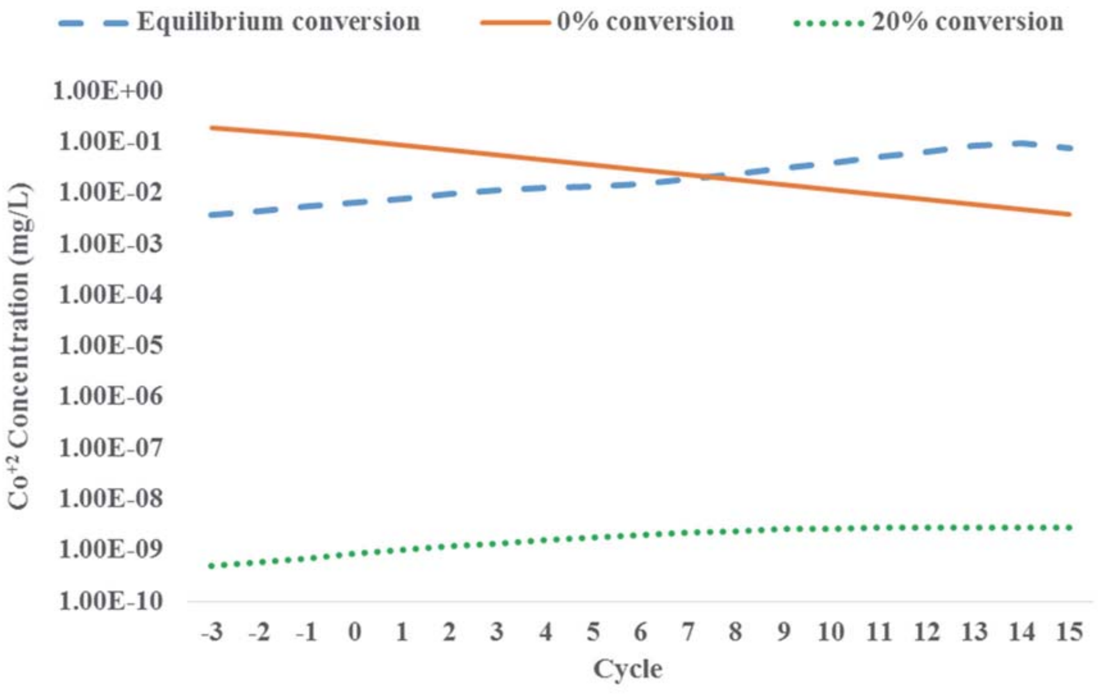

Figure 25: Soluble Co^{+2} for the AP-Model at different conversions of SO_4^{-2} to HS^-

Comparing the 0% and 100% conversions curves, the trends of soluble phase concentrations are reversed; the sulphide precipitate is a reservoir from which the metals re-solubilise into the soluble phase as the soluble sulphide is gradually washed out.

Figure 24 also shows a relationship of increased soluble phase concentration when there is no sulphide in the system. Figure 24 clearly shows the opposing relationships in the soluble phase concentration when there is no sulphide in the system and when there is, to the point of the trends crossing each other. This further emphasises the importance of knowing the sulphide concentration in the real life system, allowing it to be correctly modelled.

4.1.3 METAL PARTITIONING AT THE TWO SULPHIDE CONCENTRATION EXTREMES

This section serves to show the significance of the presence or absence of sulphide on the partitioning of the metals between the various phases, 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 sulphide in the system qualifies as a major limitation of the model to represent metal partitioning in the current system.

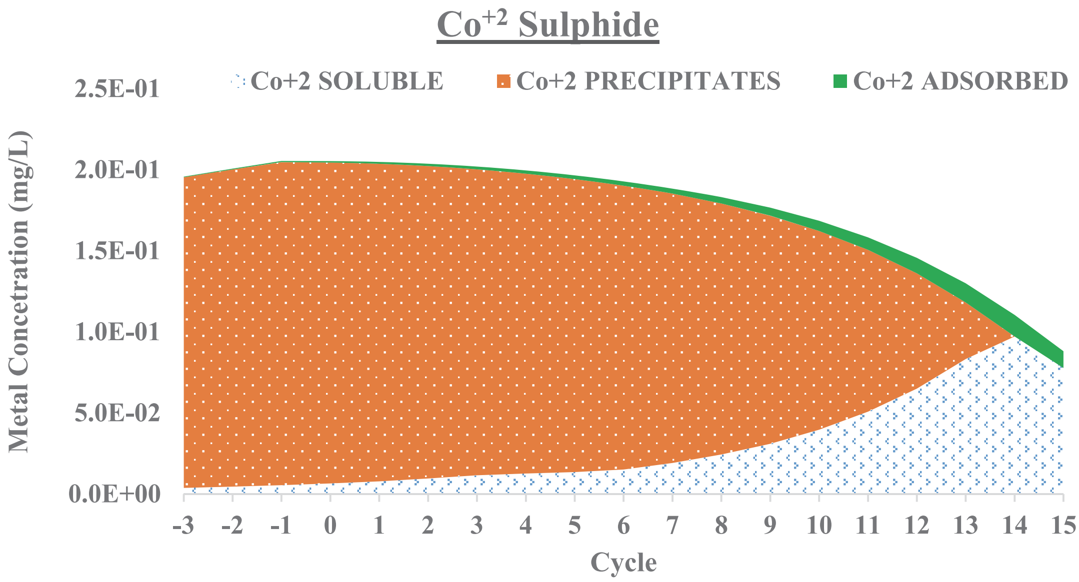

Figure 26: Partitioning of Co^{+2} in the Soluble, Precipitates and Adsorbed Phases with Sulphide present in the system

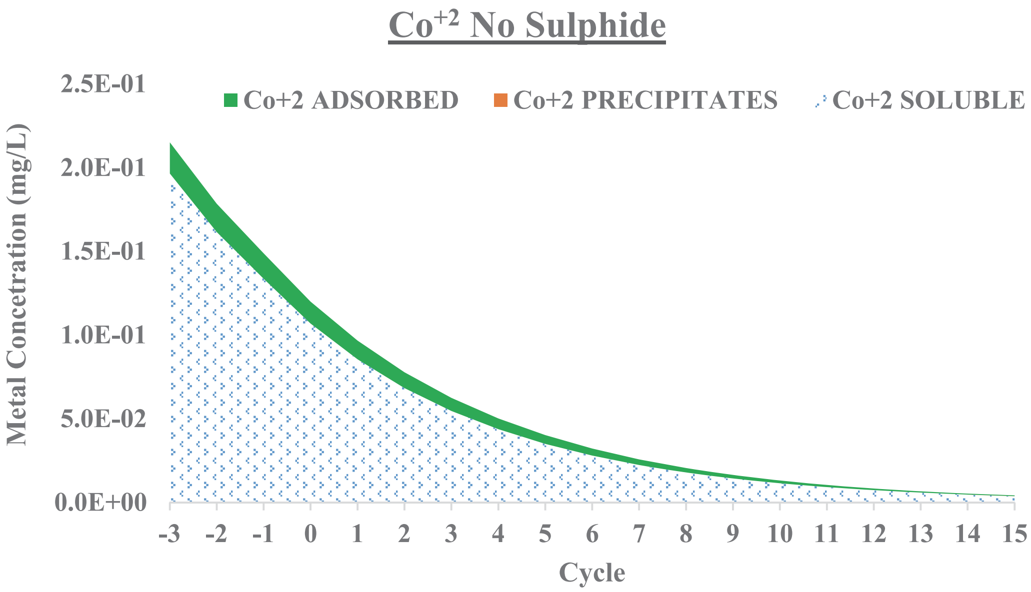

Figure 27: Partitioning of Co^{+2} in the Soluble, Precipitates and Adsorbed Phases with no Sulphide present in the system

Figure 25 and 26 show that there is significantly more Co^{+2} in the soluble phase concentration for the model without sulphide, 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 from 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, and so washed during decantation.

In the model where there is sulphide in the system, the precipitates, particularly the sulphide precipitates, are the dominant factor. However, in the model where there is no sulphide in the system, the adsorbed phase becomes more significant in retaining metals in the reactor. Appendix G shows the precipitates phase to be the controlling phase for all metals when there is sulphide present in the system.

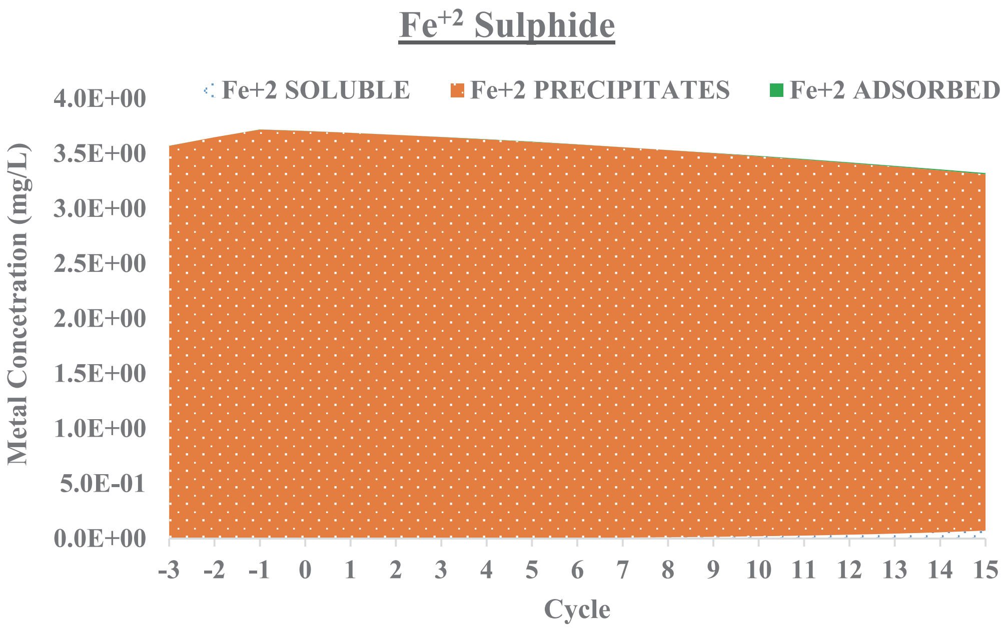

Figure 28: Partitioning of Fe^{+2} in the Soluble, Precipitates and Adsorbed Phases with Sulphide present in the system

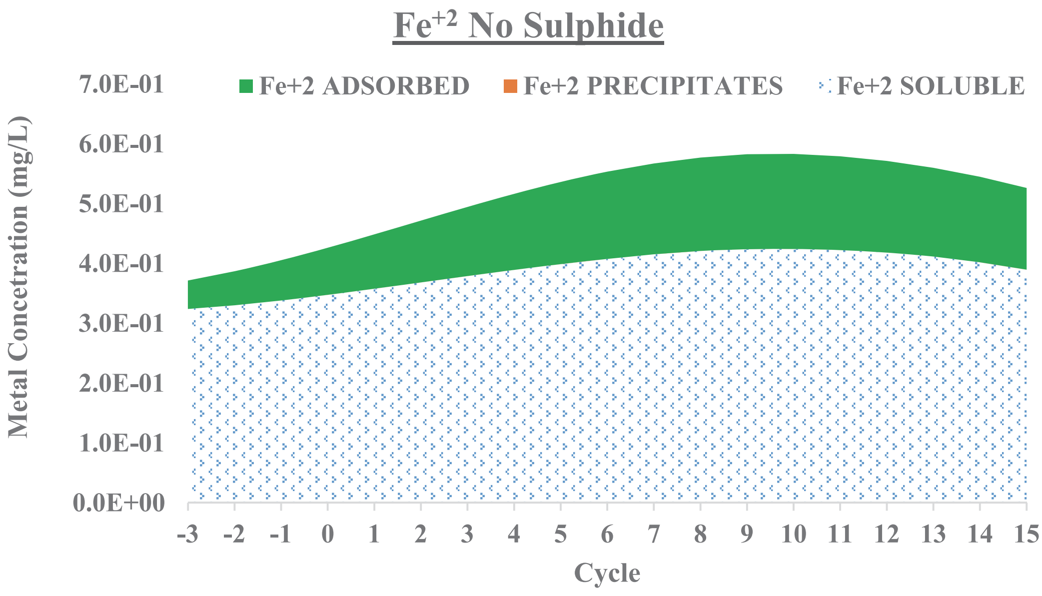

Figure 29: Partitioning of Fe^{+2} in the Soluble, Precipitates and Adsorbed Phases with no Sulphide present in the system

Figures 27 and 28 show a similar shift from the precipitates to the adsorbed phase when there is no sulphide in the system. Furthermore, Figure 28 shows the great extent to which the adsorbed Fe^{+2} increases when there is no sulphide in the system.

5 DISCUSSION AND CONCLUSIONS

The sulphide question was entirely unexpected when the project methodology was being formulated. It had also not been anticipated in the experimental investigations that provided the experimental data for the model, which explains why it was never measured.

No sulphide was fed to the system, but the possibility existed that sulphate that was present might be partially converted to sulphide by biological action. It was the model itself that drew attention to the issue. The sulphate to sulphide conversion is a redox reaction, and by default PHREEQC assumes redox equilibrium. Accordingly it predicted that some of the sulphate present in the system would be converted to sulphide. However, the equilibrium model cannot be expected to provide a reliable prediction of the conversion to sulphide, because such redox reactions are very unlikely to approach equilibrium under the conditions prevailing in a methanogenic anaerobic digester. The conversion required the action of sulphidogenic micro-organisms, and the conditions were not clearly favourable for their survival. So measurement was the only means of establishing how much sulphide was present, if any at all.

The uncertainties that remain around sulphide not only prevented the successful calibration of the model, but also constitute a major gap in understanding the dynamics of the micronutrient metals in the system, which was the ultimate goal of the project. The fact that soluble metal concentrations were not detected in most of the samples provided an indirect experimental indication that sulphide was indeed present, and that it may have constituted a strong limitation on the bioavailability of the dosed metals. This suggests that the identification of the sulphide problem may prove to be the most important outcome of the investigation.

It even seems possible, from the model results, that minimising the precipitation of micro-nutrient metal sulphides (perhaps by dosing excess iron) might turn out be the most important factor in optimising the effectiveness of micro-nutrient metal dosing. However, without any sulphide measurements to guide the model these issues could not be resolved with any certainty. The model results that have been presented are just scenarios based on assumptions, in order to give an indication the range of possibilities.

6 RECOMMENDATIONS

Modelling has exposed, but not resolved, a number of shortcomings in the experimental programmes undertaken so far, and the chief recommendation is to undertake a new experimental programme that addresses the shortcomings. Since Mathir's investigation, Sasol have developed experimental facilities that should be ideal for such an investigation, and could not conceivably be matched by the university. These are the laboratory-scale and large-scale pilot plants.

The large-scale pilot plant is fed with real factory effluent, and so is exposed to all the unexpected components that might affect the micro-nutrients. It needs a conservative, stable operating regime, but can provide large samples of material for intensive and extensive chemical analyses. So, it would be possible to undertake crystallographic analysis to confirm the presence of specific precipitates under normal operating conditions. It can also be used to provide data on the key issue of conversion of sulphate to sulphide under realistic operating conditions.

The laboratory scale pilot plant can be used for complementary experiments, particularly those which involve the risk of reaction failure, such as a nutrient wash-out experiment. This is because waste sludge from the large reactor can be used to restore it to full operation almost instantaneously. It set up for continuous operation, and comprehensively instrumented for monitoring the anaerobic digestion performance.

So the experimental programme that is envisaged is a series of nutrient washout experiments performed on the lab-scale reactor, each starting with sludge taken from the large-scale reactor during stable operation. The laboratory reactor can be operated under those same conditions as the large reactor, except for the micro-nutrient dosing. Once the anaerobic digestion performance is clearly impaired, the experiment can be stopped, and a new one initiated with sludge from the large reactor.

A series of such experiments in which different micro-nutrient components are removed from the recipe, should soon yield information which can be used to identify which ones are most critical, and eventually to optimise the formulation.

The modelling has suggested that the levels of sulphide in the system has a strong influence on the bioavailability of the micro-nutrient metals. Experiments should be designed to test this hypothesis, because if it is correct, controlling the level of sulphide in the system may prove to be a key strategy for increasing the effectiveness of micro-nutrient dosing.

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