EVALUATION OF THREE CARBON SOURCES FOR THE BIOLOGICAL TREATMENT OF ACID MINE DRAINAGE THROUGH PROCESS MODELLING

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To the memory of my father, Damoder Gopal, who passed away on the 21st of January 2005 at the age of 64 after battling for seven years with cancer. Your love and guidance made me what I am today. You will always be close to my heart and forever loved and remembered.

Hemant

ABSTRACT

South Africa is considered to be a semi arid to arid country (Harrison, 2004), hence its water resources are of great importance. In South Africa, the principal contributors to extensive sulphate pollution of ground water are the industries mining coal and metal-bearing sulphidic minerals, which gives rise to the production of acid mine drainage (AMD).

AMD is generated from both active and abandoned mining areas. The metal sulphides in the metal tailings are oxidised to produce large amounts of dissolved metals, sulphates and acids. These metals and acids constitute acid mine drainage. This natural process results from the exposure of ores to atmospheric conditions coupled with bacterial activity (Tsukamoto and Miller, 1999). Pollution by AMD can have a devastating effect on terrestrial and aquatic ecosystems. It is a long-term environmental problem since the oxidation of the metal sulphides can continue indefinitely after the closure of the mine (Tsukamoto and Miller, 1999).

The traditional method of treating AMD is by neutralisation of the acid through the addition of lime (Santos *et al.*, 2004). More recently, biological treatment of AMD has become attractive. However a concern with this method is the requirement and availability of cost effective and efficient sources of carbon and electron donors.

This thesis aims to evaluate three different substrates as sources of carbon and electron donor capacity (ethanol, molasses and primary sewage sludge) in terms of their availability and their impact on both final water quality and process economics. It seeks to determine the extent to which the carbon substrate is the limiting factor in terms of process economics. Further to the economic analysis, analysis of substrate requirements as a function of availability as well as impact of substrate used on

process complexity and water quality is reviewed. These goals are approached through use of a process model.

Data for the development of the model and its calibration has been taken from the literature. After an extensive review of the literature, a model of the anaerobic digestion process has been compiled using Excel, with the reactor being simulated using MATLAB. The program for the reactor is based on the simulation developed by Knobel (1999) in OCTAVE. The reactor was simulated as a CSTR that was well mixed and had no biomass retention.

The statistical method used to verify the fit of the model to the data was the Chi-square statistic. This is a good method of comparing the model data with literature data as it showed the degree of deviation of the model from the literature values. The values obtained from this calculation were then compared to the critical value of χ^2 at the 90% confidence level. The model was verified against four sets of anaerobic digestion data from literature with the carbon source being of various complexities.

The flowsheet developed consisted of:

- three holding tanks, one for the AMD storage, one for substrate storage, and one for hydrochloric acid,
- two continuously stirred reactors, one anaerobic (for the biological treatment of AMD) and one aerobic (to convert the sulphides to elemental sulphur),
- a mixer between the two reactors in which a buffer is added to lower the pH for optimal conditions for the aerobic reactor, and
- two settlers, one after the mixer to clarify the water by separating the biomass from it and the other after the aerobic reactor to remove sulphur from the system,

The model was then used to evaluate the treatment of three AMD sites located in South Africa with each of the three carbon sources. The sulphate concentrations at the three AMD sites assessed were:

- 1 437 mg l^{-1} for AMD site 1,
- 1 833 mg l^{-1} for AMD site 2, and
- 2 248 mg l^{-1} for AMD site 3.

The results of the mass balance showed that AMD site 3 required the highest concentration of carbon substrate owing to the highest concentration of sulphate entering the system. AMD site 3 also had the highest production of H_2S gas from both the anaerobic reactor as well as the mixer. As AMD site 3 treated the highest concentration of sulphate, it also produced the highest amounts of by-products. In the same respect, AMD site 1 treated the lowest concentration of sulphates and produced the least amount of by-products.

The simulation was set up such that the final effluent sulphate concentration met the EPA standard of 250 mg l^{-1} and a sulphide level of less than 10 mg l^{-1}. The only water parameter that needed analysis was the COD levels. The recommended COD level in the final effluent was 75 mg l^{-1} (DWAF, 1996 and Finn, 2004). Using the proposed flowsheet, only systems using ethanol as a carbon substrate approached this criterion. Both the molasses and primary sewage sludge systems failed to achieve this using the well mixed reactor system described by the model. For molasses or primary sewage sludge to meet the required COD levels, a reactor that could uncouple the hydraulic residence time and solids residence time and have high solids retention, would be required.

The capital costing of the treatment plants was based on pricing obtained by Ball and Schroeder (2001) who had previously costed similar units. A factorial method was used for the cost scaling of the units. Inflation was also taken into account. The operating cost of the system was based on the methods presented in Sinnott (2000) and Turton *et al.* (1998).

The economic results showed that using stainless steel was 16 times more expensive than using reinforced concrete as the material of construction. Hence, all further work was done on the basis of using reinforced concrete as the material of construction.

Ethanol was found to be the most economically viable choice when the cost saving on the disposal of primary sewage sludge was not taken into account. Using a complex particulate carbon source such as primary sewage sludge as the carbon substrate proved to be the most expensive option of the three where no benefit of reduced disposal costs of this complex particulate was found. However, when the savings resulting from reduced disposal requirements of primary sewage sludge from wastewater treatment were included, primary sewage sludge proved to be the most economically viable option. This was an important finding as it showed that there was a high burden reduction on the wastewater treatment works and hence should be strongly recommended for use in the treatment of acid mine drainage. As a corollary to this, the ongoing development of reactor systems exploiting the uncoupling of hydraulic and sludge residence times and maximising sludge retention is of prime importance.

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NOMENCLATURE

Abbreviations

A	Acidic compound
aFER	amino acid fermenters
AMD	acid mine drainage
aMPB	acetate utilising methanogens
aSRB	acetate utilising sulphate reducers
bACE	butyrate utilising acetogens
BOB	beta oxidising bacteria
bSRB	butyrate utilising sulphate reducers
C	concentration
CSTR	continuously stirrer tank reactor
d	day
DWAF	Department of water affairs and forestry
EPA	Environmental protection agency
eSRB	ethanol utilising sulphate reducers
G	gas phase
gFER	glucose utilising fermenters
glyFER	glycerol utilising fermenters
h	hours
HRT	hydraulic residence time
hMPB	hydrogen utilising methanogens
hSRB	hydrogen utilising sulphate reducers
Kg	kilogram
l	litre
L	liquid phase
lFER	lactate utilising fermenters
lSRB	lactate utilising sulphate reducers
M	meter
NADH	Nicotinamide Adenine Dinucleotide
NEA	National environment agency
p	partial pressure
pACE	propionate utilising acetogens
pSRB	propionate utilising sulphate reducers
SRB	sulphate reducing bacteria
t	time(hr)
T	temperature

TDS	total dissolved solids
UASB	upflow anaerobic sludge bed
VFA	volatile fatty acids

Symbols

a	interfacial area of gas liquid contact ($m^2.m^{-3}$)
D	dilution rate (hr^{-1})
H	Henry's constant
I	ionic strength
k	reaction rate constant (d^{-1})
k_d	decay rate
Ka	acid dissociation constant ($mmole.l^{-1}$)
k_L	liquid side mass transfer coefficient (ms^{-1})
$K_{I,H2}$	hydrogen inhibition constant
$K_{I,H2S}$	hydrogen sulphide inhibition constant
K_S	substrate half velocity or saturation constant ($mol.l^{-1}$)
m	gas/liquid solubility coefficient (unitless)
n	moles
N	molar flux ($mol.m^{-3}s^{-1}$)
P_{H2}	hydrogen partial pressure
Q	flow rate (m^3s^{-1})
R	universal gas constant ($m^3.atm.mol^{-1}.K^{-1}$)
R_{GL}	rate of glucose uptake
S	substrate concentration
U_o	overflow velocity (ms^{-1})
V	reactor volume (m^3)
X	biomass
Y	Yield (mg biomass/ mg substrate)
z	ionic charge

Chemical symbols

$CaCO_3$	calcium carbonate
$CH_2OHCHOHCH_2OH$	glycerol
CH_3COOH	acetate
CH_3CH_2COOH	propionate
$CH_3CH_2CH_2COOH$	butyrate
$CH_3CHOHCOOH$	lactic acid
$CH_3(CH_2)_{14}COOH$	palmitic acid
CH_4	methane
C_2H_5OH	ethanol
$C_5H_7O_2N$	proteins and biomass
$C_6H_{10}O_5$	carbohydrate
$C_6H_{12}O_6$	glucose
$C_{12}H_{22}O_{11}$	sucrose
CO_2	carbon dioxide
$C_{51}H_{98}O_6$	lipids

Fe^{3+}	ferric iron
FeS_2	iron disulphide
H^+	hydrogen ion
H_2	hydrogen
H_2CO_3	carbonic acid
HCl	hydrochloric acid
H_2O	water
H_2S	hydrogen sulphide
$Mg(OH)_2$	magnesium hydroxide
Na_2S	sodium sulphide
NaOH	sodium hydroxide
NH_3	ammonia
O_2	oxygen
S^o	elemental sulphur
SO_4^{2-}	sulphate ion

Economic symbols

C	purchased cost
CO	cost of operation
COL	cost of labour
FCR	fixed capital required
I	cost index
n	cost exponent
PPC	total physical plant cost
S	characteristic size parameter
TEC	total equipment cost

Greek symbols

μ	specific growth rate (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})
γ	activity coefficient
η	efficiency
χ^2	chi-squared statistic
α	on/off switching function
αLL	parameter in pH inhibition function
αUL	parameter in pH inhibition function

Chapter 1

INTRODUCTION

South Africa is considered to be a semi arid to arid country (Harrison, 2004), and as a result, its water resources are of great importance to it. Sulphate rich wastewater streams arise from a number of industrial processes, such as pulp and paper, leather and metal processing (Ghigliazza *et al.*, 2000). In South Africa, the principle contributors to extensive sulphate pollution of ground water are the coal-mining industries and mining operations processing metal-bearing sulphidic ores, which give rise to the production of acid mine drainage (AMD).

AMD is generated from both active and abandoned mining areas. The metal sulphides in the metal tailings are oxidised to produce large amounts of dissolved metals, sulphates and acids. These metals and acids constitute acid mine drainage. This is a natural process resulting from the exposure of the ores to atmospheric conditions coupled with bacterial activity (Tsukamoto and Miller, 1999). In abandoned mine sites, open pits, underground works and mine areas are often filled with water after closure of the mines (Christensen *et al.*, 1996). Overflow from such works also contributes significantly to acidic mine effluent. Pollution by AMD can have a devastating effect on terrestrial and aquatic ecosystems. It is a long-term environmental problem since the oxidation of the metal sulphides can continue for many year after the closure of the mine (Tsukamoto and Miller, 1999).

1.1 Problem Statement

The traditional method of treating AMD requires neutralisation of the acid by the addition of lime (Santos *et al.*, 2004). This is an expensive method (Tsukamoto and Miller, 1999) due to both the reagent costs and disposal costs to treat the large volumes of AMD produced from the various activities. More recently, biological treatment of AMD has become attractive. However a concern with this method is the requirement and availability of cost effective and efficient sources of carbon and electron donors. The choice of the carbon source affects the size of the reactor. Simple compounds require a smaller system whereas more complex carbon sources require a larger reactor and potentially more complex system. This directly affects the capital costs of the system. However, simple carbon compounds tend to be more expensive than complex carbon compounds.

1.2 Thesis Objectives

This thesis aims to evaluate three different substrates as sources of carbon and electron donor capacity in terms of their availability and their impact on both final water quality and process economics to determine if the carbon substrate is the limiting factor in terms of process economics. Further to the economic analysis, analysis of substrate requirements as a function of availability as well as impact of substrate used on process complexity and water quality is reviewed.

1.3 Hypothesis

The hypotheses proposed for this study are:

- The carbon source as well as its availability are the limiting factors in the process of sulphate reduction in terms of economics

- The applicability of the carbon source may be tested using a kinetic model of the process

1.4 Scope of the Study

Data for the development of the model and its calibration have been taken from the literature. After an extensive review of the literature, a model of the anaerobic digestion process has been compiled using Excel, with the reactor being simulated using MATLAB. The program simulating the reactor is based on the simulation developed by Knobel (1999) in OCTAVE. Improvements to the computer simulation have been made (e.g. the inclusion of ethanol as a substrate). This simulation model as well as the modelling of the units used in the anaerobic digestion process is used to form an economic evaluation of three carbon sources available in South Africa across three sites recognised for generating AMD.

The treatment of the metals in the AMD streams is not included in this study hence; the metal sulphide precipitation that would occur prior to the AMD entering the biological reactor is also not included. It is assumed that the metals are precipitated out with some of the elemental sulphur from the biological sulphide oxidation reactor and that the amount of metals entering the anaerobic reactor is negligible.

1.5 Thesis Outline

A review of the relevant literature is provided in Chapter Two. Chapter Three deals with the methodology used in performing the mass balance. The verification of the computer simulation used to simulate the reactor is presented in Chapter Four. Chapter Five deals with the calibration of the reactor simulation for the anaerobic digestion process. The results from the mass balance are presented in Chapter Six. The methodology used for the economic analysis is presented in Chapter Seven. Both the capital costing and the operating costs are considered. The results from the economic evaluation are presented in Chapter Eight. A discussion of the availability of the carbon source as a limiting factor and the impact of the carbon source on effluent quality is presented in Chapter Nine. The sensitivity of the system to changes in sulphate loading, substrate cost, disposal costs and hydraulic residence time is presented in Chapter Ten. Chapter Eleven concludes the thesis through a general discussion and conclusions drawn from the findings presented in the earlier chapters. Recommendations for further research are also presented here.

Chapter 2

LITERATURE REVIEW

The literature review provided focuses on the characterisation, formation and effects of acid mine drainage (AMD). The significance of AMD to South Africa as well as the current treatment methods for treatment of AMD are discussed.

Due to the different types of potential carbon sources available, a general degradation process starting with a complex and particulate carbon source (based on sewage) as a raw material is presented. The kinetics associated with each step in the general degradation process is also presented. A review of the different carbon sources that have been used in the study of AMD treatment at a laboratory and pilot scale is presented, followed by the discussion of criteria on which to base the selection of potential carbon sources. Finally, a review of the various reactor systems that have been proposed for AMD treatment by biological sulphate reduction is presented.

2.1 AMD, its Characterisation, Formation and Impact

In order to effectively treat AMD, it is necessary to have a basic understanding of what it is, how it is formed and what the impact of it is. This section gives a very brief overview of these factors as well as the current methods to treat it.

2.1.1 Characterisation of AMD

High sulphate wastewaters, defined as aqueous streams that have a sulphate content higher than 500 mg l^{-1} (Department of Water Affairs and Forestry, 1999), originate from various industrial activities. These include the manufacture of pulp and paper, explosives, fertilizer and other petro-chemical products as well as mining and minerals processing. An important example of such high sulphate wastewater is acid mine drainage. Acid mine drainage originates from the runoff and seepage from waste rock stockpiles and tailings or coal rejects.

***Table 2-1**: Composition of sample AMD streams. Four are taken from mines in South Africa and one each from U.S.A., Ireland and Norway.*

	South Africa[1]				USA[2]	Ireland[3]	Norway[4]
	Grootvlei	**West Rand**	**Klipspruit**	**Brugspriut**	**Anaconda**	**Avoca**	**Waalenburg**
	Gold	**Gold**	**Coal**	**Coal**	**Copper**	**Copper/Zinc**	**Copper**
PH	6.3	2.4	2.6	2.2	2.5	2.7	5.5
TDS	3 028	38 448	11 512	4 490	-	-	-
Sulphate	1 183	22 556	8 122	3 947	3 510	10 579	2 940
Sodium	291	77	1 893	129	72	-	-
Potassium	12.9	-	-	-	22	-	-
Iron	187	6 674	-	-	300	1 031	139
Zinc	77	12	-	-	155	362	34
Copper	0.5	2	-	-	29	243	2
Nickel	3	13	-	-	-	-	-
Aluminum	-	-	-	-	125	-	1
Manganese	-	5	-	-	88	-	350

All units in mg l^{-1} except pH

[1] Data obtained from Department of Mineral and Energy Affairs (1995)

[2] Data obtained from Jenke and Deibold (1983)

[3] Data obtained from Gray (1997)

[4] Data obtained from Christensen *et al.* (1996)

Table 2-1 presents the composition of seven acid mine drainage (AMD) streams. It can be seen that the major pollutants in AMD streams are sulphate, iron and sodium. The presence of other metals depends strongly on the source of the AMD. The pollutant of primary importance for this study is sulphate whose range varies considerably from 2 200 mg l^{-1} to 22 556 mg l^{-1} across these sample streams. This variable sulphate concentration is a common problem associated with AMD. Other problems associated with AMD are the high acidity that results from the high sulphate content of the wastewater and the high metal content.

2.1.2 Formation of AMD

Generation of AMD is predominately the result of bio-oxidation of the sulphide minerals and ferrous iron present in the terrain through which water drains. The resultant presence of ferric iron and acidic conditions result in drainage flow with concomitant leaching of metals (Singer and Stumm, 1970). This is a natural process resulting from the exposure of the ores to atmospheric conditions coupled with bacterial activity. The main source of AMD in abandoned mine areas are usually old waste rock dumps and rock walls in tunnels and shafts. Open pits and underground workings are often filled partly or completely with polluted water after the closure of a mine (Christensen *et al.*, 1996). An example is the oxidation of pyrite, which is accelerated by the presence of micro-organisms such as *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans, Acidithiobacillus caldus* and *Leptospirillum ferrooxidans* (Garcia *et al.*, 1996).

The oxidation of sulphide minerals is generally accepted to be mediated through the "Two-step" process proposed by Boon *et al.* (1995). This recognises that the main contribution to leaching is achieved through chemical leaching of sulphide minerals by ferric iron. The representative reaction for ferric leaching of pyrite is given in Equation 2-1. Similarly the ferric iron may leach other sulphide minerals, the case of chalcopyrite being given in Equation 2-3 (Brierlay and Brierlay, 1986). In all ferric leaching cases, the micro-organisms regenerate ferric iron through the oxidation of ferrous iron according to Equation 2-2. Further metabolism of sulphur formed by

micro-organisms such as *Acidothiobacillus thiooxidans* and *Sulfolobus acidocaldarius* further augments the resultant sulphate concentration (Rossi, 1990).

$$FeS_2 + 14\ Fe^{3+} + 8\ H_2O \rightarrow 15\ Fe^{2+} + 2\ SO_4^{2-} + 16\ H^+ \quad (2\text{-}1)$$

$$15(Fe^{2+} + \tfrac{1}{4}\ O_2 + H^+ \rightarrow Fe^{3+} + \tfrac{1}{2}\ H_2O) \quad (2\text{-}2)$$

$$CuFeS_2 + 2Fe_2(SO_4)_3 \rightarrow CuSO_4 + 5FeSO_4 + 2S \quad (2\text{-}3)$$

2.1.3 Effects of AMD

Typical features of AMD are the acidic pH of the water as well as high concentrations of metals and sulphates. This can be seen in Table 2-1. Discharge of these streams into the environment will have chemical, biological, physical and ecological effect (Elliott *et al.*, 1998). Sulphate contributes to the total dissolved solids (TDS) of water, affecting its ability to be used for drinking water, irrigation or industrial wastewater. In the environment sulphate may be biologically reduced, resulting in the formation of H_2S. This results in odour, toxicity and safety problems (Gray, 1997). If the wastewater is allowed to flow into environmental water, it will affect the respiratory, reproductive and behavioural performance of the aquatic life (Elliott *et al.*, 1998). If the pH of the sulphate containing water is low, its discharge can result in complete sterilization of the receiving water and ultimately result in the permanent ecological damage.

2.1.4 Problem of AMD in SA

South Africa is a semi-arid to arid country (Harrison, 2004), which makes its water its most limiting natural resource (Toerien and Maree, 1987). South Africa also has a large mineral processing industry. As a consequence, there are a large number of mines that are present and working, generating dumps and tailings that form AMD generation sites.

Because the generation of AMD is a natural process, further generation of AMD will continue from sites that have been abandoned (Santos *et al.*, 2004). This is

particularly important owing to the large number of mine closures planned within S.A. in the future and need to manage water levels and quality within these disused mine sites. An example is the Grootvlei Proprietary Mines Ltd which was reported to be discharging between 80 and 100 megalitres of underground water per day (Schoeman and Steyn, 2001). The water is of low quality due to the high dissolved salt concentration as well as the high metal concentrations. This water will adversely affect the water ecology of the Blesbokspruit if not treated prior to disposal into the Blesbokspruit.

Recent studies carried out by the Department of Water Affairs and Forestry have shown that if present usage patterns are maintained, then the present water supply will only be adequate till 2030 (Government Gazette, 1999). On a global scale, the same situation is predicted by 2050. Hence the culture of water treatment and re-use with South Africa needs to be nurtured as a matter of urgency.

2.1.5 Current Methods of AMD Treatment

Historically AMD has been treated by neutralisation (Santos *et al.*, 2004). Several other processes have also been investigated to treat AMD. Some of the more common methods are listed below:

- Biological treatment,
- Reverse osmosis,
- Electrodialysis,
- Crystallisation,
- Ion exchange, and
- Distillation

The methods of reverse osmosis, electrodialysis, ion exchange and distillation have been found to be uneconomical due to power and chemical reagent costs (Kuyucak and St-Germain, 1994).

The most common method used to treat AMD is neutralisation, as it is quick and effective. The conventional method uses lime as the neutralising reagent (Kuyucak and St-Germain, 1994), however this generates a high volume of sludge that needs to be relocated (Garcia *et al.*, 1996). To improve the final effluent quality and to minimise sludge disposal issues, several other neutralising reagents (e.g. $Mg(OH)_2$, Na_2S, NH_3, NaOH, $CaCO_3$) are used for treating the AMD (Kuyucak and St-Germain, 1994). Metals are then precipitated to hydroxides. This process is expensive and produces a waste sludge of gypsum and metal hydroxides that is highly contaminated with heavy metals and which must be dewatered and disposed of in landfill (Elliott *et al.*, 1998)

The advantage of this method is that it is effective in the treatment of AMD. The disadvantage is its cost. The costs arise from the expensive chemical reagents (Garcia *et al.*, 2001) and from the transporting of the wastes to landfills as well as landfill costs.

The biological sulphate reduction process is applicable as both an active and passive treatment method and has been highlighted as having potential to treat sulphate containing acidic effluents cost effectively (Colleran *et al.*, 1995). As a passive treatment system the sulphate reduction process takes place in the anaerobic zone of a wetland. As an active treatment system the sulphate reduction process occurs in bioreactors. The active process for anaerobic sulphate reduction is a promising alternative to lime neutralisation due to the inexpensive carbon source used as reducing equivalents. However, improvements in the application of the basic principle must be achieved to decrease the cost of this type of process and make it economically viable for use in the treatment of AMD (Foucher *et al*, 2001). The disadvantages of this method are:

- It requires a sophisticated process equipment; and
- It requires a sufficient continuous supply of available and economically feasible carbon sources and electron sources.

2.2 The General Degradation Process

In anaerobic environments complex organic wastes are broken down into short chain fatty acids, hydrogen, methane and carbon dioxide by fermentative bacteria, acidogens and methanogens (Gujer and Zehnder, 1983). Sulphates are reduced to sulphides with concomitant formation of carbon dioxide by sulphate-reducing bacteria. A general degradation process is presented using a complex particulate carbon source such as raw sewage as a starting point. The reaction pathway is shown in Figure 2-1 (Knobel, 1999). The component processes are described below.

2.2.1 Hydrolysis of Insoluble Compounds

In general bacteria are unable to take up particulate organic material since it first has to be broken down into soluble polymers or monomers (Gujer and Zehnder, 1983). Thus, hydrolysis is the first step in the microbial utilisation of complex biopolymers.

An example of an insoluble compound is sewage sludge. Primary sludge originates from the solid component of raw sewage settled prior to any biological treatment. The reactions that occur are (Angelidaki *et al.*, 1999):

1) hydrolysis of amide bonds of proteins to yield amino acids and peptides;
2) hydrolysis of ester bonds of lipids to yield long chain fatty acids (LCFA's) and polyols; and
3) hydrolysis of glycoside bonds of polysaccharides to yield dimeric and monomeric sugars or oligomers

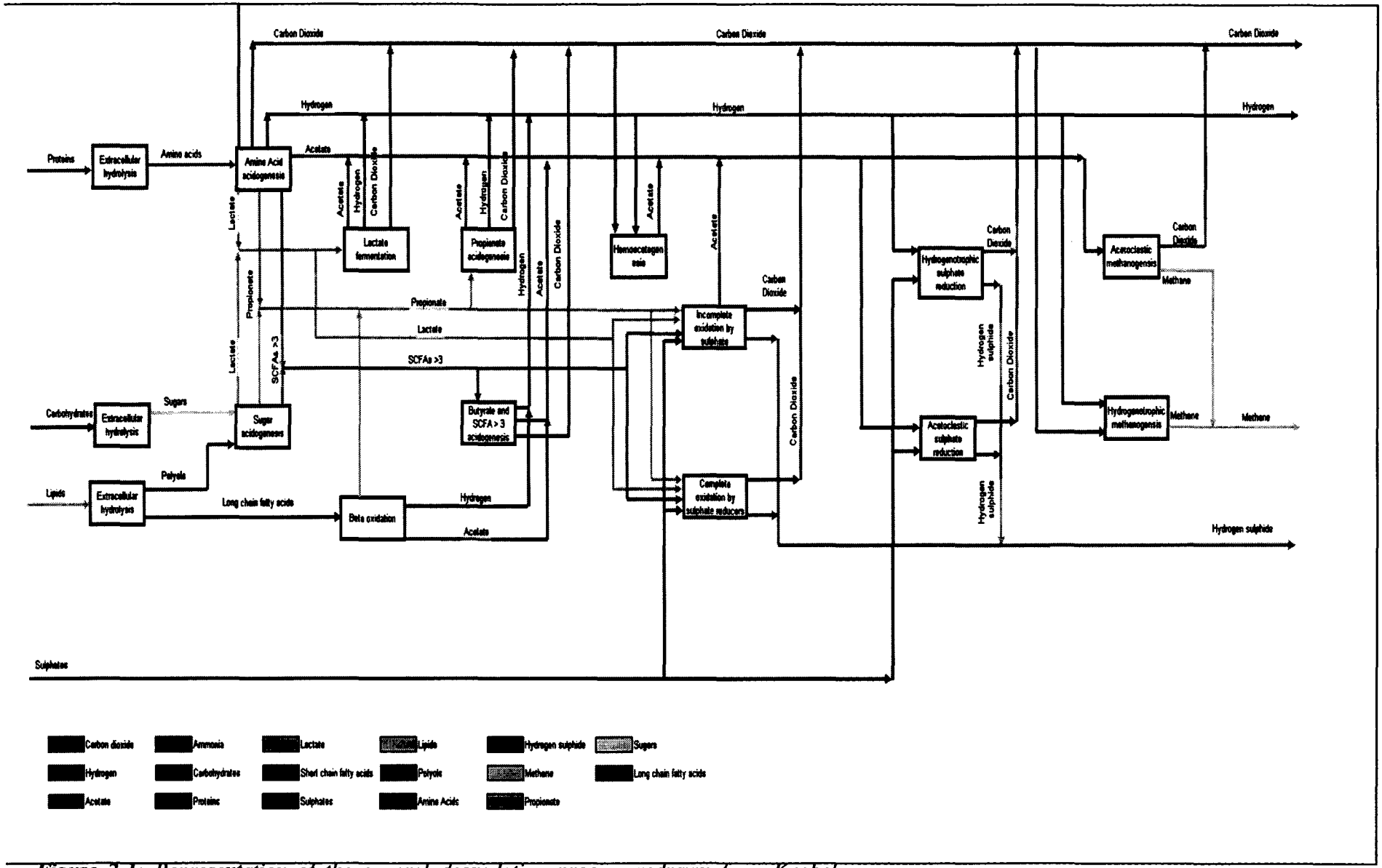

Figure 2-1: Representation of the general degradation process redrawn from Knobel.

The rate of hydrolysis is affected by temperature, pH, bacterial concentration, particle size, type of organic and soluble product concentration (Gujer and Zehnder, 1983). With respect to the subsequent fermentation and anaerobic oxidation steps, the extracellular hydrolysis step is considered to be the rate-limiting step in the anaerobic digestion of particulate organic matter (Eastman and Ferguson, 1981). Typically a first order function is used to model anaerobic sludge hydrolysis (Eastman and Ferguson, 1981; Angelidaki *et al.*, 1999). First order rate data found in literature is given in Table 2-2.

***Table 2-2**: First order rate constants for primary sludge.*

Component	T(°C)	k (d^{-1})	Reference
Sludge protein	15	0.01-0.03	O' Rourke (1968)
	20	0.01-0.08	O' Rourke (1968)
	25	0.01-0.09	O' Rourke (1968)
	35	0.01-0.10	O' Rourke (1968)
	55	1	Angelidaki *et al.* (1999)
Sludge carbohydrate	15	0.03-0.10	O' Rourke (1968)
	20	0.09-0.14	O' Rourke (1968)
	25	0.16-0.29	O' Rourke (1968)
	35	0.21-1.95	O' Rourke (1968)
	55	1	Angelidaki *et al.* (1999)
Sludge lipids	15	0	O' Rourke (1968)
	20	0-0.05	O' Rourke (1968)
	25	0-0.09	O' Rourke (1968)
	35	0.01-0.17	O' Rourke (1968)
	55	0.53	Angelidaki *et al.* (1999)

O' Rourke (1968) differentiated between degradation of lipids, cellulose and proteins in laboratory scale continuous flow digesters fed with domestic sludge. His results indicate significantly different degradation rates for the three groups of compounds. Angelidaki *et al.* (1999) fed cattle manure at 55°C with a retention time of 15 days. The first order rate constants for sludge degradation measured were confirmed with the computer simulation developed by these researchers. These confirmed the lower degradation rate of lipids with respect to proteins and carbohydrates.

2.2.2 Acidogenesis (Fermentation)

The amino acids, sugars and polyols that result from the hydrolysis reactions are broken down further by fermentation or acidogenesis (Gujer and Zehnder, 1983). The main products of this reaction are: hydrogen, carbon dioxide, and the short chain fatty acids, lactate, butyrate, propionate and acetate (Angelidaki *et al.*, 1999).

2.2.2.1 Glucose Fermentation

Acid-forming bacteria ferment glucose and other simple sugars to produce a mixture of acetic, propionic, butyric and lactic acid (Costello *et al.*, 1993). Costello *et al.* (1993) proposed Equations 2-4 to 2-6 to describe the possible fermentation routes of glucose.

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (2\text{-}4)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (2\text{-}5)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH \quad (2\text{-}6)$$

The bacteria favour the first reaction (Equation 2-4) as it provides the largest energy yield (Mosey, 1983). The other reactions occur when the hydrogen concentration is increased. Mosey (1983) theorised that the reason for this is that different cell pathways metabolising the substrate are regulated by the relative concentrations of NADH and NAD^+. The elevated hydrogen levels result in a decrease in glucose uptake.

Stamatlatou *et al.* (2003) used a glucose based synthetic medium as a feed in their work. The authors used a periodic anaerobic baffled reactor that was run at 35°C. Stamatlatou *et al.* (2003) found that Equation 2-7 stoichiometrically described their findings.

$$C_6H_{12}O_6 + 2H_2O \rightarrow 0.67\ CH_3COOH + 0.67\ CH_3CHOHCOOH + 0.33\ CH_3CH_2CH_2COOH + 2\ H_2 + 1.33\ H_2CO_3 \quad (2\text{-}7)$$

Stamatlatou *et al.* (2003) theorised that the high production of butyrate was attributed to the high concentrations of hydrogen. This theory is in line with the explanation given by Mosey (1983) that the production of butyrate and propionate increase as the hydrogen concentration increases.

The growth rate of glucose-utilising acidogens has been found to be inhibited by hydrogen concentration (Kalyuzhnyi, 1997; Stamatlatou *et al.*, 2003). The activity of the acidogenic bacteria, in general catabolising glucose by the Embden-Meyerhoff pathway, strongly depends on the ratio of NAD^+: NADH in the cells and the latter in turn is determined by hydrogen concentration. The rate of glucose uptake for energy production only, R_{GI}, is given by a non-competitive inhibition model (Mosey, 1983) as shown in Equation 2-8. Table 2-3 presents the rate data found by these authors.

$$R_{GI} = -\left(\frac{kXS}{K_S + S}\right)\left(\frac{K_{I,H_2}}{K_{I,H_2} + P_{H_2}}\right) \qquad (2\text{-}8)$$

2.2.2.2 Lactic Acid Fermentation

Lactic acid has been shown to be a major intermediate in anaerobic digestion (Zeller *et al.*, 1994). *Lactobacillus delbrueckii* and *Lactobacillus casei* are examples of bacteria that use lactate as a substrate (Lee, 2004).

In the model of Costello *et al.* (1993), lactic acid produced by glucose fermentation is broken down to different ratios of acetic acid and propionate depending on the hydrogen partial pressure. The proposed reactions are:

$$CH_3CHOHCOOH + H_2 \rightarrow CH_3CH_2COOH + H_2O \qquad (2\text{-}9)$$

$$CH_3CHOHCOOH + H_2O \rightarrow CH_3COOH + CO_2 + 2H_2 \qquad (2\text{-}10)$$

In the model of Stamatelatou *et al.* (2003) lactic acid produced by glucose fermentation is broken down according to Equation 2-11:

Table 2-3: Monod kinetic parameters for glucose-utilising fermentative organisms.

Culture	T (°C)	μ_{max} (d^{-1})	Y mg biomass/ mg substrate utilised	k_d (d^{-1})	K_s mg glucose/l	Conditions	Reference
Mixed culture	35	5.124	0.06	0.0001	170	PABR	Stamatelatou et al (2003)
Mixed culture	35	4.2	0.19278	0.03	14.60736	Batch	Kalyuzhnyi and Davlyatshina (1997)

Table 2-4: Monod kinetic parameters for lacate-utilising fermentative organisms.

Culture	T (°C)	μ_{max} (d^{-1})	Y mg biomass/ mg substrate utilised	k_d (d^{-1})	K_s mg lactate/l	Conditions	Reference
Mixed culture	35	2.552	0.13	0.0001	100	PABR	Stamatelatou et al (2003)
Desulfovibrio vulgaris	37	6			135	continuous	Zellner et al. (1994)
Clostridium propionicum	37	16.8			225	continuous	Zellner et al. (1994)

$$CH_3CHOHCOOH + 0.5\ H_2O \rightarrow 0.5\ CH_3COOH + 0.5\ CH_3CH_2COOH + 0.5\ H_2O + 1.33\ H_2CO_3 \quad (2\text{-}11)$$

Table 2-4 presents the kinetics found by various authors. Zellner *et al.* (1994) used a fluidised bed reactor to test the degradation of lactate. It was found that the *Clostridium* strain had a higher growth rate but a lower affinity (higher K_S value) for lactate than the *Desulfovibrio* strain.

2.2.3 Beta Oxidation of Long Chain Fatty Acids

According to Knoop's theory (Sawyer and McCarty, 1989), the breakdown of long chain fatty acids occurs by oxidation of the β carbon atom, resulting in the formation of acetic acid and hydrogen. While the fatty acid is shortened by two carbon atoms in each oxidation step the process is repeated. If the molecule has an even number of carbon atoms only acetic acid results. An odd number of carbon atoms in the fatty acid will result in the formation of both acetic and propionic acids. The general stoichiometry for β-oxidation (Gujer and Zehnder, 1983) is:

$$(-CH_2\text{-}CH_2\text{-}) + 2\ H_2O \rightarrow CH_3COOH + 2\ H_2 \quad (2\text{-}12)$$

If palmitic acid is taken as representative of all LCFA's present, then the overall reaction is:

$$CH_3(CH_2)_{14}COOH + 14\ H_2O \rightarrow 8\ CH_3COOH + 14\ H_2 \quad (2\text{-}13)$$

The large amount of hydrogen generated has been shown to be inhibitory to this reaction (Novak and Carlson, 1970). Rate data for long chain fatty acids taken from Novak and Carlson (1970, cited in Gujer and Zehnder, 1983) is reproduced in Table 2-5.

Table 2-5: Monod kinetic parameters for long chain fatty acid beta oxidation at 37°C. Values taken from Novak and Carlson (1970, cited in Gujer and Zehnder 1983).

Fatty Acid	μ_{max} (d^{-1})	Y (mg biomass/mg fatty acid)	k_d (d^{-1})	K_s (mg acid l^{-1})
Stearic (C-18)	0.10	0.3	0.01	143
Palmitic (C-16)	0.12	0.3	0.01	49.8
Myristic (C-14)	0.11	0.3	0.01	37.5
Oleic (C-18)	0.45	0.3	0.01	1116
Linoleic (C-18)	0.56	0.3	0.01	637

These experiments operated anaerobic enrichment cultures at 37°C. Long chain fatty acids were used as the sole carbon sources. Gujer and Zehnder (1983) reported that the yield and decay coefficients are average values for all experiments and that the yield may include biomass produced during methanogenesis. H_2 was shown to inhibit the reaction. The organisms that catalyse this reaction are related to those which degrade butyrate: obligate syntrophic bacteria (Gujer and Zehnder, 1983).

2.2.4 Acetogenesis (Anaerobic Oxidation of Short Chain Fatty Acids)

Acetogenesis is the process in which intermediate short chain fatty acids are degraded to acetate and hydrogen. A number of bacteria have been identified that can degrade butyrate and higher fatty acids. Examples of bacteria that can degrade butyrate and higher fatty acids are *Syntrophomonas wolfei* and *Syntrophomonas sapovorans* (McCarty and Mosey, 1991). However only one bacteria has been identified as being able to degrade propionate (and only propionate) and that is *Syntrophobacter wolinii* (McCarty and Mosey, 1991). Hence, for the purpose of modelling acetogenesis, these two groups are kept separate.

The reaction describing the anaerobic oxidation of propionate to yield acetic acid and the production of biomass are, respectively (Costello *et al.*, 1993; Kalyuzhnyi and Fedorovich, 1998):

$$CH_3CH_2COOH + 2\,H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 \qquad (2\text{-}14)$$

Butyrate production is represented as (Vavilin *et al.*, 1998; Costello *et al.*, 1993):

$$CH_3CH_2CH_2COOH + 2\ H_2O \rightarrow 2\ CH_3COOH + 2\ H_2 \qquad (2\text{-}15)$$

Mosey (1983) proposed that the rates of this reaction (Equations 2-14) are regulated through the availability of the co-enzyme NAD^+ and thus by the partial pressure of hydrogen. Tables 2-6 and 2-7 present some of the findings of authors.

***Table 2-6**: Monod kinetic parameters for propionate-utilising acetogens.*

T (°C)	μ_{max} (d^{-1})	Y mg biomass/ mg PrH	k_d (d^{-1})	K_s mg PrH l^{-1}	Reference
25	0.36	0.077	0.04	40	Lawrence and McCarty (1969, cited in Gujer and Zehnder, 1983)
35	0.31	0.064	0.01	758	Lawrence and McCarty (1969, cited in Gujer and Zehnder, 1983)
35	0.055	0.05	0.03	19.44	Kalyuzhnyi and Davlyatshina (1997)
33	0.155	0.025	0	163	Gujer and Zehnder (1983)
-	0.15	0.063	0.021	17.9	Maillacheruvu and Parkin (1996)
35	0.304	0.062	0.0001	72	Stamatelatou *et al* (2003)

***Table 2-7**: Monod kinetic parameters for butyrate-utilising acetogens.*

T (°C)	μ_{max} (d^{-1})	Y mg biomass/ mg BuH	k_d (d^{-1})	K_s mg BuH l^{-1}	Reference
35	0.37	0.085	0.027	7.2	Lawrence and McCarty (1969, cited in Gujer and Zehnder, 1983)
37	0.86	-	-	164	Mosey (1983)
35	0.264	0.051	0.03	96.8	Kalyuzhnyi and Davlyatshina (1997)

Laurence and McCarty (1969, cited in Gujer and Zehnder, 1983) reported on chemostat experiments for the anaerobic degradation of propionate and butyrate. These results are presented in Tables 2-6 and 2-7. Gujer and Zehnder (1983) had observed enrichment systems under continuous culture conditions for propionate degradation at 33°C. These results are presented in Table 2-6. Kalyuzhnyi and Davlyatshina (1997) had studied the anaerobic digestion of glucose under batch conditions with an initial pH of 7 and a temperature of 35°C. Maillacheruvu and Parkin (1996) studied the kinetics of propionate utilisation in anaerobic systems in batch reactors. Their studies also included the sulphide toxicity. The authors reported an inhibition constant of 26 mg H_2S l^{-1}.

2.2.5 Homoacetogenesis

Homoacetogenesis refers to the production of acetic acid from CO_2 and H_2 (McCarty and Mosey, 1991). The reaction is:

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \qquad (2\text{-}16)$$

According to Nozhevnikova and Kotsuyrbenko (1995), homoacetogenesis is only significant in relation to hydrogen consuming methanogenesis at temperatures below 20°C.

2.2.6 Methanogenesis

Methanogenesis is the process whereby low molecular weight substrates are degraded to form methane. Methanogenesis is generally the rate-limiting step in anaerobic digestion except when hydrolysis is included in the reaction pathway (Eastman and Ferguson, 1981; Vavilin and Lokshina, 1996; Gupta, 1994).

2.2.6.1 Hydrogen / Carbon Dioxide Substrate

Examples of hydrogenotrophic methanogenic bacteria are (Kalyuzhnyi and Fedorovich, 1998): *Methanobacterium formicicum* and *Methanobacterium hungatei.* Methanogenic archaea utilising H_2 and CO_2 can be represented by the following reaction (Kalyuzhnyi and Fedorovich, 1998; Isa *et al.*, 1986):

$$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O \qquad (2\text{-}17)$$

The inhibitory effect of sulphide on methanogenesis has been documented (Hilton and Oleszkiewics, 1988; Maillacheruvu and Parkin, 1996). The presence of high concentrations of unionised acetic and other volatile fatty acids has also been shown to be inhibitory.

2.2.6.2 Acetate Substrate

Examples of acetotrophic methanogenic bacteria are (Kalyuzhnyi and Fedorovich, 1998): *Methanosarcina barkeri* and *Methanothrix soehngenii.* The overall reaction is (Kalyuzhnyi and Fedorovich, 1998; Isa *et al.*, 1986):

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad (2\text{-}18)$$

Table 2-8 shows the kinetic parameters found by various authors for hydrogenotrophic methanogenic bacteria and Table 2-9 presents the parameters found by various authors for acetotrophic methanogenic bacteria. O' Flaherty *et al.* (1998) studied the growth kinetics of various methanogenic bacteria in batch reactions. These reactions were run at 30°C. Sodium molybdate was added to the reactions to inhibit sulphate reduction. Kalyuzhnyi and Davlyatshina (1997) included kinetic studies of methanogenesis in their investigation of the anaerobic digestion of glucose. Maillacheruvu and Parkin (1996) studied the kinetics of hydrogen utilisers and reported on their sulphide toxicity.

Table 2-8: Monod kinetic parameters for methanogens using hydrogen as substrate.

Culture	T (°C)	μ_{max} (d^{-1})	Y mg biomass/ mg H_2	k_d (d^{-1})	K_s µg H_2/l	KI,H_2S mg H_2S/l	Conditions	Reference
Enriched MPB	-	0.18	0.39	0.013	30	664	Batch	Maillacheruvu and Parkin (1996)
Mixed culture	35	1.392	0.2	0.03	16	-	batch	Kalyuzhnyi and Davlyatshina (1997)
Methanobacterium ivanovii	-	0.8-1.7	0.54	-	-	-	-	Jain *et al.* (1987)*
Methanobacterium formicicum	-	1.2-2.8	0.4	-	-	-	-	Schaur *et al.* (1980)*
Methanobrevibacter arboriphilus	-	1.4-3.4	0.3-0.35	-	-	-	-	Zehnder and Wuhrmann (1977)*
Methanosarcina barkeri	-	1.4	0.8	-	-	-	-	Weimer and Zeikus (1978)*
Methanosarcina barkeri	30	1.16	-	-	9	-	Batch	O' Flaherty *et al.* (1998)
Methanospirillium hungatei	30	1.25	-	-	10	-	Batch	O' Flaherty *et al.* (1998)

* Taken from Oude Elferink (1994)

Table 2-9: Monod kinetic parameters for methanogens using acetate as substrate.

Culture	T (°C)	μ_{max} (d^{-1})	Y mg biomass/ mg acetate	k_d (d^{-1})	K_s mg acetate/l	KI,H_2S mg H_2S/l	Conditions	Reference
Mixed culture	35		0.0375		6		continuous	Gupta et al.(1994)
Enriched MPB		0.14	0.041	0.013	27	117	Batch	Maillacheruvu and Parkin (1996)
not reported	25	0.25	0.054	0.011	869	-	-	Lawrence and McCarty (1969)*
not reported	30	0.27	0.058	0.037	333	-	-	Lawrence and McCarty (1969)*
not reported	35	0.357	0.04	0.015	154	-	-	Lawrence and McCarty (1969)*
not reported	37	0.16	0.02		42	-	-	Lawrence and McCarty (1969)*
Synthetic medium		0.36	0.042	0.0199	138	-	Batch	Kalyuzhnyi and Davlyatshina (1997)
Methanothrix soehngenii	30	0.15			26	-	Batch	O' Flaherty *et al.* (1998)
Methanosarcina mazei	30	0.55			112	-	Batch	O' Flaherty *et al.* (1998)

* Taken from Gujer and Zehnder (1983)

2.2.7 Sulphate Reduction

SRB's have been shown to be able to utilise a large number of substrates as electron donors and carbon sources for reducing sulphate to sulphide. These include hydrogen, volatile fatty acids up to C_{20}, alcohols, several amino acids, monomeric sugars and a large amount of aromatic compounds (Hansen, 1993). However, in the presence of fast growing fermentative bacteria, sulphate reduction of the more complex compounds plays an unimportant role (Kalyuzhnyi and Fedorovich, 1998). Monod kinetic data tend to show that SRB should outcompete acetogenic bacteria and methanogenic bacteria. SRB use sulphate as a terminal electron acceptor and concurrently the SO_4^{2-} is converted to H_2S (Foucher *et al*, 2001). Carbon dioxide as well as additional biomass is formed from the reaction. Figure 2-2 is a representation of the process.

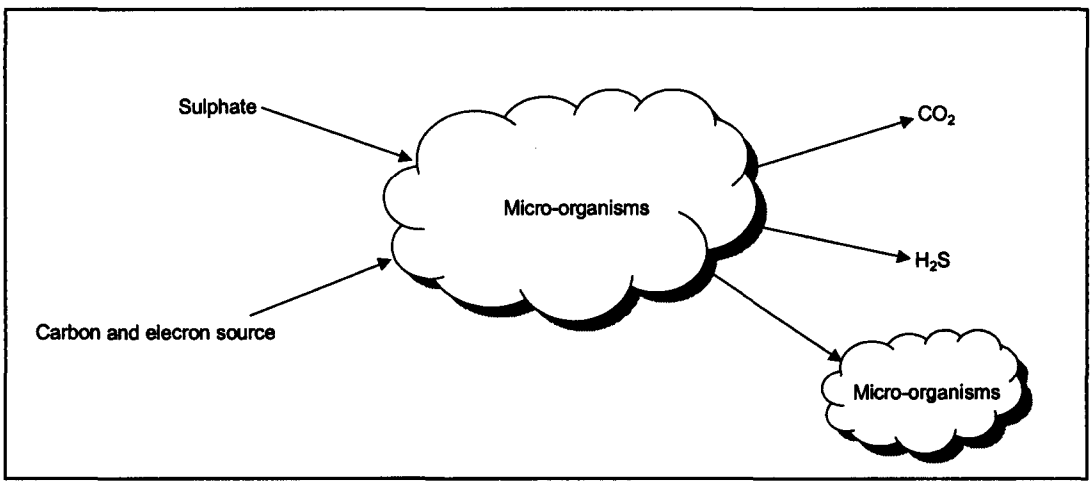

***Figure 2-2**: Representation of the sulphate reduction step.*

The general equation for sulphate reduction as given by Tsukamoto and Miller (1999):

$$4\,AH_2 + SO_4^{2-} + H^+ \rightarrow 4\,A^{2-} + HS^- + H_2O \qquad (2\text{-}19)$$

where AH_2 = carbon source (electron donor)

SO_4^{2-} = terminal electron acceptor

Along with acetate, hydrogen is probably the most important electron donor for sulphate-reducing organisms. While both incompletely and completely oxidising sulphate reducers are able to utilise hydrogen as an electron donor, only the complete oxidisers are capable of true autotrophic growth on H_2/CO_2 (Colleran *et al.*, 1995). Both H_2/CO_2 and acetate are completely oxidised. Propionate can be utilised by both completely and incompletely oxidising sulphate reducers. However thermodynamically, incomplete propionate oxidation is expected to be the preferred pathway and hence, the one of importance. Other compounds such as lactate and butyrate are only partially oxidised. Sulphate reducers compete with methanogens for H_2 and acetate. They also compete with acetogenic bacteria for butyrate and propionate (Oude Elferink *et al.*, 1994).

In order for sulphate reduction to occur, sulphate must be present in sufficient quantities. Under sulphate limiting conditions a reduced rate results. This is usually accounted for by using dual substrate kinetics (Kalyuzhnyi and Fedorovich, 1998):

$$\mu = \mu_{max}\left(\frac{[S]}{K_s + [S]}\right)\left(\frac{[S_{SO_4^{2-}}]}{K_{S,SO_4}^{2-} + [S_{SO_4}^{2-}]}\right) \qquad (2\text{-}20)$$

where [S] = concentration of substrate being oxidised

$[S_{SO_4}^{2-}]$ = sulphate concentration

K_S = half velocity constant for substrate being oxidised

K_{S,SO_4}^{2-} = half velocity constant for sulphate

Table 2-10 presents Monod kinetics for sulphate reduction for various substrates found in literature. O' Flaherty *et al.* (1998) studied the growth kinetics of various bacteria for sulphate reduction. Bromoethane sulphonic acid was added to inhibit methanogenesis. Gupta *et al.* (1994) reported findings on using acetate as a feed for sulphate-reducing bacteria in a CSTR. Maillacheruvu and Parkin (1996) reported the growth kinetics of hydrogen, acetate and propionate utilisers in a batch system. Ghigliazza *et al.* (2000) studied the use of propionate as a carbon source and electron donor for SRB. A fed-batch system was used. Erasmus (2000) did a preliminary

investigation of the kinetics of biological sulphate reduction using ethanol as a carbon source and electron donor.

2.2.8 Sulphate Reduction in Competition with Methanogenesis

In anaerobic reactors treating sulphate-containing wastewaters, both sulphate reduction and methanogenesis can be the final step in the degradation process (Kalyuzhnyi and Fedorovich, 1998). These two bacteria compete for hydrogen and acetate. In essence, it can be seen as if the methanogens are taking away substrate from the SRB and preventing them from carrying out their purpose i.e. to convert sulphates to sulphides. From this point of view it would be desirable to remove methanogens from the system to allow SRB to predominate (Esposito *et al.* 2003).

However, methanogens can play a useful role in the sulphate reduction process. In situations where there is excess hydrogen available, the MPB can reduce the hydrogen partial pressure by using it as a substrate in the production of methane. Fermenters require a low hydrogen partial pressure so that effective fermentation can occur.

Monod kinetics for SRB and MPB indicate that SRB should outcompete MB for hydrogen and acetate due to their greater affinity for the substrate (i.e. lower K_S value) (Kalyuzhnyi and Fedorovich, 1998). This can be seen by comparing K_S values in Tables 2-8, 2-9 and 2-10. Gupta *et al.* (1994) has suggested that the ratios of the respective half velocity constants may be used as an indicator of the length of time it will take one group to become extinct in a CSTR. The closer the ratio is to unity, the longer it will take for one group to become dominant. This prediction has been confirmed when using hydrogen as a substrate (Van Houten *et al.,* 1994; Esposito *et al.*, 2003). For utilisation of acetate the situation is very different. Visser *et al.* (1993) showed that SRB successfully outcompeted MPB for acetate. Others have shown that

Culture	T	μ_{max}	Y	k_d	K_s	$K_{s,SO42-}$	KI,H2S	Conditions	Reference
	(°C)	(d^{-1})	mg biomass/ mg substrate utilised	(d^{-1})	µg H_2/l	mg SO_4^{2-}/l	mg H_2S/l		
Enriched SRB	-	0.18	0.33	0.013	25	-	149	Batch	Maillacheruvu and Parkin (1996)
Desulfovibrio strain G11		1.2-1.6	0.64-0.91	-	5.28-9.24	-	-		Robinson and Tiedje (1984)*
Mixed culture		5	1.232	0.03	3.125	0.9	0.55	Batch	Kalyuzhnyi and Davlyatshina (1997)
Desulfovibrio vulgaris	30	4.22	-	-	4	0.5	-	Batch	O' Flaherty et al. (1998)
Enriched SRB	-	0.11	0.025	0.013	46.7	8.5	-	Batch	Maillacheruvu and Parkin (1996)
Mixed culture	35	-	0.0469	-	0.84	-	-	Continuous	Gupta et al. (1994)
		0.12	0.05	0.005	55	330	-		Omil et al. (1998)
Mixed culture		0.51	0.0438	0.025	22.5	19.2	285	Batch	Kalyuzhnyi and Davlyatshina (1997)
Desulfobacter postgatei	30	0.93	-	-	12	20	-	Batch	O' Flaherty et al. (1998)
Desulfotomaculum acetoxidans	30	1.5	-	-	100	40	-	Batch	O' Flaherty et al. (1998)
Desulfonema magnum	30	0.43	-	-	120	45	-	Batch	O' Flaherty et al. (1998)
Mixed acetoclastic sulphate reducing bacteria	35	1.51	0.58	-	71	-	-	CSTR	Moosa (2000)
Enriched SRB	-	0.11	0.048	-	27.2	-	206	Batch	Maillacheruvu and Parkin (1996)
Mixed culture		0.81	0.0547	0.018	190	7.4	285	Batch	Kalyuzhnyi and Davlyatshina (1997)
Desulfobulbus propionicus	30	2.75	-	-	50	3	-	Batch	O' Flaherty et al. (1998)
Mixe culture	35	0.576	0.18	-	45	38	-	Continuous	Ghigliazza et al. (2000)
Enriched SRB	-	0.11	0.048	-	27.2	-	206	Batch	Maillacheruvu and Parkin (1996)
Desulfovibrio sapovorans	30	1.5	-	-	42	0.55	-	Batch	O' Flaherty et al. (1998)
Desulfococcus multivorans	30	0.35	-	-	70	22	-	Batch	O' Flaherty et al. (1998)
Mixed culture	35	0.273	0.02	-	9.84	284	-	Continuous	Erasmus (2000)

***Table 2-10:** Monod kinetic parameters for sulphate-reducing bacteria.*

MPB successfully outcompeted SRB for acetate (Alphenaar et al., 1993; Rinzema and Schultz, 1987).

To explain the differences found, besides pure bacterial kinetics, the outcome of competition between SRB and MPB for substrate depends on the environmental conditions imposed on the bacteria (O' Flaherty, 1998). O' Flaherty (1998) states that the most important of these conditions are the pH and sulphide concentrations of the reactor mixed liquor. Since SRB and MPB have different pH optima and pH growth ranges, the reactor pH may play an important role in determining which bacterial groups become dominant. McCartney and Oleszkiewicz (1991) found that SRB are more sensitive to an increase to the total sulphide concentrations than methanogens. Maillacheruvu and Parkin (1996) have reported lower sulphide inhibition constants (i.e. will be more inhibited) for both hydrogen and acetate-utilising SRB than hydrogen and acetate-utilising methanogens. Hence, the total sulphide concentration could play a role in the competition between SRB and MPB. Other important considerations that should be considered are (Kalyuzhnyi and Fedorovich, 1998): SO_4^{2-}: COD ratio, sludge retention and nutrient limitation.

The possibility of controlling the competition between sulphate-reducing bacteria and methanogens is important for the practical application of anaerobic treatment processes (Kalyuzhnyi and Fedorovich, 1998). At low substrate conditions, the reactor environment should be setup such that SRB dominate and considerations should be made to optimising the sulphate reduction process.

2.3 Carbon Sources used for the Treatment of AMD

The literature contains extensive lists of electron donors utilised by SRB. A comprehensive list provided by Hansen (1993) is reproduced below in Table 2-11. SRB do not degrade polysaccharides, proteins or lipids but depend on the acidogenic bacteria for the supply of electron donors from these compounds. By using sulphate as an electron acceptor, the SRB can utilise reduced compounds as energy sources. The exception is when carbon monoxide or carbon dioxide is used as the carbon source.

An additional electron donor, usually hydrogen, is then required. In this section, carbon sources which have been reported for use in SRB processes, usually in the presence of fermenters and acidogens, are presented. Where possible performance data is included.

***Table 2-11**: Electron donors and carbon sources used by SRB in the presence of fermentative and acidogenic micro-organisms (Hansen, 1993).*

Class of compound	Type of compound
Aliphatic monocarboxylic acids	Formate, acetate, propionate, butyrate, isobutyrate, 2- and 3-methylbutyrate, fatty acids up to C_{20}, pyruvate, lactate
Dicarboxylic acids	Succinate, fumarate, malate, oxalate, maleinate, glutarate, pimelate
Alcohols	Methanol, ethanol, propanol-1and 2, butanol-1 and 2, isobutanol, pentanol-1, ethylene glycols 1,2- and 1,3- propanediol, glycerol
Amino acids	Glycine, serine, alanine, cysteine, threonine, valine, leucine, isoleucine, aspartate, glutamate, phenolalanine
Sugars	Fructose, glucose, mannose, xylose, rhamnose
Aromatic compounds	>35 known aromatics, including benzoate phenol, indole, resorcinol, catechol, p-cresol, quinoline, nicotinic acid, phenylacetate, vanillin, syringaldehyde, trimethoxybenzoate, etc
Miscellaneous	Very varied group including betaine, choline, furfural, acetone, cyclohexanone, etc
Inorganic compounds	H_2/CO_2

2.3.1 Sewage

Sewage sludge has been shown to be a viable organic source and electron donor for the sulphate reduction process. Maree and Strydom (1985) showed that mine water could be treated in a packed bed reactor using a raw sewage sludge effluent as the organic source. At an inlet sulphate concentration of 1.34 kg m^{-3}, 78% removal was achieved. Using a synthetic effluent supplemented with sewage sludge, Sanchez *et al.*

(1997) achieved 63% sulphate removal in a 13-litre UASB at an inlet sulphate concentration of 0.084 kg m^{-3}. Table 2-12 below summarises the work discussed.

Table 2-12: *Summary of effluents treated using sewage as the organic source and electron donor.*

Reference	Effluent treated	Percent removal of sulphate
Maree and Strydom (1985)	Mine water	78%
Sanchez *et al.* (1997)	Synthetic effluent	63%

2.3.2 Organic Acids

Colleran *et al.* (1994) treated effluent from a citric acid plant in a fixed bed reactor. They achieved 93% removal with an influent sulphate concentration of 3.4 kg m^{-3}. Omil *et al.* (1998) investigated whether sulphate reducers dominate during the treatment of volatile fatty acids. The granular upflow sludge bed reactors were run at 30°C and pH 8. In the reactors fed with volatile fatty acids, under sulphate limiting conditions, no lag phase was evident for sulphate reduction and the extent of sulphate reduction was 40%. When acetate was used, a long lag time was experienced under sulphate limitation and the maximum sulphate removal observed was 70%. In the presence of excess sulphate no lag phase was observed and the maximum removal was 38%. A further observation of this work was that sulphate reducers predominated, after prolonged periods of reactor operation, in chemostats fed with acetate. The results are produced in Table 2-13.

Table 2-13: *Performance of various organic acids for the treatment of sulphate containing wastes.*

Reference	Effluent treated	Organic utilised	Reactor type	Temp.(°C)	pH	Influent sulphate concentration (kg/m^3)	HRT (hrs)	Volumetric reduction rate ($kg/m^3.hr$)
Colleran et al. (1994)	Synthetic	Beet Molasses	USAB(lab)C	-	-	3.43	33.6	0.095
	Synthetic	Beet Molasses	USAB(full)C	-	-	4	33.6	0.112
Omil et al. (1998)	Synthetic	VFA	USAB(20L)C	30	8	1.6	8.8	0.361
	Synthetic	VFA	USAB(0.7L)C	30	8	8.3	6.4	0.783
	Synthetic	Acetic acid	USAB(10L)C	30	8	2.9	7.3	0.28
	Synthetic	Acetic acid	USAB(1.7L)C	30	8	3.5	3.4	0.625

Reactor Types: PBR,Packed Bed Reactor; USAB, Upflow anaerobic sluge bed reactor; C, Continuous

HRT, hydrolyic residence time; VFA, Volatile fatty acids

2.3.3 Complex Organics

Table 2-14 details the complex organic compounds that have been used as the organic and electron source for sulphate reduction. The treatment of mine water using sugar and pulp mill effluents as the organic source was studied by Maree and Strydom (1985) in a packed bed bioreactor. At sulphate concentrations of 2.9 and 1.4 kg m^{-3} in the mine water, 90% and 67% sulphate removal were achieved respectively.

Table 2-14: *Summary of complex organic compounds used as the organic source and electron donor for sulphate reduction.*

Reference	**Complex organic compound**	**Maximum sulphate removal achieved (%)**
Maree and Strydom (1985)	Sugar and pulp mill effluent	90
Maree (1987)	Molasses	92

In further work reported by Maree (1987) molasses was used as an organic source for sulphate reduction. Using a sludge blanket at a retention time of 15 hrs and an inlet sulphate concentration of 2.4 kg m^{-3}, the sulphate removal was 67% at a molasses

concentration of 2 ml l^{-1}. When the molasses concentration was increased to 3 ml l^{-1}, the sulphate reduction increased to 92%. Using a packed bed with dolomite pebbles as bacterial support, the sulphate removal was 42% at a retention time of 20 hrs in the presence of 2 ml l^{-1} molasses as the organic source. The packed bed was very sensitive to changes in organic and sulphate loading rates. When the retention time was decreased to 15 hrs the sulphate removal decreased to 7%. This clearly shows the dependence of the sulphate reduction process on the loading rate. The lower rate observed for the immobilised reactor system is attributed to the fact that SRB do not attach as readily as methanogens.

2.3.4 Hydrogen and Carbon Monoxide/Carbon Dioxide

Sulphate reducers are also able to utilise hydrogen and carbon monoxide as the electron donor and carbon source respectively (van Houten *et al.*, 1994). Du Preez and Maree (1994) found that at flowrates in the range 40 l d^{-1} to 120 l d^{-1} using an 825 l tank, 95% sulphate was removed from a feed containing 2.0 kg m^{-3} sulphate. In batch tests complete sulphate removal was achieved in 80 hrs.

Foucher *et al.* (2001) used H_2 and CO_2 as the energy and carbon source in a continuous fixed bed reactor at a retention time of 0.9 hrs. They achieved a volumetric reduction rate of 0.2 kg m^{-3} hr^{-1}. This value is higher than reported by most other authors. It was felt that the improvement in bacterial efficiency associated with the use of the real effluent is most likely to have been induced by traces of metallic elements.

***Table 2-15:** Summary of H_2 and CO_2 used as the organic source and electron donor for sulphate reduction.*

Reference	Reactor type	Volumetric reduction rate (kg m^{-3} hr^{-1})
Du Preez and Maree (1994)	Continuous fixed bed	0.05
Foucher *et al.* (2001)	Continuous fixed bed	0.2

2.4 Choice of Carbon Source

Various factors have to be considered when choosing a carbon source. To highlight these considerations, the differences between simple and complex organic compounds will be discussed. Table 2-16 represents the differences between simple and complex organic compounds, in terms of a set of criteria. Simple organic compounds need less time for degradation than complex organic compounds. As a consequence of this, the residence time needed for a simple compound is much lower than for a complex compound. This means that a simple compound would require a smaller reactor than a complex compound. This was shown by Omil *et al.* (1998) where they found that acetic acid would have given a better volumetric reduction rate than the mixture of volatile fatty acids under similar conditions.

***Table 2-16**: Differences between simple and complex organic compounds.*

	Simple organic compounds	Complex organic compunds
Time needed for degradation	short	longer
Reactor Size	small	larger
Residual COD after treatment	low	high
Cost of organic	high	low

Assuming sufficient micro-organisms are available, treatment with simple organic compounds leaves very little residual COD, which means that no secondary treatment of the effluent would be needed. However, after treatment with a complex organic compound, there is a high COD content remaining, implying that secondary treatment would be required. This would add to the cost of the process.

The most important factor to consider is that the cost for a simple organic compound would be higher than for a complex organic compound. The reason for this is that complex compounds can be found in the waste streams or effluents of other processes, whereas a simple compound would be synthesised industrially and then transported to site adding to the cost of the product. Hence the location of a nearby waste stream is of great importance. All these factors have to be taken into account when choosing the carbon feedstock to be used for the sulphate reduction process.

2.5 Reactor Design

The choice of reactor plays an important part in the treatment of AMD. The following five conditions have to be met by an anaerobic reactor system (Lettinga, 1995):

- High retention of viable sludge in the reactor under operational conditions;
- Sufficient contact between viable bacterial biomass and wastewater;
- High reaction rates and absence of serious transport limitations;
- The viable biomass should be sufficiently adapted and/or acclimatized; and
- Prevalence of favourable environmental conditions for all required organisms inside the reactor under all imposed operational conditions.

There have been several reactor design studies done to facilitate the anaerobic digestion process. These include:

- Upflow anaerobic sludge bed bioreactor (UASB) (e.g. Elliott *et al.*, 1998);
- Packed bed anaerobic reactors (e.g. Maree, 1987);
- CSTR digesters (e.g. Christensen *et al*, 1996);
- Gas-lift reactors (e.g. Esposito *et al*, 2003);

2.5.1 UASB reactors

The system with the widest application is undoubtedly the UASB reactor (Oude Elferink, 1994). A UASB reactor (Figure 2-3) consists of an influent distribution

system, a gas-solids separator and an effluent draw off facilities. The active biomass in the form of sludge granules is retained in the reactor by direct settling for achieving high retention times and thereby achieving highly cost effective designs. A disadvantage of this system is the long start up period required and significant wash-out of sludge during the initial phase of the process is likely (Rajeshwari *et al*, 2000). Further, at high organic loading rates, poor separation between granular (more settleable) and flocculant (less settleable) sludges may lead to sudden sludge flotation and reactor failure (Kalyuzhnyi *et al*, 1998).

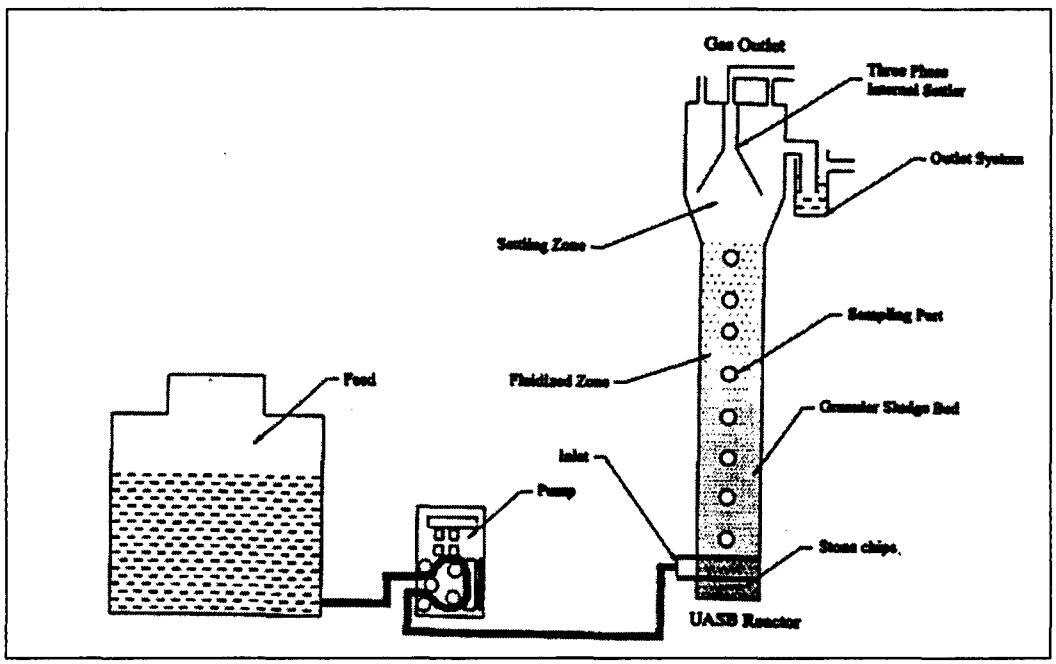

Figure 2-3: Upflow anaerobic sludge blanket reactor (taken from Rajeshwari et al., 2000).

A modification of the UASB is used in the BioPAQ process. A schematic flow diagram of this process is shown in Figure 2-4. This is considered as one of the most sophisticated sulphate reduction processes available. A UASB is used for reduction of sulphate to sulphide using ethanol as a carbon source and electron donor. This is then followed by a fixed film reactor for the conversion of sulphide to sulphur. A tilted plate settler allows the removal of the metal precipitates and other solids present in the system. A sand bed filter is used for the final purification of water prior to discharge.

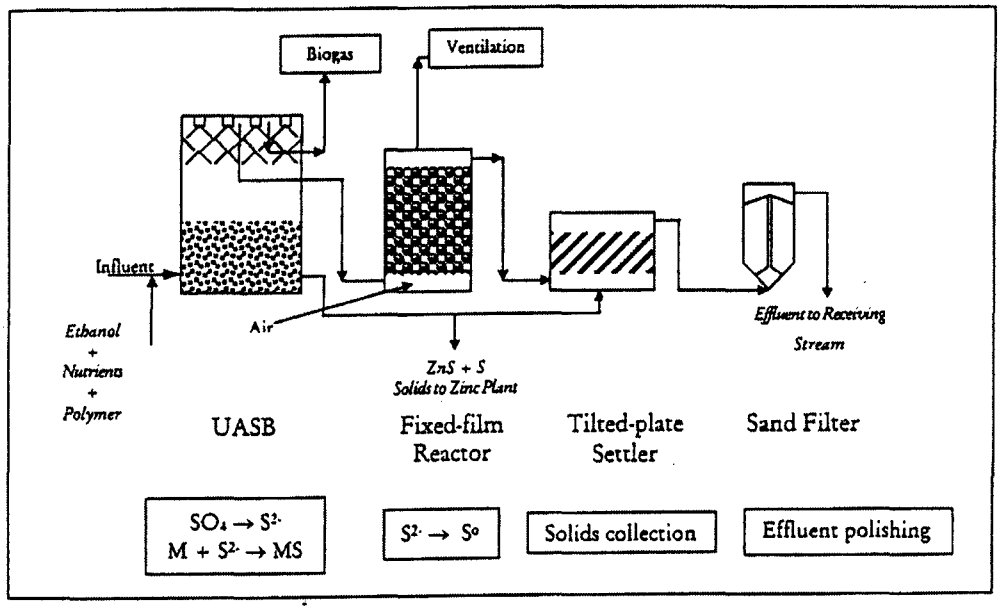

***Figure 2-4**: Schematic flow diagram of the BioPAQ process at the Budelco zinc refinery in the Netherlands (Paques Environmental Technology, 1999).*

The drawback of this system is that metal precipitates as metal sulphides within the anaerobic reactor. This results in a mixed biomass/metal sulphide sludge posing both treatment and dispersal difficulties.

2.5.2 BioSure Process

A schematic of this process is shown in Figure 2-5 (Corbett, 2001). This process uses primary sewage sludge as a carbon source and electron donor. It has two reactors in series. The falling sludge bed reactor (FSBR) followed by an anaerobic baffled reactor. In the FSBR, the mine water is mixed with primary sewage sludge. The primary role of FSBR is the hydrolysis of primary sewage sludge to short chain organics. The primary role of the baffled reactor is sulphate removal. The water is then pumped to algal ponds for precipitation of heavy metals where the algae consume residual COD.

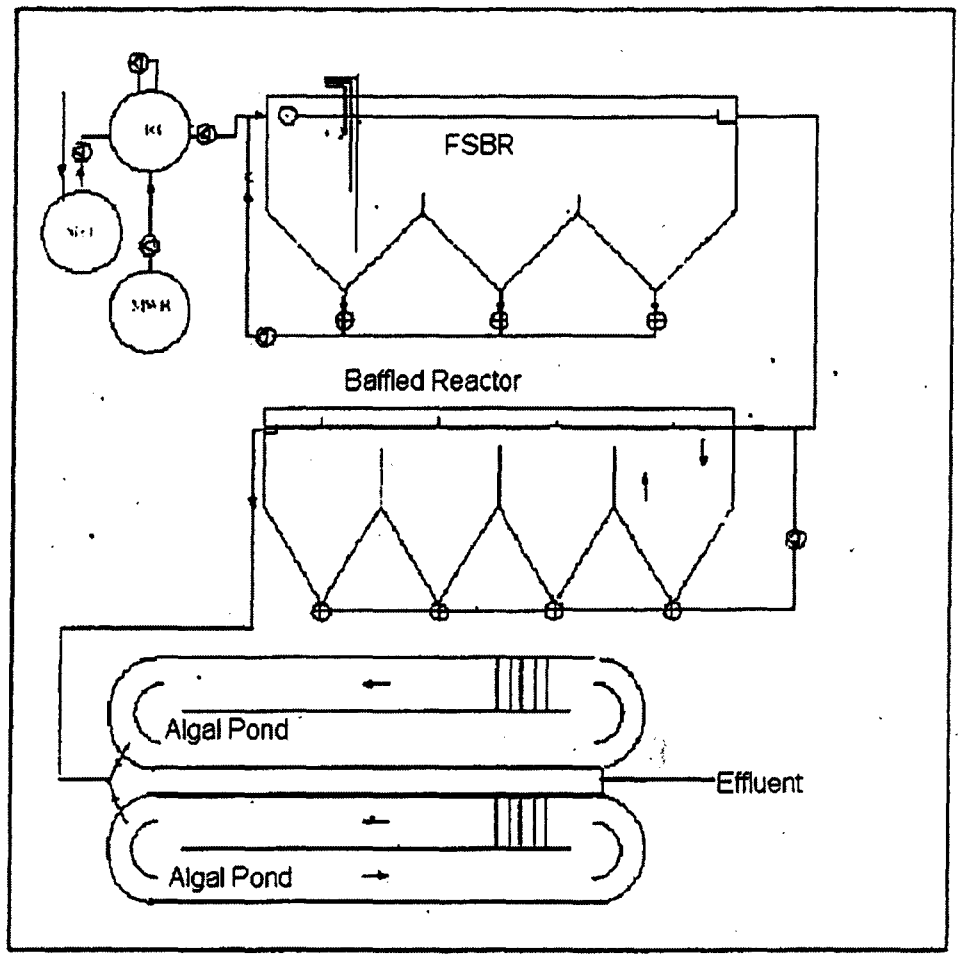

Figure 2-5: Schematic representation of the Rhodes BioSure Process (Corbett, 2001).

2.5.3 Continuously Stirred Reactors

Figure 2-6 is a schematic of an ideally continuous stirred tank reactor (CSTR). The substrate and the high sulphate stream enter the reactor via different streams. The contents are mixed. If the reactor is assumed to be ideally mixed then the concentration of the effluent exiting the reactor is assumed to have the same concentration as the liquid in the tank.

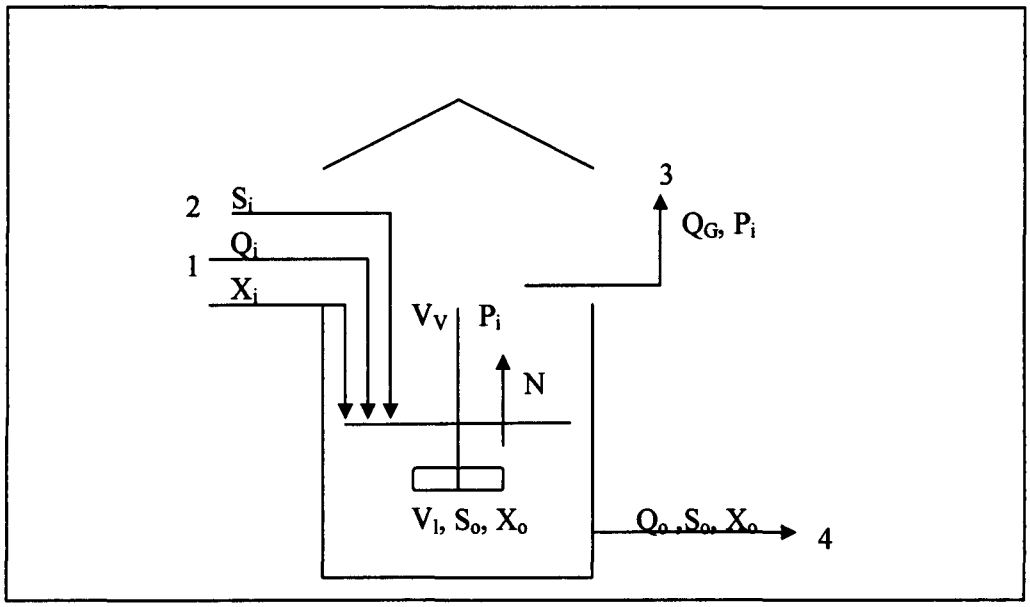

***Figure 2-6**: Diagram of an ideal continuous stirred tank reactor (two phase).*

No full-scale continuously stirred reactor was found in the literature. This is due to most systems being based on the concept of retaining high viable biomass by some mode of bacterial sludge immobilization (Rajeshwari *et al.*, 2000) to ensure cost effective systems. However, the use of CSTR's has been proven at lab scale (e.g. Gupta, 1994 a and b, Ghigliazza, 2000 and Erasmus, 2000). It provides a well defined environment for the study of sulphate reduction kinetics and for the modelling of the biological sulphate reduction process. Hence it provides a platform for expansion of process understanding.

2.6 Summary

The literature review started with the characterisation, formation and the impact of AMD. The impact of AMD on South Africa and the current methods of treatment were also presented. The literature review then moved into the general degradation process, starting with hydrolysis and ending with methanogenesis and sulphate reduction. Where possible, kinetic data has been supplied. A list of carbon sources that have been used as well as factors governing the choice of carbon source was presented. Finally, reactor types available for anaerobic digestion of AMD have been reviewed.

Chapter 3

MASS BALANCE METHODOLOGY

In this chapter, the general methodology of the mass balance is presented. Included in this section are:

- the flowsheet used,
- the design for each of the units,
- assumptions used in the mass balance, and
- sizing of each of the units.

3.1 The Process Flowsheet

A schematic of the process used in this study is presented as Figure 3-1. The process consists of

- three holding tanks, one for the AMD storage, one for substrate storage, and one for hydrochloric acid,
- two continuously stirred reactors, one anaerobic and one aerobic,
- a mixer in which a buffer is added to lower the pH, and
- two settlers, one to clarify the water by separating the biomass from it and the other to remove sulphur from the system,

It is assumed that the metals in the AMD stream (stream 1) are precipitated upstream of the holding tank. The precipitation unit is not accounted for as it falls outside the scope of this study. The mass balance is performed using a basis of 1000 $m^3 day^{-1}$ of AMD as feed into the system. The quantity of organic substrate (ethanol, molasses or sludge) used is based on the amount needed to produce a water quality level that will conform to acceptable EPA (Environmental Protection Agency, 2000) levels, defined as a residual concentration of sulphate and sulphide of 250 and 10 mg l^{-1} respectively. The levels of total dissolved solids and sulphate for reuse of water as outlined by the EPA, DWAF (Department of agriculture and Forestry, 1996) and NEA (National Environmental Agency, 2004) are presented in Table 3-1.

***Table 3-1**: Industrial effluent standards for reuse of water.*

Constituent	**pH**	**Total dissolved solids (mg l^{-1})**	**Sulphate (mg l^{-1})**
U.S. Environmental Protection Agency (2000)	6.5-8.5	500	250
South African Department of Water Affairs and Forestry (1996)	6-9	450	200
Singapore National Environmental Agency (2004)	6-9	2000	500

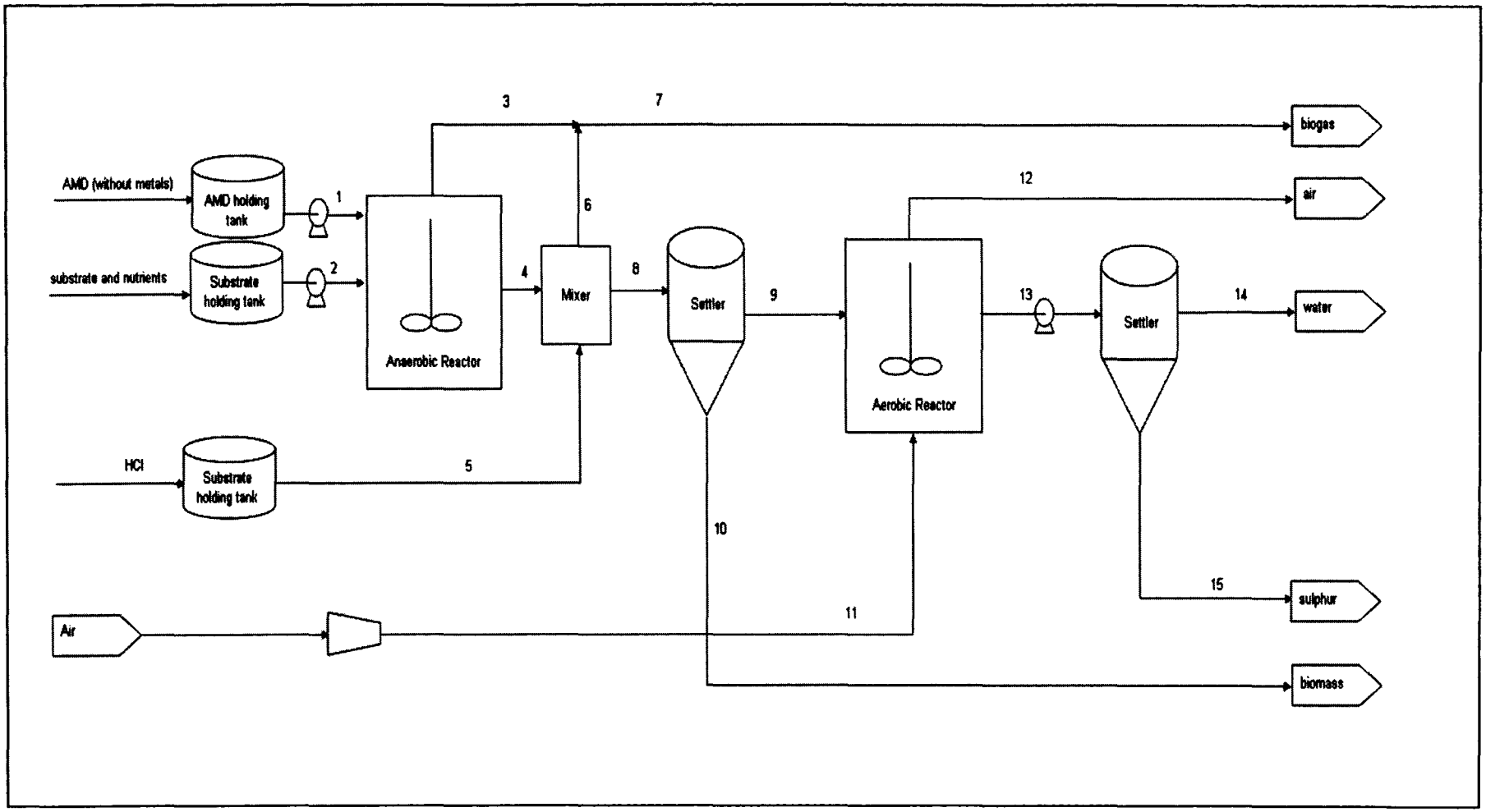

***Figure 3-1**: A schematic representation of the biological treatment process used in this study.*

3.2 Composition of Organic Substrates

Based on their availability to the Southern African mining industry, three carbon sources have been selected for evaluation as electron donors for AMD treatment. These are:

- ethanol
- molasses
- primary sewage sludge

3.2.1 Ethanol

Low grade ethanol obtained from Triangle Solvents (2004) has a 92% mass content of ethanol. Impurities in the ethanol could include a maximum of 8.0% iso-Propanol and 0.4% water content.

3.2.2 Molasses

The United States Sugar Corporation (2004) gave a sugar content of 48.3% for cane molasses. Sucrose accounted for 36% of the molasses whereas fructose and glucose accounted for 5.6% and 2.6% respectively. It was assumed that the sugar content was the primary fermentative component present in the molasses. The unaccounted 4.1% of sugars is assumed not to be fermentable. The unaccounted 51.7% of the molasses was reported as 23.5% water, 6.3% proteins, 16% ash, 4.2% potassium and the remainder as 1.2% as various other metals and salts. The 6.3% proteins were accounted for in the mass balance; however the water, ash and potassium were not. The amount of water contained in the molasses can be considered negligible when compared to the influent water flow rate. The amount of ash and potassium is also low enough to be considered as having a negligible effect on the system. The program developed for this study does not account for sucrose as a component; however each mole of sucrose can be modelled as two moles of glucose as shown in the Equation 3-1, based on enzymatic conversion in the presence of invertase and glucose isomerase (Vu *et al.*, 1995).

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow 2C_6H_{12}O_6 \qquad (3\text{-}1)$$

3.2.3 Primary Sewage Sludge

The composition for primary municipal sludge given by Eastman and Ferguson (1981) was used for this project and is shown in Table 3-2. This has subsequently been confirmed by Ristow (1999). Work done by Ristow *et al.* (2004) showed that primary sewage sludge has a 33.45% undegradable fraction.

Table 3-2: *The composition of primary municipal sludge as given by Eastman and Ferguson.*

Component	Mole Percentage
Proteins	52
Carbohydrates	25.4
Lipids	5.4
Acetate	17.2

3.3 Anaerobic Reactor Specification

For the purposes of this work the reactor design used was a CSTR. This choice was based on using a simple reactor system to compare the organic substrates. The operating conditions of this reactor were set at standard temperature and pressure of 25°C and 1 atm and the pH was set at 7.41. This pH value was chosen as it is in the middle of the optimum pH range for sulphate reduction, which is 7.0-7.8 (Visser, 1995, cited in Knobel, 1999). A diagrammatic representation of the reactor is shown as Figure 2-6. The reactor has two feed streams (streams 1 and 2) and two outlet streams (streams 3 and 4). Stream 1 is the AMD feed stream to the reactor and stream 2 the organic carbon feed. Stream 3 is the biogas outlet stream, and can consist of gaseous hydrogen, methane, carbon dioxide and hydrogen sulphide. The gas component (stream 3) is dependent on which organic source is used in stream 2. Stream 4 is the liquid effluent from the reactor and is sent to the settling tank for separation. The volume of the reactor was set at 10 000 m^3 to provide a reactor

residence time of 10 days. The high residence time was chosen to accommodate the slow rates of reaction for hydrolysis. For the sake of continuity, this residence time was maintained for each of the substrates.

The components found in the reactor can be divided into the following groups:

- Insoluble components suspended in the liquid phase,
- Non-dissociating soluble components in the liquid phase,
- Dissociating soluble components in the liquid phase, and
- Components reporting to the gas phase

These components are listed in Table 3-3 to Table 3-6. Table 3-7 represents the microbial groups present in the reactor. The stoichiometric equations and rate constants used in the modelling of the reactor can be found in Appendix A.

Table 3-3: *Insoluble components in the liquid phase.*

Generic insoluble proteins and dead biomass	$C_5H_7O_2N$	Assumed
Generic insoluble carbohydrates	$C_6H_{10}O_5$	Angelidaki *et al.* (1999)
Generic insoluble lipids	$C_{51}H_{98}O_6$	Assumed
Sulphur	S^o	Assumed

Table 3-4: *Non-dissociating soluble components in the liquid phase.*

Generic amino acids	$C_5H_9O_3N$	Assumed
Glucose (representing mono and disaccharides	$C_6H_{12}O_6$	Angelidaki *et al.* (1999)
Glycerol	$CH_2OHCHOHCH_2OH$	Angelidaki *et al.* (1999)
Palmitic acid	$CH_3(CH_2)_{14}COOH$	Gujer and Zehnder (1983)

Table 3-5: *Dissociating soluble components in the liquid phase.*

Hydrogen	H_2 and H^+	Costello *et al.*, 1991
Hydrogen sulphide	H_2S, HS^- and S^{2-}	Costello *et al.*, 1991
Sulphate species	H_2SO_4, HSO_4^- and SO_4^{2-}	Costello *et al.*, 1991
Acetic acid	CH_3COOH and CH_3COO^-	Costello *et al.*, 1991
Lactate	$CH_3CHOHCOOH$ and $CH_3CHOHCOO^-$	Costello *et al.*, 1991
Propionate	CH_3CH_2COOH and $CH_3CH_2COO^-$	Costello *et al.*, 1991
Butyrate	$CH_3CH_2CH_2COOH$ and $CH_3CH_2CH_2COO^-$	Costello *et al.*, 1991
Carbon dioxide	CO_2	Costello *et al.*, 1991
Carbonic acid	H_2CO_3, HCO_3^- and CO_3^{2-}	Kalyuzhnyi and Fedorovich (1998)
Methane	CH_4	Costello *et al.*, 1991
Ammonia	NH_3 and NH_4^+	Angelidaki *et al.* (1999)
Ethanol	C_2H_5OH	Erasmus (2000)

Table 3-6: *The components that are used in the gas phase.*

Hydrogen	H_2	Costello *et al.* (1991)
Hydrogen Sulphide	H_2S	Kalyuzhnyi and Fedorovich (1998)
Methane	CH_4	Costello *et al.* (1991)
Carbon Dioxide	CO_2	Costello *et al.* (1991)

Table 3-7: The microbial groups used in the reactor.

Fermenters
Glucose utilising fermenters (gFER)
Amino acid utilising fermenters (aFER)
Glycerol utilising fermenters (glyFER)
Lactate utilising fermenters (lFER)
Beta oxidising bacteria (BOB) utilizing long chain fatty acids
Acetogens
Butyrate utilising acetogens (bACE)
Propionate utilising acetogens (pACE)
Methanogens
Hydrogen utilising methanogens (hMPB)
Acetate utilising methanogens (aMPB)
Sulphate reducers
Hydrogen utilising sulphate reducers (hSRB)
Acetate utilising sulphate reducers (aSRB)
Lactate utilising sulphate reducers (lSRB)
Propionate utilising sulphate reducers (pSRB)
Butyrate utilising sulphate reducers (bSRB)
Ethanol utilising sulphate reducers (eSRB)

3.3.1 Anaerobic Reactor Mass Balance

In order to carry out the mass balance, various assumptions were made. The assumptions are as follows:

- Gas and liquid phase of the reactor are ideally mixed.
- The liquid flow rate entering the reactor is constant.
- The gas-side mass transfer resistance is negligible. This is generally true for gases of low solubility like hydrogen and hydrogen sulphide (van Houten *et. al.*, 1994).
- External mass transfer limitations (i.e. around biofilms or granules) are neglected. With an external mass transfer film thickness typically in the order of 10 μm for aqueous systems, this assumption usually will be valid (van Houten *et. al.*, 1994).
- The ideal gas law will hold.

The mass balance can loosely be divided into two sections: liquid phase and gas phase. For the liquid phase, the general mass balance is as follows:

$$V_L \frac{dC_{j,Lout}}{dt} = Q_{L,in} C_{j,Lin} - Q_{L,out} C_{j,Lout} + k_L a \left(\frac{C_{j,Gout}}{m} - C_{j,Lout} \right) V_L + R_j V_L \qquad (3\text{-}2)$$

where	V	=	Reactor volume (m^3)
	C_j	=	Concentration of species j ($mol.m^{-3}$)
	Q	=	flow rate ($m^3.s^{-1}$)
	k_L	=	liquid side mass transfer coefficient ($m.s^{-1}$)
	a	=	interfacial area of gas-liquid contact ($m^2.m^{-3}$)
	m	=	gas/liquid solubility coefficient (unitless)
	R_j	=	rate of reaction of component j ($mol.m^{-3}.s^{-1}$)
Subscripts	G	=	gas phase
	L	=	liquid phase
	in	=	incoming stream
	out	=	outgoing stream
	j	=	component j

The flux term can simply be written as:

$$k_L a \left(\frac{C_{j,Gout}}{m} - C_{j,Lout} \right) = N_j \qquad (3\text{-}3)$$

Assuming that $Q_{in} = Q_{out}$, the equation for the liquid phase mass balance can then be simplified to:

$$\frac{dC_{j,Lout}}{dt} = \frac{Q_{in}}{V_L}\left(C_{j,Lin} - C_{j,Lout}\right) + R_j + N_j \qquad (3\text{-}4)$$

The general mass balance for the component j over the gas phase is:

$$V_G \frac{dC_{j,Gout}}{dt} = Q_{G,in}C_{j,Gin} - Q_{G,out}C_{j,Gout} - k_L a\left(\frac{C_{j,Gout}}{m} - C_{j,Lout}\right)V_L + R_j V_G \qquad (3\text{-}5)$$

For systems using a complex organic source as a carbon and electron donor, there is no gas feed to the process (i.e. $Q_{G,in} = 0$). Examples of gaseous sources of carbon and electron donor are the CO/H_2 and CO_2/H_2 systems. Since no reactions are assumed to be occurring in the gas phase, R_j is set as zero. Hence, the mass balance over the gas phase in systems using dissolved carbon sources is written as:

$$V_G \frac{dC_{j,Gout}}{dt} = -\frac{Q_{G,out}}{V_G}C_{j,Gout} - N_j \frac{V_L}{V_G} \qquad (3\text{-}6)$$

Henry's Law states that:

$$H_j = \frac{p_j}{C_j} \qquad (3\text{-}7)$$

where H_j = Henry's Law constant for component j ($atm.m^3.mol^{-1}$)

p_j = Partial pressure of component j (atm)

Using Henry's law (Equation 3-7) and multiplying the gas phase mass balance (Equation 3-6) by H_j gives Equation 3-8:

$$\frac{dp_{j,Gout}}{dt} = -\frac{Q_{G,out}}{V_G} p_{j,out} - N_j \frac{V_L}{V_G} H_j \qquad (3\text{-}8)$$

Assuming that the ideal gas law given in Equation 3-9 holds, it can be rearranged to give Equation 3-10:

$$p_j V = n_j RT \qquad (3\text{-}9)$$

where n_j = number of moles of component j (mols)

R = universal gas constant (m^3.atm.mol^{-1}.K^{-1})

T = temperature of the system (K)

$$p_j = \frac{n_j}{V} RT = C_j RT \qquad (3\text{-}10)$$

$$\frac{p_j}{C_j} = H = RT \qquad (3\text{-}11)$$

Substituting Equation 3-11 into the gas phase mass balance, given in Equation 3-8, yields:

$$\frac{dp_{j,Gout}}{dt} = -\frac{Q_{G,out}}{V_G} p_{j,out} - N_j \frac{V_L}{V_G} RT \qquad (3\text{-}12)$$

where at steady state

$$Q_{G,out} = V_L RT \sum N_i \qquad \text{at 1 atm} \qquad (3\text{-}13)$$

For the cases where a gaseous feed is used to provide the carbon and electron donor source, $Q_{G,in}$ cannot be made to equal zero. The resultant gas phase mass balance equation is written as:

$$\frac{dP_{j,Gout}}{dt} = \frac{Q_{G,in}P_{j,in} - Q_{G,out}P_{j,out}}{V_G} - N_j \frac{V_L}{V_G} RT \qquad (3\text{-}14)$$

3.3.2 Equilibrium Calculation

Most of the microbial reactions are inhibited by undissociated components of hydrogen sulphide and volatile fatty acids e.g. methanogenesis is inhibited by undissociated hydrogen sulphide (Maillacheruvu and Parkin, 1996). Hence, to account for the inhibition caused by these components, it is necessary to calculate the undissociated concentration of these components. The following simple generic equation is used to represent the acid dissociation reaction:

$$HA \Leftrightarrow A^- + H^+ \qquad (3\text{-}15)$$

where H = hydrogen
A = acid compound

Acid and base ionization reactions are assumed to be in equilibrium. A detailed list of the acid and base ionization reactions can be found in Appendix A. The state of dissociation is calculated from an activity based expression, given in Equation 3-16:

$$K_a = \frac{(\gamma_{A-}C_{A-})(\gamma_{H+}C_{H+})}{\gamma_{AH}C_{AH}} \qquad (3\text{-}16)$$

where γ_i = activity coefficient of species i
K_a = acid dissociation constant (mmole.l^{-1})

The activity coefficient is calculated from the Davies equation (Van Haandel and Lettinga, 1994, cited by Knobel and Lewis, 2002)

$$\log_{10}(\gamma_i) = -Az_i^2\left[\frac{\sqrt{I}}{1+\sqrt{I}} - 0.3I\right] \quad (3\text{-}17)$$

where A = constant
z = ionic charge
I = ionic strength

The temperature dependency of A is given as:

$$A = 1.82\text{x}10^6(78.3\text{T})^{-1.5} \quad (3\text{-}18)$$

where T = temperature (K)

The ionic strength of the solution is calculated from:

$$I = \frac{1}{2}\Sigma\, C_i z_i^2 \quad (3\text{-}19)$$

These equations, together with the charge balance can be solved to calculate the amount of each component of each species in the reactor system.

3.3.3 Using MATLAB as an Analytical Tool for the Anaerobic Reactor Design

The development of the model for the reactor was based largely on the work of Knobel (1999). Knobel had completed the reactor model in OCTAVE whereas this model was developed in MATLAB. The protocol setup in MATLAB to solve the mass balance of the reactor is presented in Figure 3-2. The equations used in the model have been presented in Appendix A. The user inputs into the program can be seen in Figure 3-2. A detailed list of the constants used in the model (sourced from the literature) and a listing of the computer code can be found in the Appendix B and C respectively.

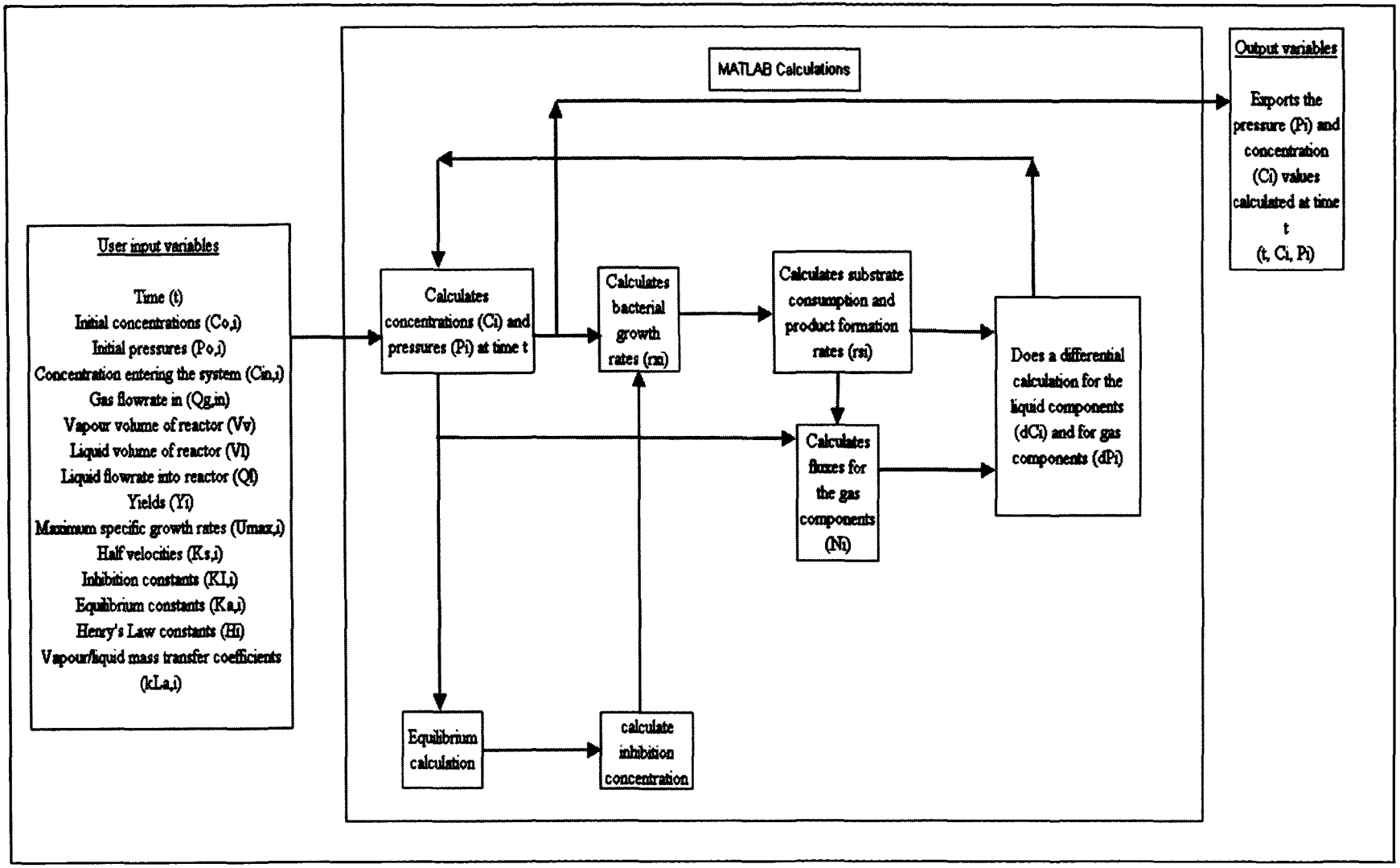

Figure 3-2: Schematic representation of the calculations performed by MATLAB.

Using these inputs, a MATLAB program has been constructed to calculate the concentrations of each component as well as the pressure for each of the biogas components. The MATLAB programme then used the equations from the Section 3.3.2 to calculate the inhibitor concentrations in the system. The pH, calculated concentrations and pressures as well as the user inputs were then used to calculate the substrate consumption rates and product formation rates of the various components i.e. the rates of formation and consumption of the components (Section 2.2). The fluxes of the gaseous components into and out of the liquid phase were also calculated at this point (Equation 3-3). Once these values were all known, the MATLAB programme used the differential form of the material balance equations (Equations 3-4 and 3-12) to calculate the new concentrations of the components specified as well as the partial pressures of the biogas components. The process then repeated itself with the new concentrations being used as the start concentrations. This process was repeated until the final time was reached. For each iteration of the MATLAB programme, the values for the concentrations and pressures calculated were exported from the program as output variables.

3.4 Aerobic Reactor

The purpose of the aerobic reactor was to convert the sulphide that was formed in the anaerobic reactor to elemental sulphur. The excess sulphide not sent for metal precipitation can thus be easily removed as insoluble sulphur from the system through solid-liquid separation. Stream 9, which has a high concentration of hydrogen sulphide, enters the reactor. The reactor liquid exit stream (stream 13) has a much lower concentration of hydrogen sulphide and a high concentration of sulphur. Again, a simple CSTR was employed to allow for a simple comparison amongst the various carbon sources.

The reaction for the production of elemental sulphur is:

$$2\ H_2S + O_2 \rightarrow 2\ S^o + 2\ H_2O \qquad (3\text{-}20)$$

Kuhn *et al.* (1983) suggested that the oxidation of sulphide along the oxidation chain shown in Figure 3-3 depended on the ratio of molecular oxygen and sulphide concentrations. At higher levels of O_2, H_2S is completely oxidised to produce SO_4^{2-}.

$$H_2S \xrightarrow{O_2} S^o \xrightarrow{O_2} S_2O_3^{2-} \xrightarrow{O_2} SO_3^{2-} \xrightarrow{O_2} SO_4^{2-}$$

***Figure 3-3**: Schematic representation of possible valence states of sulphur in aqueous media (Kuhn et al., 1983, cited in Mamashela, 2002).*

It is assumed for the purposes of this project that enough O_2 is added to system for the production of S^o and that the concentration of O_2 would be low enough so that the sulphur will not further oxidise along the chain. The operating conditions for the reactor will be at standard temperature and pressure of 25°C and 1 atm with the pH at 7.05. Chen and Morris (1972, cited in Mamashela, 2002) had found the pH maxima to be around a pH 7.00.

The oxygen content needed in the reactor is calculated using Equation 3-20. Air enters the reactor from the atmosphere via a compressor. Air has a composition of 21% oxygen and 79% nitrogen. Work done by Sublette (1989) found that the composition of the oxygen exiting the reactor was at 20.9%. This was confirmed by unpublished data at the University of Cape Town. Air enters the reactor via stream 11 and exits through stream 12.

3.4.1 Aerobic Reactor Mass Balance

In order to perform the reactor mass balance, the following assumptions were made:

- Reactor is perfectly mixed i.e. the concentration in the reactor is the same as the concentration exiting the reactor.
- The liquid flow rate entering the reactor is constant.
- Bacteria follows Monod kinetics

The general mass balance equation (Equation 3-2) is reproduced below:

$$V_L \frac{dC_{j,Lout}}{dt} = Q_{L,in} C_{j,Lin} - Q_{L,out} C_{j,Lout} + k_L a \left(\frac{C_{j,Gout}}{m} - C_{j,Lout} \right) V_L + R_j V_L \qquad (3\text{-}2)$$

When considering the biomass mass balance, biomass is not continuously added to the system and hence $C_{j,Lin}$ is set to zero. Also, the system is continuous and in steady state, thus $\frac{\partial Cj, Lout}{\partial t}$ is set to zero. There is no flux term for bacteria, hence $k_L a \left(\frac{C_{j,Gout}}{m} - C_{j,Lout} \right)$ is also set to zero. Equation 3-2 can now be simplified to:

$$QC_X + r_X V = 0 \qquad (3\text{-}21)$$

where x = biomass

The growth rate of bacteria (μ) is defined as:

$$\mu = \frac{r_X}{C_X} \qquad (3\text{-}22)$$

The dilution rate (D) is defined as flow rate over volume:

$$D = \frac{Q}{V} \qquad (3\text{-}23)$$

Substituting Equations 3-20 and 3-21 into 3-19 yields:

$$C_X(\mu - D) = 0 \qquad (3\text{-}24)$$

Since C_x can not equal zero, it follows that:

$$\mu = D \qquad (3\text{-}25)$$

Assuming the growth rate follows the Monod Equation:

$$\mu = \frac{\mu_{max} C_S}{K_S + C_S} = D \tag{3-26}$$

where S = substrate

Rearranging Equation 3-26 and solving for C_S produces:

$$C_S = \frac{DK_S}{\mu_{max} - D} \tag{3-27}$$

Hence, the concentration of substrate leaving the reactor is dependent on the dilution rate, the maximum growth rate of the bacteria and the half velocity constant.

Starting with Equation 3-2 and performing a substrate mass balance results in:

$$V\frac{\partial C_S}{\partial t} = QC_{Sf} - QC_S + r_S V = 0 \tag{3-28}$$

where C_{Sf} = the initial substrate concentration

The substrate reaction rate is related to the biomass reaction rate via the yield coefficient, which is defined as:

$$\frac{1}{Y_{SX}} = -\frac{r_S}{r_X} \tag{3-29}$$

Substituting Equation 3-29 and Equation 3-23 into 3-28:

$$DC_{sf} - DC_s - \frac{r_X}{Y_{S/X}} = 0 \tag{3-30}$$

Rearranging 3-22 and substituting Equation 3-25:

$$r_X = \mu C_x = DC_x \tag{3-31}$$

Substituting Equation 3-31 into Equation 3-30 and rearranging:

$$C_x = Y_{S/X}(C_{sf} - C_s) \tag{3-32}$$

The substrate concentration exiting the reactor can be calculated from Equation 3-27 and the biomass concentration from Equation 3-32.

Kinetic data was taken from Mamashela (2002) who used a mixed culture to convert sulphide to elemental sulphur. The kinetic data found by Mamashela (2002) is produced in Table 3-8.

Table 3-8: *Kinetic constants for a mixed culture converting sulphide to sulphur.*

Variable	Value	Units
Ks	2.941	mmole l^{-1}
Y	0.0512	mmole $mmole^{-1}$
μ_{max}	1.272	day^{-1}

Applying this data to Equation 3-27 and setting the exit substrate concentration to 8.5 mg l^{-1}, a dilution rate of approximately 10 days is calculated. The volume of liquid entering the reactor is almost 1000 m^3, hence the volume of the tank will be 10000 m^3.

3.5 Mixer

The mixer was placed between the two reactors with the intention of lowering the pH of the effluent from the anaerobic reactor from 7.41 to 7.05 by the addition of hydrochloric acid (stream 5). This is done to achieve optimal conditions for the aerobic reactor. The liquid enters the mixer via stream 4. The liquid exits the mixer

via stream 8. The lowering the pH causes some of the hydrogen sulphide in the liquid to enter the gas phase. The hydrogen sulphide in the gas phase (stream 6) then joins the biogas produced from the anaerobic reactor (stream 3). The combined gas stream (stream 7) is then taken to the precipitation unit where it is used to remove metals from the AMD stream.

To calculate the amount of H_2S converted to gas in the mixer, the amount of H_2S and its species in the liquid needs to be known. Applying Equation 3-16 for the two dissociating reactions for H_2S and rearranging results in:

$$\frac{[H^+]}{K_{s1}} = \frac{[H_2S]}{[HS^-]} \tag{3-33}$$

$$\frac{K_{s2}}{[H^+]} = \frac{[S^{2-}]}{[HS^-]} \tag{3-34}$$

The total species balance for H_2S is:

$$[H_2S]_t = [H_2S] + [HS^-] + [S^{2-}] \tag{3-35}$$

Dividing by $[HS^-]$ and substituting Equations 3-33 and 3-34 into 3-35 results in:

$$\frac{[H_2S]_t}{[HS^-]} = 1 + \frac{[H^+]}{K_{s1}} + \frac{K_{s2}}{[H^+]} \tag{3-36}$$

Solving for Equation 3-36 for $[HS^-]$:

$$[HS^-] = \frac{[H_2S]_t}{1 + \frac{[H^+]}{K_{s1}} + \frac{K_{s2}}{[H^+]}} \tag{3-37}$$

Thus $[H_2S]$ and $[S^{2-}]$ can be solved:

$$[H_2S] = \frac{[HS^-][H^+]}{K_{s1}} \tag{3-38}$$

$$[S^{2-}] = \frac{K_{s2}[HS^-]}{[H^+]} \tag{3-39}$$

The concentrations of the H_2S species in the liquid are described by Equations 3-37 to 3-38. Hence the concentration of H_2S in the liquid at both pH levels can be calculated. The amount of gas leaving the system is then calculated using a modified version of Equation 3-3.

$$N = -k_L a\left(\frac{P_i}{H_i} - C_i\right) \tag{3-40}$$

To calculate the amount of HCl that is needed to lower the pH, the amount of H^+ ion needed to be calculated. The equilibrium reactions that are affected can be found in Appendix A.

The concentrations of each of the species at both pH values are calculated in a similar manner as that of H_2S (Equations 3-31 to 3-37). The equations above show that for each mole of ion that is converted, one mole of H^+ is required. H^+ is obtained by the dissociation of hydrochloric acid. Hydrochloric acid is a strong acid and can be assumed to completely dissociate by Reaction 3-41.

$$HCl \Rightarrow H^+ + Cl^- \tag{3-41}$$

The reaction shows that for each mole of HCl added, one mole of H^+ is produced. Hence it follows that for each mole of ion that is converted; one mole of HCl is required.

The mixer was sized to be 1 m^3. This value is based on the assumption that the change in pH will occur almost instantaneously. Hence a residence time of 86.4 seconds suffices.

3.6 Settling Tank

A settling tank employs energy dissipating devices and gravity settling principles at reduced flow conditions to induce water-solid separation. Two settling tanks are employed in this system (Figure 3-1). The first settler is placed after the anaerobic reactor. Its purpose is to lower the high concentrations of the insoluble components (Table 3-3) leaving the anaerobic reactor. By removing the solids from the water, the settler also thickens the sludge. This is important since it was proposed that the sludge be sent to a landfill. Thickening the sludge reduces the landfill capacity needed, thus lowering the operating cost of the process. The second settler is placed after the aerobic reactor. This settler serves primarily to remove the sulphur from the effluent stream. Rossle and Pretorius (2001) presented a review of the characterisation requirements for in-line prefermenters used in water care works with primary sludge. Typical values of removal efficiencies for a settling tank, taken from Rossle and Pretorius (2001), are presented in Table 3-9.

Sincero and Sincero (1996) gave typical removal efficiencies for suspended solids in settling tanks as a function of overflow rate. This is presented in Table 3-10.

***Table 3-9:** Performance of a typical settling tank (Rossle and Pretorius, 2001)*

Function	Performance Level
Removal of settleable solids	90-95%
Removal of suspended solids	50-80%
Removal of COD	30-50%
Removal of total Kjeldahl nitrogen and total phosphorus	15-25%

***Table 3-10**: Typical removal efficiency of primary settling tanks (Sincero and Sincero, 1996)*

Overflow rate	Percent Removal
20	70
30	65
40	58

Ristow *et al.* (2004) tested the settleability of primary sewage sludge after it had gone through digestion in a sulphate-reducing system and a methanogenic system. Effluent from the digesters were placed in a 500 ml measuring cylinder and allowed to settle for 30 min. A sample was drawn 8 cm from the liquid surface after the 30 min. The height where the sample was drawn was always above the height of the settled solids zone. The authors had found that 29.0 ± 6.7 % of the total effluent particulate COD concentration of the methanogenic system had remained suspended and 55.9 ± 0.6 % had remained for the sulphate-reducing system. Hence, 71% and 44% of the particulate COD would settle in the methanogenic and sulphate-reducing systems respectively.

Based on the three studies presented above, it is tentatively assumed that primary sewage sludge would have a 65% removal rate and sludge produced from the molasses and ethanol systems would have a 44.1% removal rate. It is assumed for the purposes of this study that the insoluble components as presented in Table 3-3, with the exception of sulphur, will have an equivalent removal efficiency to the particulate COD. It is also assumed that biomass from the anaerobic reactor will settle at the same rate as sewage sludge exiting the anaerobic reactor.

The Paques process has a removal efficiency of 90% for sulphur (Rein, 2004). However this requires the addition of $FeCl_3$ as a coagulant as well as a flocculant. Janssen *et al.* (1999) found a removal efficiency of 90% of sulphur at a velocity greater than 25 m h^{-1}. Biologically produced sulphur is hydrophilic and the buoyant density of S^0 produced by *Chromatium* has been determined to be 1.22 gcm^{-3} (Janssen *et al.*, 1999)

The area of the settler was determined by the overflow rate, according to the Equation 3-42 proposed by Ekama *et al.* (1984):

$$U_o = \frac{Q_i}{A} \tag{3-42}$$

where U_o = overflow velocity

Q_i = influent flow rate

A = area

3.7 Influent Sulphate Concentration

The influent sulphate concentration was based on real figures from AMD sites in Far East Rand and Witbank, which are both located in South Africa. The influent sulphate concentrations for these sites are shown in Table 3-11. Sites one and two are located in Far East Rand and the third site is located in Witbank.

***Table 3-11**: Influent sulphate concentration for each of the AMD sites.*

AMD site	Sulphate level (mg l^{-1})
Site 1	1437
Site 2	1833
Site 3	2248

3.8 Summary

This chapter presented the general methodology of the mass balance. Three carbon sources selected for comparison were:

- Ethanol
- Molasses
- Primary sewage sludge

The composition of each of the carbon sources was given. The flowsheet proposed for this study consisted of two reactors, a mixer and two settling tanks.

A description of the mass balance of the anaerobic reactor was presented. This included the definition of each of the components and compounds in the reactor as well as the bacteria present. The formulation of the mass balance and equilibrium reactions was presented. An explanation of the MATLAB simulation model was presented.

A description of the mass balance around the aerobic reactor was also presented. This included the formulation of the mass balance equations for the substrate and the biomass.

A mixer was included in the system with the main purpose of lowering the pH of the effluent from the anaerobic reactor to the optimal range for the aerobic reactor. This was done by the addition of hydrochloric acid. Lastly, a description of the performance of the settling tanks was presented.

Chapter 4

MODEL VERIFICATION

Chapter 4 demonstrates the strength of the model when compared to data from the literature. The first section of the chapter presents the statistical approach used to verify the goodness of fit of the model with data from the literature. The second section statistically confirms the model with data from literature.

The model needed to be verified when only SRB were present. This was attempted in the first two scenarios. In the first scenario, propionate was used as the carbon source and acetate in the second. To test a more complex carbon source, glucose was chosen as the carbon source for the third scenario. The fourth scenario tested a complex mixture of acetate, propionate and sucrose.

4.1 Statistical Approach to Model Verification

The statistical method used to verify if the model had fitted the data was the Chi-square statistic. The Chi-squared statistic is given as (Davies, 1961):

$$\chi^2 = \sum_{i=1}^{k} \frac{(O_i - E_i)^2}{E_i} \tag{4-1}$$

where O_i and E_i are the observed and expected frequencies in class i respectively. χ^2 is a general measure of deviation from expected values (Davies, 1961). This is a good method of comparing the model data with literature data as it quantifies the degree of deviation of the model from the literature values. The values obtained from this calculation were then compared to the critical value of χ^2 at the 90% confidence level.

Critical values of χ^2 were taken from Davies (1961). If the calculated χ^2 value was less than the critical value of χ^2, then the curve was found to be significant at that confidence level and hence fitted the data.

4.2 Using Propionate as a Carbon Source for Sulphate Reduction

The study of Ghigliazza *et al.* (2000) was focused on understanding the role of the ratio of propionate to sulphate on the degradation process. The authors had selected a culture that was constituted largely of SRB from an anaerobic treatment plant at an urban sewage treatment plant. The working conditions had been tested in order to avoid substrate competition and suppress MPB growth. Since the study centred on the propionate interactions with sulphate, propionate was used as a carbon source for sulphate reduction. A fed-batch 1.5 litre reactor was operated at a constant temperature of 35°C and a pH of 7.86. The comparison of the prediction of the simulation model presented in this thesis with the experimental data collected by Ghigliazza *et al.* (2000) is shown in Figures 4-1 and 4-2.

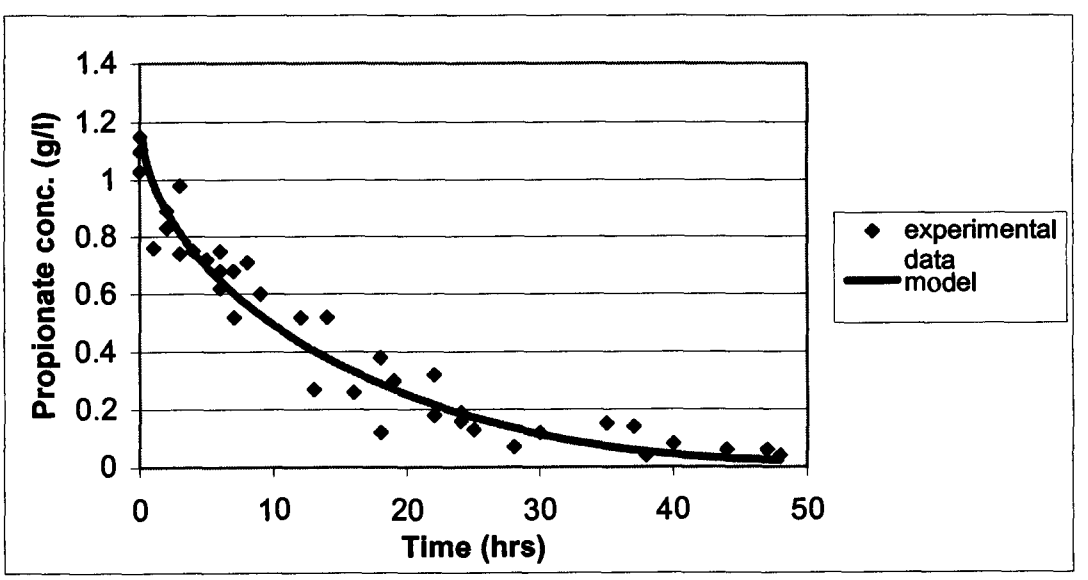

Figure 4-1: *A comparison of the model prediction and experimental data of propionate consumption using a 1.5 litre fed batch reactor from Ghigliazza et al. (2000).*

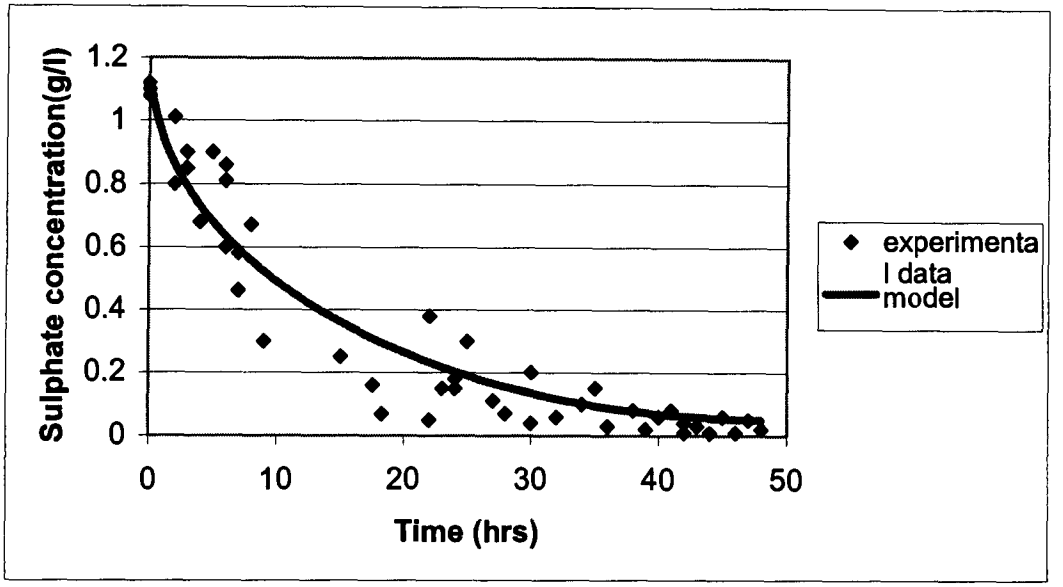

Figure 4-2: A comparison of the model prediction and experimental data of sulphate consumption using propionate as a carbon source in a 1.5 litre fed batch reactor from Ghigliazza et al. (2000).

Visually the model seemed to fit the data reasonably. This was confirmed by the statistical analysis of the model results with that of the experimental data obtained by Ghigliazza *et al.* For the propionate curve, the calculated χ^2 value was 1.2 compared to a critical value of 48.3. For the sulphate curve, the χ^2 value was calculated as being 1.45 compared with the critical value of 56.3. In both instances the critical values given were at the 90% confidence level. These results show that statistically the model fitted the data excellently.

4.3 Using Acetate as a Carbon Source for Sulphate Reduction

Moosa (2000) investigated the kinetic effects of sulphate and temperature on the anaerobic sulphate reduction process using both batch and continuous processes. In the continuous experiments, acetate in conjunction with acetate utilising SRB was used to treat sulphate at various hydraulic residence times. The microbial culture was sourced from sewage works and was enriched for SRB. Acetate was chosen over longer chain organic sources to eliminate the effect of the acid-producing and methane-producing bacteria. Acetate favours growth of sulphate-reducing bacteria

over acid-producing and methane-producing bacteria (Moosa, 2000). A laboratory scale 1 litre continuously stirred tank reactor (CSTR) was used and the temperature and pH were kept constant at 25°C and pH 7.8 respectively. The model was tested against data obtained by Moosa at hydraulic residence times of four and six days. Because the study was focused on effect of sulphate, acetate was added in excess. A graphical comparison of the results from the model and the results obtained by Moosa are shown in Figures 4-3 and 4-4.

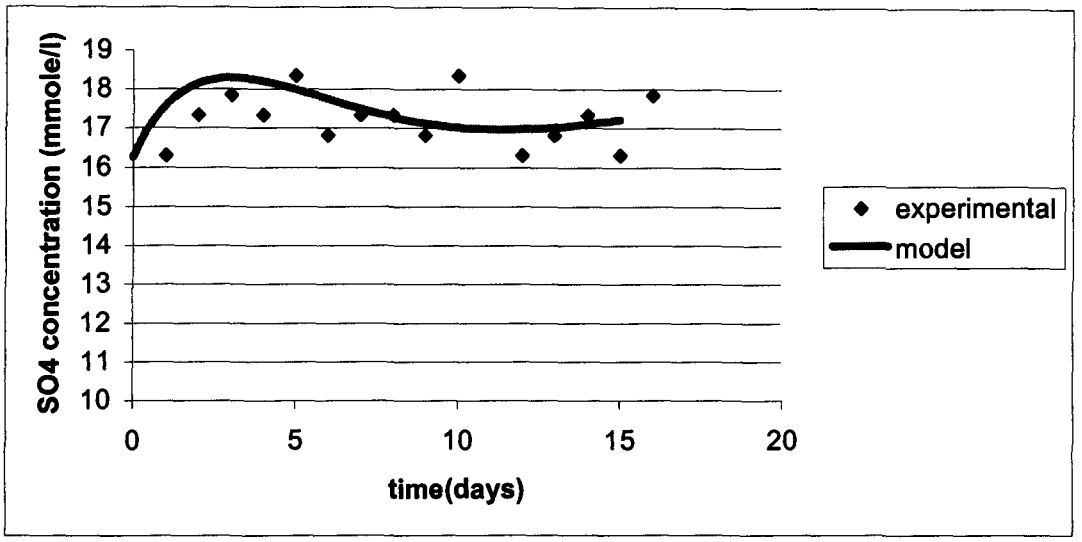

Figure 4-3: A comparison of the model prediction and experimental data of sulphate consumption in a CSTR at a hydraulic retention time of 4 days from Moosa (2000).

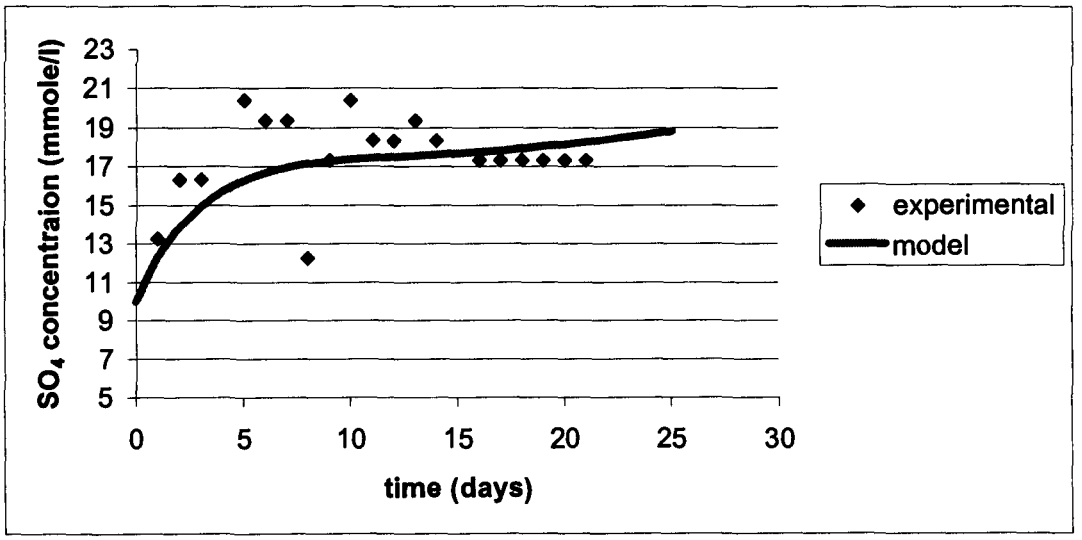

Figure 4-4: A comparison of the model prediction and experimental data of sulphate consumption in a CSTR at a hydraulic retention time of 6 days from Moosa (2000).

Again, visually the model appears to fit the data well. This was confirmed by the statistical analysis. Statistically comparing data from Figure 4-3 where the hydraulic residence time is 4 days, the calculated χ^2 value was 1.46, whereas the critical value was 21.1 at the 90% confidence level. In Figure 4-4, the χ^2 value was found to be 4.83 and the critical value was 27.2 at the 90% confidence level. Again, statistically the model fitted the data extremely well.

4.4 Using Glucose as the Feed Source for Anaerobic Digestion

Grauer (1986, cited in Costello *et al.*, 1991) used glucose as a feed for anaerobic digestion. The experiments did include sulphate reduction. The 60 litre fluidised bed reactor, operating at 35°C, was shocked loaded for 1 hour and then allowed to settle. Most of the readings were taken in the first 10 hours with the last reading being taken after 24 hours. The only significant quantities of acid recorded during the experiment were acetic and propionic acid. The comparison of the model results and the experimental results are shown in Figures 4-5 and 4-6.

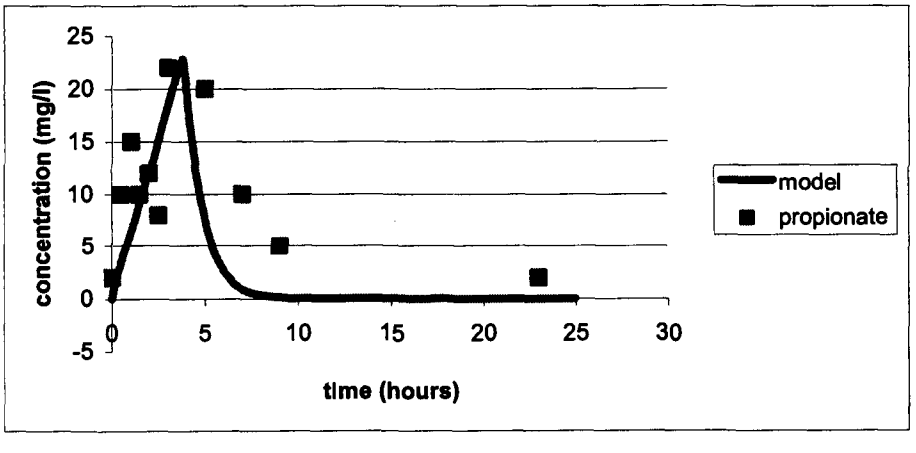

Figure 4-5: *A comparison of the model prediction and experimental data of propionate concentration from Grauer (1986, cited in Costello et al., 1991).*

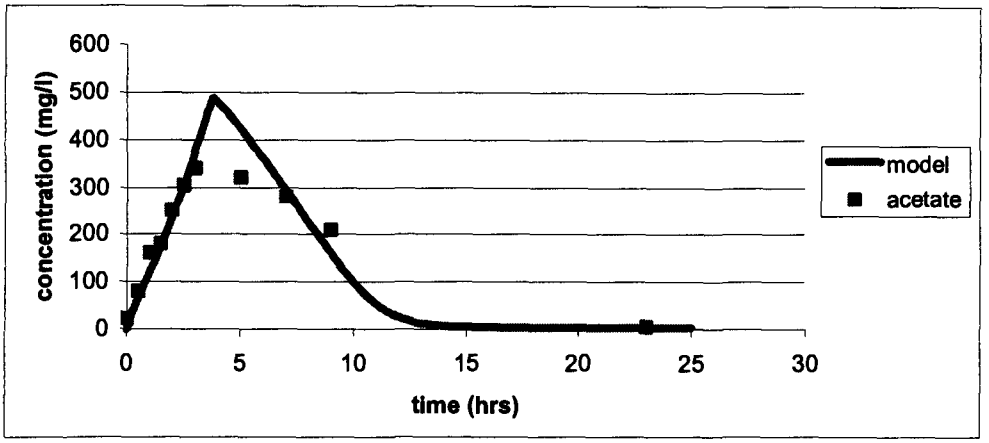

Figure 4-6: A comparison of the model prediction and experimental data of acetate concentration from Grauer (1986, cited in Costello et al., 1991).

Analysis of propionate as a function of time gave a χ^2 value of 0.2, while analysis of acetate as a function of time gave a χ^2 value of 3.07. The critical χ^2 values for both these graphs are 16.0 at the 90% confidence level. This shows that the model is considered to be statistically significant.

4.5 Using a Mixed Feed as a Feedstock for Anaerobic Digestion and for Sulphate Reduction

Alphenaar *et al.* (1993, cited in Kalyuzhnyi and Fedorovich, 1998) used sucrose in combination with acetate and propionate as their carbon feedstock for sulphate reduction. The simulation model used in this study and verified against literature data accounts for sucrose as the equivalent of two glucose molecules, as shown in Equation 4-2.

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow 2C_6H_{12}O_6 \qquad (4\text{-}2)$$

Alphenaar *et al.* used a UASB and a CSTR with recycle. The mixing regime was considered to be that of a CSTR (Kalyuzhnyi and Fedorovich, 1998). The reactor volume was 6.1 litres and the experiment ran for 150 days. The influent flow had a ratio of acetate: propionate: sucrose of 5:4:1. The comparison of the model and the

experimental data on intermediate carbon sources obtained by the authors are shown in Figures 4-7 to 4-9.

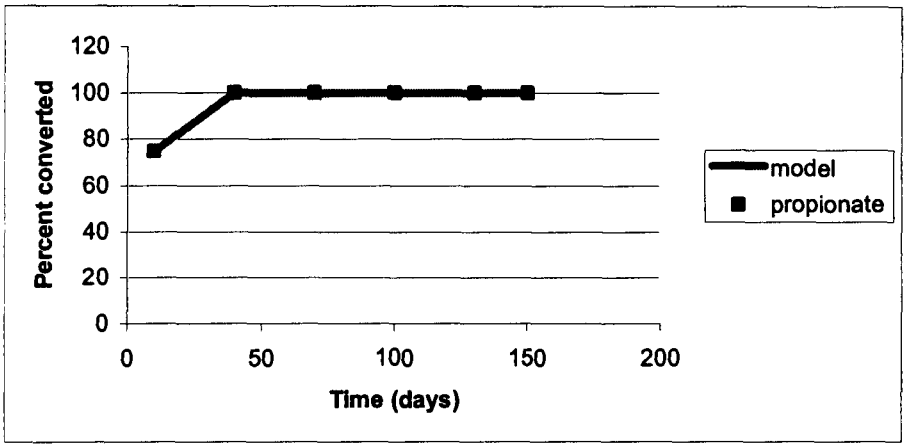

Figure 4-7: *A comparison of the model prediction and experimental data for propionate conversion by SRB from Alphenaar et al. (1993, cited in Kalyuzhnyi and Fedorovich, 1998).*

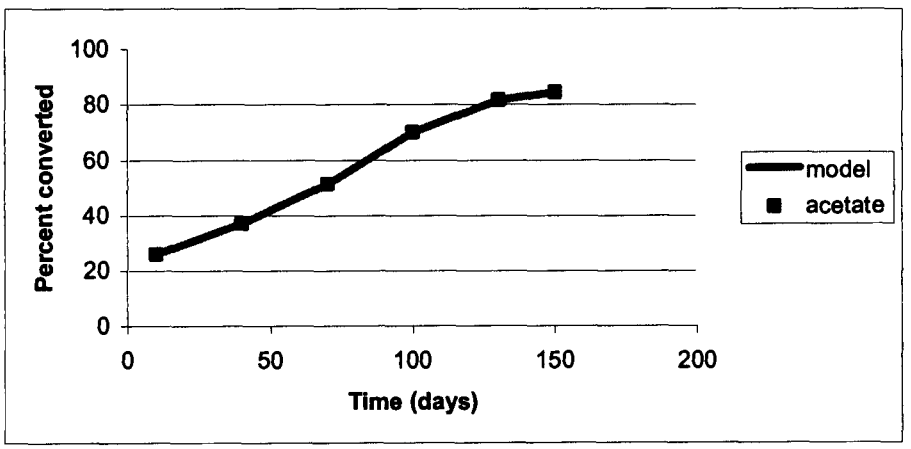

Figure 4-8: *A comparison of the model prediction and experimental data for acetate conversion by SRB from Alphenaar et al. (1993, cited in Kalyuzhnyi and Fedorovich, 1998).*

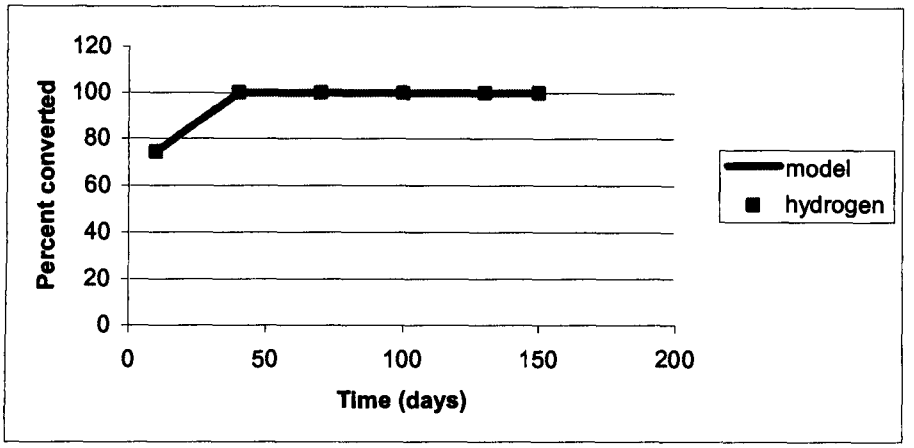

Figure 4-9: *A comparison of the model prediction and experimental data for hydrogen conversion by SRB from Alphenaar et al. (1993, cited in Kalyuzhnyi and Fedorovich, 1998).*

The χ^2 values for the comparison of the propionate, acetate and the hydrogen data with the relevant model are 1.31, 6.49 and 0.18 respectively. The critical χ^2 value for all these graphs is 9.23 at the 90% confidence level. These values show that the model is significant at the 90% confidence level and that statistically the model fits the data very well.

4.6 General Comments About Using the Model to Predict Experimental Results

Table 4-1 is a summary of the calculated χ^2 values for the above scenarios as well as the critical values at both the 90% and 95% confidence levels.

When comparing the critical values of both the 90% and 95% confidence levels, it is clear that the 90% confidence level produces a much more significant fit. In all of the above cases, the χ^2 value is lower than the 90% critical value. This meant that in all cases the model fitted the data extremely well. The implication of this is that the model is significant.

Table 4-1: *Summary of calculated χ^2 values and critical values*

Feed	Compound monitored/modelled	χ^2	Critical χ^2 values at 90% confidence level*	Critical χ^2 values at 95% confidence level*	Source
propionate	propionate	1.12	48.3	52.2	Ghigliazza *et al.* (2000)
	sulphate	1.45	56.3	60.5	Ghigliazza *et al.* (2000)
acetate	acetate HRT=4days	1.46	21.1	23.7	Moosa (2000)
	acetate HRT=6days	4.83	27.2	30.1	Moosa (2000)
glucose	propionate	0.23	16.0	18.3	Grauer (1986, cited in Costello *et al.*, 1991)
	acetate	3.07	16.0	18.3	Grauer (1986, cited in Costello *et al.*, 1991)
sucrose, acetate, propionate (1:5:4)	Propionate	1.31	9.24	11.07	Alphenaar *et al.* (1993, cited in Kalyuzhnyi and Fedorovich, 1998)
	acetate	6.49	9.24	11.07	Alphenaar *et al.* (1993, cited in Kalyuzhnyi and Fedorovich, 1998)
	hydrogen	0.18	9.24	11.07	Alphenaar *et al.* (1993, cited in Kalyuzhnyi and Fedorovich, 1998)

* Taken from Davies (1961)

Chapter 5

MODEL CALIBRATION

Chapter 5 deals with the method of calibration used to establish the rate constants used in the model. The development of any simulation model involves numerous simplifications concerning the processes that take place in the system (Vavilin and Lokshina, 1996). With regards to the kinetics of all the steps, with the exception of the hydrolysis step, the following assumptions have been made (Knobel, 1999):

- The rate of growth of each of the bacteria follows Monod kinetics (Kalyuzhnyi and Fedorovich, 1998).
- All bacterial steps have been assumed to be pH dependent. Because the pH inhibition occurs outside a certain pH range, an on/off switching function has to be included and is as follows:

$$\xi_{pH} = (1 + e^{-\alpha_{LL}(pH - pH_{LL})})^{-1}(1 + e^{\alpha_{UL}(pH - pH_{UL})})^{-1} \quad (5\text{-}1)$$

  α_{LL} and α_{UL} quantify quickly the inhibition that comes into effect and pH_{LL} and pH_{UL} are the upper and lower pH limits.
- All reactions are effectively rate controlled, i.e. the effects of diffusion limitations of biomass aggregates are constant and incorporated into the kinetic term (Kalyuzhnyi and Fedorovich, 1998).
- Biomass is represented by the formula $C_5H_7O_2N$ (Angelidaki *et al.*, 1999; Keshtkar *et al.*, 2001; Mosey, 1983).

The hydrogen inhibition coefficient was used to relate the ratio of "reduced-to-oxidised" NAD^+ to the partial pressure of hydrogen in the biogas, using the Nernst equation (Mosey, 1983; Costello, 1991), given as Equation 5-2:

$$\frac{[NADH]}{[NAD^+]} = \alpha P_{H_2} \quad \log(\alpha) = 7 - \frac{1139}{T + 273} \qquad (5\text{-}2)$$

where α = 1 500 at 25°C (Costello, 1991)
= hydrogen inhibition parameter

5.1 Hydrolysis

The first order approach has been shown to fit the experimental hydrolysis data quite well (Angelidaki *et al.*, 1999). Hence, Equation 5-3 will suffice for this step.

$$r_X = -k_h(X - X_\infty)$$
$$= -k_h X_{deg} \qquad (5\text{-}3)$$

where k_h = overall hydrolysis rate constant (d^{-1})
r_X = rate of degradation of a component of the sludge (mg l^{-1} d^{-1})
X = total concentration of sludge component (mg l^{-1})
X_∞ = concentration of "nondegradable" fraction (mg l^{-1})
X_{deg} = concentration of degradable fraction (mg l^{-1})

Definite stoichiometry for hydrolysis is difficult to write due to the poorly defined nature of sludge. A possible solution to this problem is to represent each fraction with a generic molecule.

5.1.1 Proteins

A simple average of 20 amino acids gives a generic formula of $(C_5H_9O_3N)_n$, which was used in the model. A better method would be to use a weighted amino acid fraction. Angelidaki *et al.* (1999) used the formula composition of gelatin

($CH_{2.03}O_{0.6}N_{0.3}S_{0.001}$) as a representative of the average amino acid composition, which is representative of many animalic proteins. The reaction for the formation of amino acids from the simple average of proteins can then be written as:

$$(C_5H_7O_2N)_n + n\ H_2O \rightarrow n\ C_5H_9O_3N \qquad (5\text{-}4)$$

5.1.2 Carbohydrates

In a similar manner to proteins $(C_6H_{10}O_5)_n$ is used as the generic formula for the carbohydrate fraction assumed to be a polymer of monosaccharides composed of six carbon atoms such as glucose and fructose. The reaction can then be written as:

$$(C_6H_{10}O_5)_n + n\ H_2O \rightarrow n\ C_6H_{12}O_6 \qquad (5\text{-}5)$$

5.1.3 Lipids

Palmitic acid is used to represent the product of the lipid fraction. The reaction to produce glycerol and palmitic acid from lipids can then be represented by:

$$C_{51}H_{98}O_6 + 3\ H_2O \rightarrow CH_2OHCHOHCH_2OH + 3\ CH_3(CH_2)_{14}COOH \qquad (5\text{-}6)$$

5.1.4 Rate constants for hydrolysis

From Table 2-2, it is seen that only one range of substrate utilisation rate constants is reported in the literature for each of the hydrolysis steps at 25°C. These values that were chosen for the model, represent the maxima in each range and are presented in Table 5-1

Table 5-1: 1st order degradation rate constants for hydrolysis used in the model (O' Rourke, 1968).

1st order degradation rate constant	day-1
$k_{Proteins}$	0.09
$k_{Carbohyrates}$	0.29
k_{Lipids}	0.09

5.2 Fermentation

Fermentation of four compounds was considered i.e. glucose, lactate, amino acids and glycerol.

5.2.1 Glucose Fermentation

In the two schemes presented for the stoichiometry for glucose fermentation in Section 2.2.2.1 (Equations 2-4 to 2-6 represents the first scheme and Equation 2-7 the second), the first is a theoretical approach whereas the second is based on experimental data. For this reason, work done by Stamatlatou *et al.* (2003) has been chosen to represent this.

Using the work from Stamatlatou *et al.* (2003), the stoichiometric equation representing the degradation of glucose is written as:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 0.67\ CH_3COOH + 0.67\ CH_3CHOHCOOH + 0.33\ CH_3CH_2CH_2COOH + 2\ H_2 + 1.33\ H_2CO_3 \quad (2\text{-}8)$$

The reaction describing the production of biomass from glucose is:

$$5\ C_6H_{12}O_6 + 6\ NH_3 \rightarrow 6\ C_5H_7O_2N + 18\ H_2O \quad (5\text{-}7)$$

The rate of glucose uptake for energy production only, $R_{Glucose}$, is given by a non-competitive hydrogen inhibition model (Mosey, 1983). However due to the effects of inhibition by undissociated fatty acids (Costello *et al.*, 1991), pH inhibition

(Kalyuzhnyi and Fedorovich, 1998) and H_2S inhibition (Hilton and Oleszkiewicz, 1988), the reaction is refined to the following:

$$R_{Glucose} = -\frac{1}{Y}\left(\frac{\mu_{max} XS}{K_S + S}\right)\left(\frac{K_{I,H_2}}{K_{I,H_2} + P_{H_2}}\right)\left(\frac{K_{I,VFA}}{K_{I,VFA} + [I_{VFA}]}\right)\left(\frac{K_{I,H_2S}}{K_{I,H_2S} + [I_{H_2S}]}\right)\xi_{pH} \qquad (5\text{-}8)$$

where
- Y = yield coefficient
- μ_{max} = maximum growth rate of bacteria
- X = biomass concentration (mg l^{-1})
- K_S = Monod half velocity constant (mg l^{-1})
- S = substrate concentration (mg l^{-1})
- K_I = inhibition constant (mg l^{-1})
- I = total concentration of inhibitor (mg l^{-1})
- P_{H_2} = hydrogen partial pressure (atm)
- ξ_{pH} = pH inhibition on/off switching function

The rate constants of Stamatlatou *et al.* (2003) used in this study are presented in Table 5-2. The inhibition by volatile fatty acid was taken from Costello *et al.* (1991) and the inhibition constant for undissociated H_2S was taken from Alphenaar *et al.*, (1993).

***Table 5-2**: Rate constants for glucose fermentation used in the model.*

μ_m (day^{-1})	Y ($mM\ mM^{-1}$)	K_S (mmole l^{-1})	k_d (day^{-1})	$K_{I,VFA}$ (mmole l^{-1})	$K_{I,H2S}$ (mmole l^{-1})
5.124[1]	0.112[1]	0.082[1]	0.0001[1]	10[2]	17.19[3]

1 Stamatlatou *et al.* (2003)
2 Costello *et al.* (1991)
3 Alphenaar *et al.* (1993)

5.2.2 Lactate Fermentation

Using the same argument as for glucose, the stoichiometric equation used for the fermentation of lactate is taken from Stamatlatou *et al.* (2003):

$$CH_3CHOHCOOH + 0.5\ H_2O \rightarrow 0.5\ CH_3COOH + 0.5\ CH_3CH_2COOH + 0.5\ H_2O + 1.33\ H_2CO_3 \quad (2\text{-}11)$$

The biomass synthesis reaction from lactic acid is:

$$5\ CH_3CHOHCOOH + 3\ NH_3 \rightarrow 3\ C_5H_7O_2N + 9\ H_2O \quad (5\text{-}9)$$

The same argument presented for the rate of glucose uptake can be used to consider the rate of lactate uptake for energy production, and hence a similar rate equation is expressed by Equation 5-10:

$$R_{Lactate} = -\frac{1}{Y}\left(\frac{\mu_{max} XS}{K_S + S}\right)\left(\frac{K_{I,H_2}}{K_{I,H_2} + P_{H_2}}\right)\left(\frac{K_{I,VFA}}{K_{I,VFA} + I_{VFA}}\right)\left(\frac{K_{I,H_2S}}{K_{I,H_2S} + I_{H_2S}}\right)\xi_{pH} \quad (5\text{-}10)$$

The rate constants of Stamatlatou *et al.* (2003) used in this study are presented in Table 5-3. The inhibition of volatile fatty acid was taken from Costello *et al.* (1991) and the inhibition constant for undissociated H_2S was taken from Knobel (1999) who had assumed this value.

***Table 5-3**: Rate constants for lactate fermentation used in the model.*

μ_m (day^{-1})	Y ($mM\ mM^{-1}$)	K_S ($mmole\ l^{-1}$)	k_d (day^{-1})	$K_{I,VFA}$ ($mmole\ l^{-1}$)	$K_{I,H2S}$ ($mmole\ l^{-1}$)
2.552[1]	0.1[1]	1.11[1]	0.0001[1]	10[2]	3.12[3]

1 Stamatlatou *et al.* (2003)
2 Costello *et al.* (1991)
3 Values assumed by Knobel (1999)

5.2.3 Amino Acids Fermentation

Assuming that the formula $C_5H_9O_3N$ is a valid approximation for the average of all amino acids produced in the hydrolysis step (Knobel, 1999), the reactions for the amino acids are:

$$C_5H_9O_3N + 3H_2O \rightarrow 2CH_3COOH + CO_2 + 2H_2 + NH_3 \quad (5\text{-}11)$$

$$C_5H_9O_3N + 3H_2O \rightarrow CH_3CH_2COOH + 2CO_2 + 3H_2 + NH_3 \quad (5\text{-}12)$$

$$C_5H_9O_3N + H_2O \rightarrow CH_3CH_2CH_2COOH + CO_2 + NH_3 \quad (5\text{-}13)$$

$$C_5H_9O_3N + 4H_2O \rightarrow CH_3CHOHCOOH + 2CO_2 + 4H_2 + NH_3 \quad (5\text{-}14)$$

Eastman and Ferguson (1981) studied the hydrolysis and acidogenesis of primary sludge. It was found that only acetate and propionate were formed and that the production was relatively equal on a COD basis. It is assumed that this ratio will also be valid here.

The relevant biomass synthesis equation is:

$$C_5H_9O_3N \rightarrow C_5H_7O_2N + H_2O \quad (5\text{-}15)$$

No data for the kinetics of amino acid acetogenesis are available in the literature. A rate similar to that of glucose fermentation was tentatively assumed by Knobel (1999). Eastman and Furguson (1981) assumed a yield of 0.48 g cell COD/g COD utilised. Based on a cell COD of 1.41 and a generic amino acid COD of 1.22, this corresponds to a yield of 0.55 g biomass/g amino acid utilised.

The rate equation for amino acids used in the model is:

$$R_{a\,\mathrm{min}\,oacid} = -\frac{1}{Y}\left(\frac{\mu_{\max} XS}{K_S + S}\right)\left(\frac{K_{I,H_2}}{K_{I,H_2} + P_{H_2}}\right)\left(\frac{K_{I,VFA}}{K_{I,VFA} + I_{VFA}}\right)\left(\frac{K_{I,H_2S}}{K_{I,H_2S} + I_{H_2S}}\right)\xi_{pH} \quad (5\text{-}16)$$

Rate data was taken from Knobel (1999), who had assumed each of these constants except for the yield constant, which was taken from Eastman and Furguson (1981).

***Table 5-4**: Rate constants for amino acid fermentation used in the model.*

μ_m (day^{-1})	Y ($mM\ mM^{-1}$)	K_S ($mmole\ l^{-1}$)	k_d (day^{-1})	$K_{I,VFA}$ ($mmole\ l^{-1}$)	$K_{I,H2S}$ ($mmole\ l^{-1}$)
1.5[1]	0.57[2]	0.153[1]	0.0001[1]	10[1]	3.12[1]

1 Values assumed by Knobel (1999)
2 Eastman and Furguson (1981)

5.2.4 Glycerol Fermentation

No data for glycerol was found in the literature. Knobel (1999) assumed that acetate was the main product formed from the fermentation of glycerol. The same assumption is used here.

$$CH_2OHCHOHCH_2OH + H_2O \rightarrow CH_3COOH + 3\ H_2 + CO_2 \qquad (5\text{-}17)$$

The biomass equation is:

$$2\ CH_2OHCHOHCH_2OH + NH_3 \rightarrow C_5H_7O_2N + CO_2 + 2\ H_2O + 4H_2 \qquad (5\text{-}18)$$

The rate equation describing the fermentation of glycerol is shown in Equation 5-19.

$$R_{glycerol} = -\frac{1}{Y}\left(\frac{\mu_{max} XS}{K_S + S}\right)\left(\frac{K_{I,H_2}}{K_{I,H_2} + P_{H_2}}\right)\left(\frac{K_{I,VFA}}{K_{I,VFA} + I_{VFA}}\right)\left(\frac{K_{I,H_2S}}{K_{I,H_2S} + I_{H_2S}}\right)\xi_{pH} \qquad (5\text{-}19)$$

As no data was found in the literature with regards to glycerol fermentation, Knobel's (1999) assumptions for the rate constants have been retained.

Table 5-5: *Rate constants for glycerol fermentation used in the model (Values assumed by Knobel, 1999).*

μ_m (day^{-1})	Y ($mM\ mM^{-1}$)	K_S ($mmole\ l^{-1}$)	k_d (day^{-1})	$K_{I,VFA}$ ($mmole\ l^{-1}$)	$K_{I,H2S}$ ($mmole\ l^{-1}$)
10	0.4	0.25	0.02	10	3.12

5.3 Beta Oxidation

Knobel (1999) assumed that palmitic acid is representative of all long chain fatty acids; this assumption has been retained for this model.

The relevant cell synthesis reaction is proposed as:

$$5\ CH_3(CH_2)_{14}COOH + 16\ NH_3 + 22\ H_2O \rightarrow 16\ C_5H_7O_2N + 70\ H_2 \qquad (5\text{-}20)$$

A similar rate expression to that used in fermentation is used here. The rate constants from Novak and Carlson (1970, cited in Gujer and Zehnder, 1983) are used. Inhibition constants for volatile fatty acids and hydrogen sulphide were assumed by Knobel (1999) and retained here.

***Table 5-6**: Rate constants for beta oxidation used in the model.*

μ_m (day^{-1})	Y ($mM\ mM^{-1}$)	K_S ($mmole\ l^{-1}$)	k_d (day^{-1})	$K_{I,VFA}$ ($mmole\ l^{-1}$)	$K_{I,H2S}$ ($mmole\ l^{-1}$)
0.12[1]	0.674[1]	0.19[1]	0.01[1]	10[2]	3.12[2]

1 Novak and Carlson (1970, cited in Gujer and Zehnder, 1983)
2 Values assumed by Knobel (1999)

5.4 Acetogenesis

Equations 2-14 and 2-15 describe the formation of acetate from butyrate and propionate. These equations have been maintained in the model.

The biomass equations for propionate and butyrate are as follows:

$$5\ CH_3CH_2COOH + 3\ NH_3 \rightarrow 3C_5H_7O_2N + 4H_2O + 5H_2 \qquad (5\text{-}21)$$

$$5\ CH_3CH_2CH_2COOH + 4\ NH_3 \rightarrow 4C_5H_7O_2N + 2H_2O + 10H_2 \qquad (5\text{-}22)$$

Equation 5-23, describing the specific growth rate of the acetogenic bacteria, includes a term for competitive volatile fatty acid inhibition, (Costello, 1991), hydrogen inhibition (Mosey, 1983), a competitive inhibition term for volatile fatty acids (Costello *et al.*, 1991), pH inhibition (Kalyuzhnyi and Fedorovich, 1998) and a non-competitive term for sulphide inhibition (Vavilin and Lokshina, 1996).

$$r_S = -\frac{\mu_{max}}{Y}\left(\frac{XS}{K_S\left(1+\frac{[VFA]}{K_{I,VFA}}\right)+S}\right)\left(\frac{K_{I,H_2S}}{K_{I,H_2S}+[H_2S]}\right)\left(\frac{K_{I,H_2}}{K_{I,H_2}+P_{H_2}}\right)\xi_{pH} \quad (5\text{-}23)$$

Rate kinetics from Lawrence and McCarty (1969) were used for the propionate data. Rate kinetics from Kalyuzhnyi and Davlyatshina (1997) were used for the butyrate data. Inhibition constants for volatile fatty acid inhibition were taken from Costello *et al.*, (1991). Inhibition constants for hydrogen sulphide were taken from Maillacheruvu and Parkin (1996).

***Table 5-7:** Rate constants for glucose acetogenesis used in the model.*

	μ_m (day^{-1})	Y ($mM\ mM^{-1}$)	K_S ($mmole\ l^{-1}$)	k_d (day^{-1})	$K_{I,VFA}$ ($mmole\ l^{-1}$)	$K_{I,H2S}$ ($mmole\ l^{-1}$)
Propionate	0.36[1]	0.03[1]	0.5[1]	0.01[1]	3[2]	0.83[3]
Butyrate	0.264[4]	0.04[4]	1.1[4]	0.01[4]	30[2]	0.81[3]

1 Lawernce and McCarty (1969)
2 Costello *et al.* (1991)
3 Maillacheruvu and Parkin (1996)
4 Kalyuzhnyi and Davlyatshina (1997)

5.5 Methanogenesis

Cell synthesis for hydrogen and acetate can be represented by Equations 5-24 and 5-25:

$$5CO_2 + 10H_2 + NH_3 \rightarrow C_5H_7O_2N + 8H_2O \quad (5\text{-}24)$$

$$5CH_3COOH + 2NH_3 \rightarrow 2C_5H_7O_2N + 6H_2O \quad (5\text{-}25)$$

Equation 5-26, describing the specific growth rate of the methanogenic bacteria, includes a term for non-competitive volatile fatty acid inhibition (Vavilin and Lokshina, 1996), pH inhibition (Kalyuzhnyi and Fedorovich, 1998) and an uncompetitive term for sulphide inhibition (Maillacheruvu and Parkin, 1996).

$$r_S = -\frac{\mu_{max}}{Y}\left(\frac{XS}{K_S + S\left(1+\frac{[H_2S]}{K_{I,H_2S}}\right)}\right)\left(\frac{K_{I,VFA}}{K_{I,VFA}+[VFA]}\right)\xi_{pH} \qquad (5\text{-}26)$$

Rate data was taken from Kalyuzhnyi and Fedorovich (1998). Inhibition constants for volatile fatty acids were taken from Costello *et al.* (1991). Hydrogen sulphide inhibition was taken from Maillacheruvu and Parkin (1996).

***Table 5-8**: Rate constants for methanogenesis used in the model.*

	μ_m (day^{-1})	Y (mM mM^{-1})	K_S (mmole l^{-1})	k_d (day^{-1})	$K_{I,VFA}$ (mmole l^{-1})	$K_{I,H2S}$ (mmole l^{-1})
H_2	1[1]	0.002[1]	0.008125[1]	0.01[1]	3[2]	20.71[3]
Acetate	0.36[1]	0.0127[1]	0.875[1]	0.01[1]	10[2]	3.65[3]

1 Kaluzhnyi and Fedorovich (1998)
2 Costello *et al.* (1991)
3 Maillacheruvu and Parkin (1996)

5.6 Sulphate Reduction

An addition to the model developed by Knobel (1999) is the inclusion of ethanol as a substrate for sulphate reduction. To simplify the model only complete oxidation of ethanol was considered.

$$C_2H_5OH + H_2SO_4 \rightarrow H_2S + 2\,CO_2 + 2H_2 \qquad (5\text{-}27)$$

The biomass equations for sulphate reduction are:

$$5\,CO_2 + 10\,H_2 + NH_3 \rightarrow C_5H_7O_2N + 8\,H_2O \qquad (5\text{-}28)$$

$$CH_3COOH + 2\,NH_3 \rightarrow 2\,C_5H_7O_2N + 6\,H_2O \qquad (5\text{-}29)$$

$$5\,CH_3CHOHCOOH + 3\,NH_3 \rightarrow 3\,C_5H_7O_2N + 9\,H_2O \qquad (5\text{-}30)$$

$$CH_3CH_2COOH + 3\,NH_3 \rightarrow 3\,C_5H_7O_2N + 4\,H_2O + 5\,H_2 \qquad (5\text{-}31)$$

$$5\,CH_3CH_2CH_2COOH + 3\,NH_3 \rightarrow 4\,C_5H_7O_2N + 2\,H_2O + 2.5\,H_2 \qquad (5\text{-}32)$$

$$5\,C_2H_5OH + 2\,NH_3 \rightarrow 2\,C_5H_7O_2N + 5\,H_2 \qquad (5\text{-}33)$$

Equation 5-34, describing the specific growth rate of the sulphate reducing bacteria includes a term for non-competitive volatile fatty acid inhibition (Reis *et al.*, 1990), pH inhibition (Hilton and Oleszkiewicz, 1988), and an uncompetitive term for sulphide inhibition (Maillacheruvu and Parkin, 1996).

$$r_S = -\frac{\mu_{max}}{Y}\left(\frac{XS}{K_S + S\left(1+\frac{[H_2S]}{K_{I,H_2S}}\right)}\right)\left(\frac{[SO_4^{2-}]}{K_{S,SO_4}+[SO_4^{2-}]}\right)\left(\frac{K_{I,VFA}}{K_{I,VFA}+[VFA]}\right)\xi_{pH} \qquad (5\text{-}34)$$

Reaction kinetics for hydrogen, acetate and propionate were taken from Kalyuzhnyi and Fedorovich (1998). Reaction kinetics for ethanol was taken from Erasmus (2000). Reaction kinetics for lactate was taken from Traore (1982), which was cited in Knobel (1999). Rate data for butyrate were taken from Schauder (1986), which was cited in Knobel (1999).

***Table 5-9**: Rate constants for sulphate reduction used in the model.*

	μ_m (day^{-1})	Y (mM/ mM)	K_S ($mmole\ l^{-1}$)	$K_{S,SO4}$ ($mmole\ l^{-1}$)	k_d (day^{-1})	$K_{I,VFA}$ ($mmole\ l^{-1}$)	$K_{I,H2S}$ ($mmole\ l^{-1}$)
H_2[1]	5	0.021	0.0015	0.0093	0.013	100	4.65
Acetate[1]	0.51	0.023	0.375	0.2	0.013	10	4.75
Lactate[2]	2.5	0.02	0.0488	0.00877	0.02	10	7.83
Propionate[1]	0.81	0.03	2.56	0.077	0.02	10	8.89
Butyrate[3]	0.41	0.04	0.309	0.17	0.02	10	15.6
Ethanol[4]	0.8	0.02	0.124	5.39	0.02	10	5.6

1 Kalyuzhnyi and Fedorovich (1998)
2 Traore (1982, cited in Knobel, 1999)
3 Schauder (1986, cited in Knobel, 1999)
4 Erasmus (2000)

5.7 Summary

This chapter summarises the data used in the MATLAB model. Data from various sources were used. In cases where no literature was found, values assumed by Knobel (1999) were retained. Ethanol was added to the model as an extra substrate. Data taken from Erasmus (2000) was used for ethanol.

Chapter 6

RESULTS OF THE MASS BALANCE

The mass balance was performed at each of the three AMD sites using each of the carbon substrates (ethanol, molasses and primary sewage sludge). The chapter starts with an example of the mass balance performed using primary sewage sludge as the carbon source and electron donor for AMD site 2 in which the mass balance for each unit in the system is detailed. Presentation of the example is concluded by showing the full mole and mass balance for AMD treatment at site 2 based on primary sewage sludge as the carbon source and electron donor.

The full mole and mass balances of AMD treatment at site 2 using molasses and ethanol as carbon substrates are presented in Sections 6.2 and 6.3 respectively. In Section 6.4, comparisons across the three AMD sites are presented using each of the three substrates. Section 6.5 gives a summary of the chapter and its findings.

6.1 Treatment of AMD from AMD Site 2 Using Primary Sewage Sludge as Carbon Source: Detailed Example of Mass Balance

In presenting the mass balance for the treatment of acid mine drainage at the second AMD site, using primary sewage sludge as the carbon source as a detailed example, the results of the mass balance across each unit are presented individually prior to the presentation of the composite mass balance.

6.1.1 Anaerobic Reactor

The basis for the mass balance was 1 000 m^3 AMD entering the process per day in each case. The AMD stream had a sulphate content of 1 830 mg l^{-1} at the second AMD site. This translated to an inflow of 19 100 moles of sulphate entering the system each day. The MATLAB simulation of the reactor was run at a residence time of ten days on a trial and error basis until a steady state was reached at which a residual sulphate concentration of 250 mg l^{-1} was achieved. The results of the mole balance around the reactor are shown in Table 6-1. Stream 1 is the influent AMD stream. This stream has been pretreated to ensure a negligible concentration of metals. Stream 2 is the carbon substrate, primary sewage sludge. The composition of the sewage sludge is presented in Table 3-2. Some 33.45% was not biodegradable. Stream 3 is the exit gas stream and stream 4 is the liquid effluent stream. The reactor was operated at the conditions specified in Section 3.3 i.e. 25°C, 1 atm and pH 7.4. The reactions simulated to occur in the reactor are detailed in Appendix A and the constants used in the simulation model is presented in Appendix B. Appendix C presents the MATLAB script files used.

Some reduction in the insoluble compounds (Table 3-3) occurred in the reactor; however a large amount of insoluble solids remained in the effluent, due to about one third of the insoluble components being unreactive as well as the low rates of reaction for hydrolysis.

There is an 87% reduction of the sulphate that enters the reactor, resulting in its conversion to sulphide. The exit sulphate concentration in the liquid effluent was 248 mg l^{-1}. This is below the target concentration of 250 mg l^{-1}; hence the reactor had achieved its primary aim of reducing the sulphate levels to acceptable limits.

There was no production of methane. It could be explained by sulphate reducers outcompeting methanogens for hydrogen and acetate. No hydrogen evolved from the system. This could be explained by hydrogen being the limiting reactant and reacted completely. Carbon dioxide and hydrogen sulphide left the system as gas.

***Table 6-1**: Results of the mole balance around the reactor for AMD site 2 using primary sewage sludge as the carbon source and electron donor (all results in moles day^{-1}) (Streams: 1- AMD, 2- carbon substrate, 3- exit gas, 4- liquid effluent).*

Stream No.	1	2	3	4
Proteins	0	13700	0	9750
Carbohydrates	0	6690	0	3380
Lipids	0	1420	0	970
Amino acids	0	0	0	32.3
Glucose	0	0	0	2.86
Glycerol	0	0	0	6.71
Palmitic	0	0	0	48.5
Hydrogen	0	0	0	0
Acetate	0	4530	0	149
Lactate	0	0	0	3.39
Propionate	0	0	0	518
Butyrate	0	0	0	162
Sulphate	19100	0	0	2580
Hydrogen sulphide	0	0	1850	14800
Carbon dioxide	0	0	8400	21800
Methane	0	0	0	0
Ammonia	0	0	0	520
Ethanol	0	0	0	0

6.1.2 Mixer

The effluent from the anaerobic reactor was sent to a mixer where hydrochloric acid was added to decrease the pH from 7.41 to 7.05. Only the liquid solubility of hydrogen sulphide was assumed to be affected by the decrease in pH. CO_2 and NH_3 leaving the mixer were assumed to be negligible, based on the very low solubilities of $CO_2(aq)$ and $NH_3(aq)$ at these pH's and the small differences in their concentrations. Of the 14 800 moles day^{-1} of aqueous hydrogen sulphide entering the mixer, 7 050 moles day^{-1} left the mixer as gas (for recycle to precipitate metals prior to sulphate reduction). The remaining amount of 7 750 moles day^{-1} remained in the aqueous form.

To calculate the amount of hydrochloric acid needed to lower the pH from 7.41 to 7.05, the concentrations of carbonate, sulphide, acetate, propionate, butyrate and lactate ions were required as a function of pH. These were calculated similarly to Equations 3-33 to 3-39 and the results are shown in Table 6-2. While a decrease in the

concentration of each ion was shown on decreasing pH, HCO_3^- and HS^- were the most strongly affected. In this example, 4 735 moles HCl per day was needed to effect the pH change.

***Table 6-2**: The total dissolved sulphide concentration of the liquid effluent stream from the anaerobic reactor at pH of 7.41 and 7.05 using primary sewage sludge as the carbon source and electron donor at AMD site 2 (all results in moles day^{-1} except pH).*

pH	7.41	7.05	Change in content
	Moles day^{-1}	Moles day^{-1}	Mole day^{-1}
HCO_3^-	19800	17900	1900
CO_3^{2-}	26.8	10.7	16.0
HS^-	10200	7370	2850
S^{2-}	0.0355	0.0355	0
Ac^-	148	148	0.4
Pr^-	517	515	2
Bu^-	161	160	0.5
La^-	3.39	3.39	0.00121

The density of liquid hydrochloric acid is 1 193 kg m^{-3} (Sinnott, 2000). Using the density and the molar mass of HCl, it was calculated that 145 l day^{-1} of hydrochloric acid is needed. Using a concentration of 32 % hydrochloric acid, the total volume of acid required is 454 l day^{-1}.

6.1.3 Settler

The removal efficiency of insoluble compounds by the settler was estimated at 65% for systems that utilise primary sewage sludge and 44% for the molasses and ethanol systems (Ristow, 2004). The sludge cake produced was assumed to have a 20% wet solids content (Sincero and Sincero, 1996 and Toll, 2004). Hence, the water content of the sludge was calculated.

The soluble components of the system were assumed to have the same split as water. The mass balance across the settler is shown in Table 6-3. Stream 8 is the mixer effluent entering the settler. Stream 9 is the settler overflow stream. Stream 10 is the concentrated sludge stream sent for disposal in landfills. Less than 0.8% of the water entering the settler in the mixer effluent is disposed with the biomass sludge. The total insoluble component of the sludge stream, comprising 20% of the stream, is

1890 kg day^{-1}. The settler resulted in the removal of approximately 60 % of the COD from the liquid stream.

***Table 6-3**: Results of the mass balance around the settling tank for AMD site 2 using sewage sludge as the carbon source and electron donor (results in kg day^{-1}) (Streams: 8-liquid entering the settler, 9- liquid overflow, 10-sludge).*

Stream No.	8	9	10
Proteins	1110	389	723
Carbohydrates	548	192	356
Lipids	787	275	511
Amino acids	4.24	4.20	0.04
Glucose	0.515	0.511	0.004
Glycerol	0.618	0.613	0.005
Palmitic acid	12.4	12.3	0.1
Hydrogen	0	0	0
Acetate	8.93	8.86	0.07
Lactate	0.305	0.303	0.002
Propionate	38.4	38.1	0.3
Butyrate	14.3	14.1	0.2
Sulphates	248	246	2
Hydrogen sulphide	254	252	2
Carbon dioxide	959	952	7
Methane	0	0	0
Ammonia	8.86	8.79	0.07
Ethanol	0	0	0
Hydrochloric acid	173	172	1.3
Sulphur	0	0	0
Bacteria	466	163	303
Water	1000000	992000	7600
COD	5660	2310	3350

6.1.4 Aerobic Reactor

The aim of the aerobic reactor is to decrease the soluble sulphide concentration in the liquid effluent by converting it to elemental sulphur. Setting the reactor volume at 10 000 m^3 gave a dilution rate of 0.1 day^{-1} (Equation 3-23). The exit concentration of H_2S was calculated using Equation 3-27 and the data used presented in Table 3-8 and reproduced here. A final dissolved sulphide concentration of 8.46 mg l^{-1} was achieved. The amount of elemental sulphur produced followed Equation 3-20 i.e. 7 140 mol day^{-1} of H_2S was converted to produce 228 kg day^{-1} elemental sulphur.

The amount of oxygen used, calculated from Equation 3-20, was 431 moles day^{-1}. The oxygen was supplied as air. Based on current research at the University of Cape Town, the percentage O_2 leaving the system would be 20.9% O_2 (Searby, 2004). Using a general mass balance for the air and assuming the amount of nitrogen dissolved was negligible, the total amount of air required was calculated as 2 820 000 moles day^{-1}.

***Table 3-8:** Kinetic constants for a mixed culture converting sulphide to sulphur (Mamashela, 2002).*

Variable	Value	Units
K_s	2.941	mmole l^{-1}
Y	0.0512	mmole $mmole^{-1}$
μ_{max}	1.272	day^{-1}

6.1.5 Sulphur Settling Tank

In the second settling tank, the elemental sulphur formed in the aerobic reactor was recovered. It was assumed that 90% of the sulphur settled (Janssen, 1999 and Ryan, 2004) and the biomass sludge settled at the same efficiency as in the biomass settling tank (i.e. 65% for primary sewage sludge and 44% for the ethanol and molasses systems). The water content of the sludge produced was assumed to be 80% (Sincero and Sincero, 1996 and Toll, 2004). The results of the mass balance across the sulphur settling tank are shown in Table 6-4. Stream 13 represents the effluent from the aerobic reactor entering the settler. Stream 14 is the overflow from the settler, which is the final effluent of the process disposed to the river. Stream 15 is the sludge produced from the sulphur settling tank, forming a potential elemental sulphur product.

Less than 0.4% of the water that entered the sulphur settler was removed with the sludge. The soluble components were assumed to have the same split as that of the water. Of the COD that entered the settler, 64% reported to the sludge stream and was removed from the effluent. Sulphur made up 4.6% of the sludge stream.

Table 6-4: Results of the mass balance around the sulphur settling tank for AMD site 2 using sewage sludge as the carbon source and electron donor (results in kg day^{-1}) (Streams: 13-liquid entering the settler, 14- liquid overflow, 15- sulphate sludge).

	13	14	15
Proteins	389	136	253
Carbohydrates	192	67	125
Lipids	275	96	179
Amino acids	4.20	4.19	0.01
Glucose	0.511	0.510	0.001
Glycerol	0.613	0.611	0.002
Palmitic acid	12.3	12.3	0.03
Hydrogen	0	0	0
Acetate	8.86	8.84	0.02
Lactate	0.303	0.302	0.001
Propionate	38.1	38.0	0.1
Butyrate	14.1	14.1	0.05
Sulphates	246	245	0.9
Hydrogen sulphide	8.42	8.39	0.03
Carbon dioxide	952	949	3.5
Methane	0	0	0
Ammonia	8.79	8.76	0.3
Ethanol	0	0	0
Hydrochloric acid	172	171	1
Sulphur	228	22.8	206
Bacteria	204	71.7	133
Water	992000	990000	3580
COD	2370	850	1520

6.1.6 Complete Mole and Mass Balance Using Primary Sewage Sludge

The complete mole and mass balances across the proposed flowsheet for AMD treatment at AMD site 2 using primary sewage sludge as a substrate are presented in Tables 6-5 and 6-6.

The tables show that there was a high reduction of insoluble components in the effluent. Proteins were reduced by 91%, carbohydrates by 93% and lipids by 91%. The concentration of the sulphides in the final effluent stream (Stream 14) was at a significantly lower concentration than that produced from the reactor. A final H_2S concentration of 8.5 mg l^{-1} was achieved. 173 kg of hydrochloric acid was needed each day to lower the pH from 7.41 to the optimal levels for sulphide oxidation.

Analysis of these results and the results presented in Section 6.2 and 6.3 are presented in Section 6.4.

***Table 6-5**: Mole balance of AMD site 2 using primary sewage sludge as substrate (results in moles day^{-1}) (Streams: 1-AMD, 2-carbon substrate, 3-anaerobic reactor exit gas, 4-anaerobic liquid effluent, 5-HCl, 6-mixer exit gas, 7-combined exit gas, 8-liquid effluent from the mixer, 9-liquid overflow from settler, 10-sludge, 11-air, 12-aerobic gas exit, 13-liquid entering the sulphur settler, 14-liquid overflow of sulphur settler, 15-sulphate sludge).*

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	13700	0	9800	0	0	9800	0	3400	6300	3400	0	0	1200	2200
Carbohydrates	0	6700	0	3400	0	0	3400	0	1200	2200	1200	0	0	400	800
Lipids	0	1400	0	1000	0	0	1000	0	300	600	300	0	0	100	200
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	4500	0	100	0	0	100	0	100	0	100	0	0	100	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	500	0	0	500	0	500	0	500	0	0	500	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	19100	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	1900	14800	0	7400	7400	9200	7400	100	200	0	0	200	0
Carbon dioxide	0	0	8400	21600	0	0	21800	8400	21600	200	21600	0	0	21500	100
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	500	0	0	500	0	500	0	500	0	0	500	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4700	0	4700	0	4700	0	4700	0	0	4700	0
Sulphur	0	0	0	0	0	0	0	0	0	0	7100	0	0	700	6400
Oxygen	0	0	0	0	0	0	0	0	0	0	0	592800	589200	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2230000	2230000	0	0
Bacteria	0	0	0	4100	0	0	4100	0	1400	2700	1800	0	0	600	1200
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.51E+07	4.21E+05	5.51E+07	0	0	5.49E+07	1.99E+05

***Table 6-6**: Mass balance at AMD site 2 using primary sewage sludge as substrate (results in kg day^{-1}) (Streams: 1-AMD, 2-carbon substrate, 3-anaerobic reactor exit gas, 4-anaerobic liquid effluent, 5-HCl, 6-mixer exit gas, 7-combined exit gas, 8-liquid effluent from the mixer, 9-liquid overflow from settler, 10-sludge, 11-air, 12-aerobic gas exit, 13-liquid entering the sulphur settler, 14-liquid overflow of sulphur settler, 15-sulphate sludge).*

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1560	0	1110	0	0	1110	0	390	720	390	0	0	140	250
Carbohydrates	0	1090	0	550	0	0	550	0	190	360	190	0	0	70	120
Lipids	0	1150	0	790	0	0	790	0	280	510	280	0	0	100	180
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	270	0	10	0	0	10	0	10	0	10	0	0	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	40	0	0	40	0	40	0	40	0	0	40	0
Butyrate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
sulphates	1830	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	60	500	0	250	250	310	250	0	10	0	0	10	0
Carbon dioxide	0	0	370	960	0	0	960	370	950	10	950	0	0	950	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	170	0	170	0	170	0	170	0	0	170	0
Sulphur	0	0	0	0	0	0	0	0	0	0	230	0	0	20	210
Oxygen	0	0	0	0	0	0	0	0	0	0	0	18970	18850	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	62440	62440	0	0
Bacteria	0	0	0	470	0	0	470	0	160	300	200	0	0	70	130
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	9.92E+05	7580	9.92E+05	0	0	9.89E+05	3580

6.2 Treatment of AMD at AMD Site 2 Using Molasses as Carbon Source: Results of the Mass Balance

The composition of molasses was presented in Section 3.2. The primary fermentative sugar in molasses is sucrose and each mole of sucrose was modelled in the reactor as two moles of glucose (following action of invertase and glucose isomerase). Only the fermentative and non-fermentative sugars and proteins were represented in the mass balance. The water content and salts were considered to be negligible. The water content is justifiable as it would only add 690 l day^{-1} to the system, based on molasses having a 23.5% water content (United States Sugar Corporation, 2004). The results of the mole and mass balances using molasses as the carbon source and electron donor for the treatment of acid mine drainage at AMD site 2 are shown in Tables 6-7 and 6-8 respectively.

Of the glucose entering the system, 91.3% reacted i.e. 95.3% of the fermentative sugars reacted. The sulphate level was decreased to less than 250 mg l^{-1} in the effluent stream. The sulphide concentration in the exit effluent stream was 8.5 mg l^{-1}. A hydrochloric acid addition of 169 kg was used to reduce the pH from 7.41 to 7.05. No hydrogen was evolved from the anaerobic reactor owing to hydrogen being the limiting reagent when H_2 and CO_2 react. No methane was produced as sulphate reducers outcompeted methanogens in the presence of sulphate. Of the 220 kg day^{-1} of elemental sulphur produced, 198 kg was removed from the system each day.

***Table 6-7**: Mole balance of AMD site 2 using molasses as substrate (results in moles day^{-1})*

(Streams: 1-AMD, 2-carbon substrate, 3-anaerobic reactor exit gas, 4-anaerobic liquid effluent, 5-HCl, 6-mixer exit gas, 7-combined exit gas, 8-liquid effluent from the mixer, 9-liquid overflow from settler, 10-sludge, 11-air, 12-aerobic gas exit, 13-liquid entering the sulphur settler, 14-liquid overflow of sulphur settler, 15-sulphate sludge).

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1800	0	1800	0	0	1800	0	1000	800	1000	0	0	500	400
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	7500	0	700	0	0	700	0	700	0	700	0	0	700	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	19100	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
Hydrogen sulphide	0	0	2500	14200	0	7000	7100	9600	7100	0	300	0	0	300	0
Carbon dioxide	0	0	12200	22000	0	0	22000	12200	22000	0	22000	0	0	22000	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	100	0	100	0	0	100	0	100	0	100	0	0	100	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4600	0	4600	0	4600	0	4600	0	0	4600	0
Sulphur	0	0	0	0	0	0	0	0	0	0	6900	0	0	700	6200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	6.E+05	6.E+05	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2.E+06	2.E+06	0	0
Bacteria	0	0	0	1200	0	0	1200	0	700	500	1000	0	0	800	500
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	33400	5.55E+07	0	0	5.55E+07	66500

***Table 6-8**: Mass balance of AMD site 2 using molasses as substrate (results in kg day^{-1})*

(Streams: 1-AMD, 2-carbon substrate, 3-anaerobic reactor exit gas, 4-anaerobic liquid effluent, 5-HCl, 6-mixer exit gas, 7-combined exit gas, 8-liquid effluent from the mixer, 9-liquid overflow from settler, 10-sludge, 11-air, 12-aerobic gas exit, 13-liquid entering the sulphur settler, 14-liquid overflow of sulphur settler, 15-sulphate sludge).

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	180	0	200	0	0	0	200	110	90	0	0	110	80	50
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	1350	0	120	0	0	0	120	120	0	0	0	120	120	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	10	0	0	0	10	10	0	0	0	10	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	10	0	0	0	10	10	0	0	0	10	10	0
sulphates	1830	0	0	250	0	0	0	250	250	0	0	0	250	250	0
hydrogen sulphide	0	0	90	480	0	450	530	40	40	0	0	0	10	10	0
Carbon dioxide	0	0	540	970	0	0	540	970	970	0	0	0	970	970	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	170	0	170	0	0	0	170	170	0	0	0	170	170	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	170	0	0	170	170	0	0	0	170	170	0
Oxygen	0	0	0	0	0	0	0	0	0	0	2170	2160	0	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	7150	7150	0	0	0
Sulphur	0	0	0	0	0	0	0	0	0	0	0	0	30	0	20
Bacteria	0	0	0	140	0	0	0	140	80	60	0	0	80	50	40
Water	1000000	0	0	1000000	0	0	0	1000000	999400	600	0	0	999400	999000	440

6.3 Treatment of AMD at AMD Site 2 Using Ethanol as Carbon Source: Results of the Mass Balance

The results of the mole and mass balances using ethanol as the carbon source and electron donor for the treatment of acid mine drainage at AMD site 2 are shown in Tables 6-9 and 6-10 respectively. These results demonstrate that low levels of insoluble compounds were produced when using ethanol. Sulphate levels in the effluent stream were reduced to below 250 mg l^{-1}. The high sulphide levels produced in the anaerobic reactor were reduced to 8.5 mg l^{-1} in the final effluent stream. The rates of biomass sludge disposed and sulphur produced were 187 kg per day and 257 kg per day respectively. Of the latter, 232 kg sulphur was removed via the sulphur settling tank. Some 182 kg of hydrochloric acid was needed each day to lower the pH from 7.41 to 7.05.

***Table 6-9**: Mole balance at AMD site 2 using ethanol as substrate (results in moles day^{-1})*
(Streams: 1-AMD, 2-carbon substrate, 3-anaerobic reactor exit gas, 4-anaerobic liquid effluent, 5-HCl, 6-mixer exit gas, 7-combined exit gas, 8-liquid effluent from the mixer, 9-liquid overflow from settler, 10-sludge, 11-air, 12-aerobic gas exit, 13-liquid entering the sulphur settler, 14-liquid overflow of sulphur settler, 15-sulphate sludge).

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Protein	0	0	0	100	0	0	100	0	100	0	100	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	19100	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	0	16500	0	8200	8300	8200	8300	0	300	0	0	300	0
Carbon dioxide	0	0	0	21100	0	0	21100	0	21100	0	21100	0	0	21000	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	1000	0	300	0	0	300	0	300	0	300	0	0	300	0
Ethanol	0	12100	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	5000	0	5000	0	5000	0	5000	0	0	5000	0
Sulphur	0	0	0	0	0	0	0	0	0	0	8000	0	0	800	7200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	667900	663900	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2512700	2512700	0	0
Bacteria	0	0	0	600	0	0	600	0	400	300	800	0	0	400	300
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	8320	5.55E+07	0	0	5.55E+07	8.07E+04

Table 6-10: *Mass balance at AMD site 2 using ethanol as substrate (results in kg day^{-1})*

(Streams: 1-AMD, 2-carbon substrate, 3-anaerobic reactor exit gas, 4-anaerobic liquid effluent, 5-HCl, 6-mixer exit gas, 7-combined exit gas, 8-liquid effluent from the mixer, 9-liquid overflow from settler, 10-sludge, 11-air, 12-aerobic gas exit, 13-liquid entering the sulphur settler, 14-liquid overflow of sulphur settler, 15-sulphate sludge).

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	10	0	0	10	0	10	0	10	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	1830	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	0	560	0	280	280	280	280	0	10	0	0	10	0
Carbon dioxide	0	0	0	930	0	0	930	0	930	0	930	0	0	930	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	560	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	180	0	180	0	180	0	180	0	0	180	0
Sulphur	0	0	0	0	0	0	0	0	0	0	280	0	0	30	230
Oxygen	0	0	0	0	0	0	0	0	0	0	0	21370	21250	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	70360	70360	0	0
Bacteria	0	0	0	70	0	0	70	0	40	30	90	0	0	50	40
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	1.00E+06	150	1.00E+06	0	0	9.99E+05	1090

6.4 Combined Results for the Three Substrates Across the Three AMD Sites

For each of the AMD sites considered, the material balance was performed on a basis of 1 000 m^3 of AMD being treated per day. The difference in the quality of effluent being treated was the concentration of sulphate present. It was assumed that these AMD streams are largely metal free owing to their prior precipitation as metal sulphides. The concentrations of sulphate found in the AMD effluents at each of the AMD sites are shown in Table 6-11. Comparisons across the three AMD sites with the three carbon sources are made in terms of substrate requirements, sludge produced, H_2S evolved as a gas, HCl required and COD produced. Results of the material balances for the three AMD sites using the three carbon sources are presented in Appendix D (Tables D-1 to D-18).

Table 6-11: *Influent sulphate concentration for each of the AMD sites.*

AMD site	Sulphate level (mg l^{-1})
Site 1	1 437
Site 2	1 833
Site 3	2 248

6.4.1 Carbon Substrate Required as Carbon Source and Electron Donor

The first important comparison is the substrate requirements across the three different AMD sites using the three substrates. The mass of substrate required is shown in Table 6-12. For each carbon source used at the three different sites, the third AMD site having the highest influent sulphate concentration, needed the greatest amount of carbon substrate. For any particular AMD site, the lowest mass of carbon substrate required was ethanol and the highest was sewage sludge.

Table 6-12: Mass of substrate required (results in kg day^{-1}).

AMD site	Ethanol	Molasses	Primary sewage sludge
Site 1	420	2 120	3 130
Site 2	557	2 800	4 070
Site 3	703	3 510	5 110

On average, one mole of carbon is needed to convert one mole of sulphate to sulphide (Equation 2-19). Based on this, it was expected that more moles of ethanol would be required than either molasses or primary sewage sludge, due to ethanol only having two carbon atoms per molecule. Table 6-13 shows a comparison of the moles of substrate required. Molasses is not included in this comparison due to the difficulty in finding the molar mass of molasses or the ash found in the molasses. Sewage sludge was modelled to have a carbon content of seven atoms per molecule (Section 3.2). Hence it would be expected that 3.5 times less sewage sludge would be needed than ethanol on a molar basis. Taking into account the fact that sewage sludge contained a 33.45 % non- degradable portion, it was still expected that more ethanol would be needed than primary sewage sludge, however primary sewage sludge metabolised slower than ethanol. Primary sewage sludge was the only carbon source analysed that underwent hydrolysis. When hydrolysis is included in the reaction scheme, it is generally the rate limiting step and it slows the process down quite considerably (Eastman and Ferguson, 1981). To compensate for the reduction in the rate of substrate consumption, a larger amount of substrate was needed.

Table 6-13: Moles of substrate required at each AMD site (moles day^{-1}).

AMD site	Ethanol	Primary sewage sludge
Site 1	9 120	20 300
Site 2	12 100	26 400
Site 3	15 300	33 100

6.4.2 Biomass Sludge Produced

The waste stream (stream 10) from the first settling tank consisted of biomass, un-used substrate, intermediatory substrates and unconverted sulphates and sulphides. All waste that was extracted from this settler was taken to a landfill for disposal. Waste production in the biological treatment of acid mine drainage is of great importance as it adds significantly to the cost of the operation due to the high landfill costs. Table 6-14 shows the amount of waste produced in kilograms per day and the COD removed from the settler using different carbon sources at the three AMD sites. The results showed that the more complex the carbon source, the more sludge it produced i.e. systems using primary sewage sludge produced the most sludge whereas systems using ethanol produced the least. The high amounts of waste produced when using primary sewage sludge as the carbon substrate can be attributed to incomplete hydrolysis as well as the non-degradable fraction of the carbon substrate. Because of the low rates of reaction for hydrolysis, most of the degradable sludge did not break down and hence added to the insoluble components that settled.

Systems using ethanol as the carbon substrate had the lowest removal of COD, whereas systems using primary sewage sludge had the highest. The increasing removal rate of COD for increasing complexity of carbon substrate was expected, as the more complex the carbon source is, the higher the influent COD concentration to the system and hence the higher the removal rate. The burden reduction potential for the wastewater treatment works by using primary sewage sludge as a carbon source is discussed in Section 8.4.

Table 6-14: Mass of sludge disposed and COD removed from the first settler each day (kg day^{-1}).

AMD site	Ethanol		Molasses		Primary sewage sludge	
	Sludge	COD	Sludge	COD	Sludge	COD
Site 1	141	40	568	161	7290	2577
Site 2	187	53	752	213	9 480	3352
Site 3	236	67	945	267	11 900	4207

6.4.3 H_2S Removal from the System

Hydrogen sulphide is a toxic substance and needs to be removed from the effluent, at least to acceptable limits. H_2S was removed from the system at three different points. The first was from the anaerobic reactor as gas that was evolved, the second was from the mixer, again as a gas, and the third was on its conversion to and removal as elemental sulphur. Gaseous H_2S that is removed from the system was taken to the precipitation unit and hence was not treated any further.

Sulphides were formed in the anaerobic reactor as products of sulphate reduction. Once the water became saturated with the $H_2S(aq)$, the H_2S then formed an equilibrium between the gaseous and dissolved state and some H_2S left the system as a gas. The amount of H_2S gas that left the system from the anaerobic reactor for each of the systems is shown in Table 6-15.

In three cases there was no H_2S gas produced. This can be attributed to the water not being saturated with H_2S in its aqueous form. AMD site 3 produced the most gaseous H_2S as it has the highest concentration of sulphates entering the reactor. AMD site 1 produced the least owing to the lower concentration of sulphates entering the reactor.

Table 6-15: Amount of H_2S gas leaving reactor (in moles day^{-1}).

AMD site	Ethanol	Molasses	Primary sewage sludge
Site 1	0	436	0
Site 2	0	2 510	1 850
Site 3	2 070	5 350	4 580

The second point in the system where H_2S was removed as a gas was the mixer. The amount of H_2S gas leaving each of the systems from the mixer is shown in

Table 6-16. The results show that for each of the substrates the AMD site 1 evolved the least amount of H_2S gas and AMD site 3 the most.

***Table 6-16**: Amount of H_2S gas leaving the mixer (in moles day^{-1}).*

AMD site	Ethanol	Molasses	Primary sewage sludge
Site 1	6 180	5 970	6 160
Site 2	8 210	7 050	7 370
Site 3	9 450	7 920	8 370

The H_2S gas from both the anaerobic reactor as well as the mixer are combined and then sent to the precipitation unit. The total amount of H_2S gas produced from both the anaerobic reactor and the mixer is shown in Table 6-17. The results showed that each AMD site produced a similar amount of H_2S gas across the different carbon sources being used. As expected, AMD site 1 produced the lowest amount of H_2S and AMD site 3 the most.

***Table 6-17**: Amount of H_2S gas sent to precipitation unit (in moles day^{-1}).*

AMD site	Ethanol	Molasses	Primary sewage sludge
Site 1	6 180	6 400	6 160
Site 2	8 220	9 560	9 220
Site 3	11 500	13 300	13 000

The production of sulphur arises from the aerobic oxidation of sulphides. The sulphur settling tank removed 90% of the sulphur produced in the aerobic reactor. The amount of sulphur produced from each of the systems is shown in Table 6-18. The results show that the amount of sulphur produced was a function of the influent sulphate concentration i.e. AMD site 1 produced the least amount of sulphur and AMD site 3 the most. The sulphur produced has some monetary value associated with it i.e. if it is recovered in a sufficiently concentrated form, it can form a product rather than a disposal cost.

***Table 6-18**: Amount of sulphur removed from effluent in the sulphur settling tank (in kg day^{-1}).*

AMD site	Ethanol	Molasses	Primary sewage sludge
Site 1	172	166	170
Site 2	232	197	205
Site 3	267	223	234

Apart from sulphur removal, the sulphur settling tank removed COD and sludge from the water prior to the effluent being released into the river. Table 6-19 shows the amount of sludge as well as the COD removed from the effluent. The results follow a similar trend to that of the first settling tank with the more complex carbon substrates systems removing a higher amount of COD.

Comparing Table 6-18 and Table 6-19, sulphur formed 17% of the sludge stream when ethanol was used as the carbon substrate, 13% when molasses was used and 4% when primary sewage sludge was used. The assumption that the sludge produced from the sulphur settling tank may have a monetary value associated with it is invalid owing to the low percentages of sulphur in the sludge. If the biomass was retained and the COD used more efficiently, then a higher concentration of sulphur would be obtained. A disposal cost associated with this stream should be accounted for in future work and compared to the process costs of improving the sulphur purity.

***Table 6-19:** Mass of sludge disposed and COD removed from the sulphur settler each day (kg day^{-1}).*

AMD site	Ethanol		Molasses		Primary sewage sludge	
	Sludge	COD	Sludge	COD	Sludge	COD
Site 1	1 020	303	1 220	360	3 520	1 190
Site 2	1 370	406	1 500	393	4 480	1 520
Site 3	1 590	472	1 740	512	5 490	1 870

6.4.4 HCl Utilised

HCl was needed to lower the pH from 7.41 to 7.05 to achieve optimal conditions for the aerobic reactor. Table 6-20 shows the amount of HCl needed for each plant using the various substrates. As with the amount of H_2S gas produced, the amount of HCl required was shown to be a function of the influent sulphate concentration i.e. AMD site 3 required the highest amount of HCl of the three AMD sites regardless of which substrate was used and AMD site 1 the least.

The choice of the carbon substrate impacted the amount of HCl required from 0.6% to 14%. The sulphate concentration of the AMD site impacted the amount of HCl required from 7% to 31%. These results show that the HCl required was more

sensitive to changes in influent sulphate concentrations rather than the choice in carbon substrate.

Table 6-20: Amount of HCl required (in kg day^{-1}).

AMD site	Ethanol	Molasses	Primary sewage sludge
Site 1	137	156	157
Site 2	182	169	173
Site 3	198	179	185

6.4.5 COD Reduction

The system was designed such that the sulphate concentration leaving the system in the effluent was below EPA levels of 250 mg l^{-1} and the sulphide concentration was below 10 mg l^{-1}, as above this level health issues arise in the drinking of this water (Washington State Department of Health, 2001). The final water quality parameter to be addressed was the COD concentration in the final effluent.

On adding carbon source to the AMD, COD is added to the effluent. The more complex the carbon source, the higher was the concentration of COD introduced into the system. Table 6-21 shows the percent removal of COD from the system and Table 6-22 shows the concentration of the COD in the final effluent stream.

The results show that although there was a high removal of COD from all the systems (the lowest being 75.4%), the concentration of COD remaining in the treated water stream when using molasses and primary sewage sludge is high. The generally accepted COD level in the effluent from wastewater treatment works is 75 mg l^{-1} (DWAF, 1996 and Finn, 2004). Only the ethanol systems were able to approach this requirement in the way in which this flowsheet was configured. To use molasses or primary sewage sludge as the carbon source, the COD levels have to be reduced. This could be achieved by using a reactor system with a high retention of viable biomass eg. UASB or BioSure systems as well as improved solids removal systems. Modification of this analysis to include these systems is recommended in future studies.

Table 6-21: Percentage COD removed from the system.

AMD site	Ethanol	Molasses	Primary sewage sludge
Site 1	87.2	75.4	87.3
Site 2	88.3	76.8	88.0
Site 3	89.6	77.8	88.4

Table 6-22: Concentration of COD in the final effluent (mg l^{-1}).

AMD site	Ethanol	Molasses	Primary sewage sludge
Site 1	116	315	1 200
Site 2	140	394	1 540
Site 3	155	473	1 890

6.5 Summary

This chapter has presented an example of the mass balance using primary sewage sludge as the substrate at AMD site 2. Each unit was considered individually. The example was then concluded by presenting the mole and mass balances over the entire plant. The mass balance of AMD site 2 using ethanol and molasses as substrates were then presented.

Detailed mass balances for the three AMD sites using the three carbon substrates are presented in Appendix D. Comparisons across all three AMD sites using each of the substrates were made. The results showed that AMD site 3 required the highest concentration of substrate owing to the highest concentration of sulphate entering the system. AMD site 3 also had the highest production of H_2S gas from both the anaerobic reactor as well as the mixer. As AMD site 3 treated the highest concentration of sulphate, it also produced the highest amounts of by-products. In the same respect, AMD site 1 treated the lowest concentration of sulphates and produced the least amount of by-products.

It was assumed that the sludge produced from the sulphur settler would have a monetary value associated to it. However, when comparing the percentage of sulphur in the sulphur sludge stream it was apparent that this assumption is not correct for the configuration used. Systems using ethanol as the carbon substrate had a sulphur content in the sulphur sludge stream of 17%, whereas systems using molasses and

primary sewage sludge had a sulphur content of 13% and 4% respectively. A system that has a high biomass retention and uses COD more efficiently would possibly achieve this.

The simulation was set up such that the final effluent would have a sulphate concentration of less than the EPA standard of 250 mg l^{-1} and a sulphide level of less than 10 mg l^{-1}. The recommended COD level in the final effluent was 75 mg l^{-1}. Only systems using ethanol as a carbon substrate approached this criterion with the treatment configuration used. Both the molasses and primary sewage sludge systems failed to achieve this. For molasses or primary sewage sludge to be used, a reactor that could uncouple the hydraulic residence time and solids residence time and have high solids retention would be required. Examples of these undergoing assessment for practical implementation are the UASB and falling sludge bed reactor (Ristow, 1999, Molwantwa *et al.*, 2004). Modification of the reactor simulation model to account for this is recommended.

Chapter 7

METHODOLOGY OF ECONOMIC ANALYSIS

A primary aim of the project was to compare the economic viability of the use of a range of potential carbon sources and electron donors in biological sulphate reduction in order to treat the required volumes of AMD at specific sites. Further aims addressed the feasibility of each substrate in terms of its availability, proximity and impact on resultant water quality and waste disposal. To address the former, an economic analysis of the process using each organic compound proposed was performed. Carbon sources considered include ethanol, molasses and sewage sludge.

A common basis was required to allow an effective comparison between the different carbon sources. A volumetric flow rate of AMD entering the system of 1 000 $m^3 day^{-1}$ and a reactor size of 10 000 m^3 for both reactors were chosen as the basis for assessment, thereby setting the hydraulic residence time in the anaerobic reactor at 10 days. Influent sulphate concentrations for each of the three AMD sites are shown in Table 7-1.

***Table 7-1**: Water Quality Parameters for the Mine Effluent Sources (in mg l^{-1}).*

Water Quality Parameter	AMD site 1	AMD site 2	AMD site 3
Sulphate	1 437	1 833	2 248

This section presents the methodology used for the economic analysis. In Section 7.1, the capital costing associated with the construction of the plant is detailed. Section 7.2 defines the predicted operating costs of the process.

7.1 Capital Cost

The capital cost includes those costs associated with the construction of a new plant and its ancillaries (Turton *et al.*, 1998). According to the process flow sheet presented in Figure 3-1, the major components of the plant are: three holding tanks, one for the acid mine drainage, one for HCl and the other for the organic substrate; two continuously stirred reactors, one anaerobic and one aerobic; two settlers; and a mixer. For the capital costing of the plant, each of these units is sized and then costed. The sizing of the units is presented in Section 7.1.2. Further the three pumps defined in the flow sheet and the associated pipe work are specified and costed.

7.1.1 Materials of Construction

Prior to costing the various units, the materials of construction needed to be selected. Two types of materials were considered:

1) Stainless steel, and
2) Type 5 concrete with appropriate lining.

The 316 stainless steel is an austenitic type of stainless steel containing 18 to 20 percent chromium and a nickel content of higher than 7 percent (Sinnott, 2000). Molybdenum is added to this alloy to improve its corrosion resistance under reducing conditions.

The concrete is reinforced with carbon steel ripple bar at half meter intervals both horizontally and vertically. The lining used may either be epoxy polyurethane or calcium aluminate to resist corrosion (Sinnott, 2000).

7.1.2 Sizing of Equipment

The sizes of the two reactors were selected to be 10 000 m^3 each. This provided a 10 day hydraulic residence time in the anaerobic reactor. The mixer was sized to be 1 m^3 providing a residence time of 8.64 seconds. However, the sizing of the settling tanks and the holding tanks needed to be calculated.

7.1.2.1 Settling Tank

The sizing of the settling tank was based on the method given by Sincero and Sincero (1996). Rossle and Pretorius (2001) reviewed the characterisation requirements for in-line prefermenters and stated that the overflow rate should be less than 2 m h^{-1} to ensure that settleable particles are able to gravitate out of suspension. The overflow rate was set at 1.5 m h^{-1}. The overflow area could then be found by dividing the flow rate by the overflow rate. This also provides the settling zone diameter. The detention time was set to 1.5 h (Sincero and Sincero, 1994). The settling zone of the tank was calculated by multiplying the detention time with the flowrate. The height of the settling zone is the quotient of the settling zone of the tank and the overflow area. The diameter of the tank is then calculated by adding the settling zone diameter to the inlet and outlet zones. The inlet and outlet zones are each equal to the settling zone depth. The depth of the tank is calculated by adding the settling zone depth with the free board and sludge zone. Both the free board and sludge zone can be assumed to have lengths of 0.5 m (Sincero and Sincero, 1994).

7.1.2.2 Holding tanks

Three holding tanks are needed for the acid mine drainage, carbon substrate and hydrochloric acid. To ensure continuous flow of AMD to the system, the AMD holding tank was set to 6 000 m^3 so that the system could operate for six days at an inlet flowrate of 1 000 m^3 day^{-1} if there is a blockage of the AMD water into the system.

The size of the substrate tank was based on the amount of substrate required from the mass balance and the density of the substrate. The densities of the substrates are shown in Table 7-2.

For primary sewage sludge, the substrate holding tank volume was to hold a three day feed volume. Both molasses and ethanol substrate holding tanks were calculated to hold a feed volume of 28 days. This was based on delivery of these substrates to the site at least once every four weeks. For ethanol, nutrients will be added to this tank.

***Table 7-2:** Densities of carbon substrates.*

Substrate	Density (kg l^{-1})	Reference
Primary sewage sludge	1.1	Assumed
Molasses	1.438	United States Sugar Corporation (2004)
Ethanol	0.789	Triangle Solvents (2004)

From the results of the mass balance, the amount of hydrochloric acid needed was always less than 200 kg day^{-1}. This value was chosen as the basis for the sizing of the HCl tank. The density of HCl is 1193 kg m^{-3} (Sinnott, 2000). This amounts to a volume of 167.6 l day^{-1}. Assuming that HCl will be transported every four weeks, the volume of the tank can be calculated to be 4.7 m^3.

7.1.3 Costing of Major Equipment

Turton *et al.* (1998) states that: "The most accurate estimate of the purchased cost of a piece of major equipment is provided by a current price quote from a suitable vendor. The next best alternative is to use cost data on previously purchased equipment of the same type". Ball and Schroeder (2001) had costed similar units from D.B Fabricators, an engineering firm located in Kwa-Zulu Natal, and DEL Cut and Supply (Pty) Ltd., located in the Gauteng region. The quotes for the major equipment from these

suppliers differed by less than half a percent. These were used as a basis for costing the vessels. The costing of the major units occurred in two steps. Firstly, the cost of the unit was scaled on a volume basis using the findings of Ball and Schroeder (2001) to the cost of the volume of the unit used in this study, using Equation 7-1 provided by Turton *et al.* (1998):

$$\frac{C_a}{C_b} = \left(\frac{A_a}{A_b}\right)^n \tag{7-1}$$

where A = Equipment cost attribute
C = Purchased cost
n = Cost exponent

subscripts a = equipment with required attribute
b = equipment with base attribute

Secondly, the costs were adjusted to take inflation into account over the time period from 2001 to the present. This was done using the Marshall & Swift Equipment Cost Index, applied according to Equation 7-2 (Turton *et al*, 1998):

$$C_2 = C_1\left(\frac{I_2}{I_1}\right) \tag{7-2}$$

where C = Purchased cost
I = Cost index

subscripts 1 = base time when cost is known
2 = time when cost is not known

This method was used to calculate the cost of the holding tanks, reactors, settlers, and the mixer. It was assumed that the mixing tank could be costed from the reactor due to the similarities in equipment.

7.1.4 Costing of Ancillary Equipment

The ancillary equipment used in this system included three pumps and a compressor. Costing of the pumps was estimated in the same manner as that of the major pieces of equipment.

The cost of the compressor was calculated based on the factorial method, according to Equation 7-3 (Sinnott, 2000). Inflationary effects were taken into account in the same manner as for the major equipment pieces, using Equation 7-2.

$$C_e = CS^n \tag{7-3}$$

where C_e = purchased equipment cost
S = characteristic size parameter
C = cost constant
n = index for that type of equipment

Following costing of major and ancillary equipment, the remainder of the total capital cost was calculated based on costing factors provided in Turton *et al.* (1998) and Sinnott (2000). These factors used are reproduced in Table 7-3. The total equipment cost (TEC) is found by summing the cost of the individual units of equipment. The remainder of the capital cost of the plant is calculated based on this value such that the total capital investment can be calculated.

Table 7-3: Factors Used For Capital Cost Estimation.

Factor	Multiplying factor used in economic evaluation
Total Equipment Cost	TEC
Erection, foundations and minor structural work[1]	0.45TEC
Piping and fitting[1]	0.5TEC
Instrumentation[1]	0.15TEC
Electrical power[1]	0.1TEC
Site development[2]	0.1TEC
Process buildings[2]	0.1TEC
Total Physical Plant Cost (PPC)	2.4 TEC
Design and engineering[1]	0.25PPC
Contractors fee[1]	0.05PPC
Contingency[2]	0.2PPC
Total Fixed Capital required (FCR)	1.5 PPC
Cost of Land[2]	0.06FCR
Total Capital (FCI)	1.06FCR

1 – Sinnott (2000)
2 – Turton *et al.* (1998)

7.2 Operating Costs

As stated by Sinnott (2000), "An estimate of the operating costs, the cost of producing the product, is needed to judge the viability of the project, and to make choices between possible alternative processing schemes". In terms of this project, the operating cost is calculated based on an operating performance, which allows the sulphate concentration to be reduced to below EPA standards of 250 mg l^{-1}.

Operating costs can be divided into two groups, namely:

1) Fixed operating costs: These are costs that do not vary with treatment rate. They are expenses incurred that are independent of the quantity produced.
2) Variable operating costs: These are costs that are dependent on the amount of effluent treated.

7.2.1 Fixed Operating Costs

The fixed operating costs consist of insurance, patents and royalties, maintenance and repairs as well as labour. These were calculated using the factorial method. Factors defined by Turton *et al.* (1998) and Sinnott (2000) are given in Table 7-4.

Table 7-4. *Factorials Used to Calculate the Fixed operating costs.*

Factor	**Multiplication factor used in economic evaluation**
Total Fixed Capital Investment	FCI
Insurance[2]	0.03FCI
Patents and royalties[1]	0.02FCI
Maintenance and repairs[1]	0.01FCI
Labour	COL
Total Fixed Operating Costs (FOC)	0.06FCI+COL

1 – Sinnott (2000)
2 – Turton *et al.* (1998)

To calculate the cost of labour it was assumed that the plant required two operators working in shifts as well as two supervisors. It was assumed that the operators would be paid an annual salary of R53 750 each whereas the supervisors would be paid R88 000 each annually (Gunning, 2004). Minimal management time would be needed for this plant and it was not considered in the labour calculations.

7.2.2 Variable Operating Costs

Four major variable contributions to operating costs need to be considered. They are:

1) Cost of the substrate;
2) Cost of hydrochloric acid;
3) Cost of utilities (primarily electricity); and
4) Disposal costs.

7.2.2.1 Cost of Substrate

The price quoted for ethanol was R4.95 per litre including the cost of transport (Triangle Solvents, 2004). The quality of the ethanol would be a minimum of 92% ethanol by mass. Impurities in the ethanol include iso-propanol and water which would have a maximum content of 8.0% and 0.4% by mass respectively.

Molasses can be obtained from the Voermol Feeds (du Plessis, 2004) at a price of R1367.8 per ton. This price includes the cost of transport. Animal grade molasses was used, as it is the cheapest molasses available.

Using primary sewage sludge as a carbon substrate would incur no raw material costs or transport costs, provided the location of a sewage plant is nearby to the plant site. Construction of a pipe line between the sewage plant and the plant site would be needed, contributing to the capital expenses. It was assumed that this expense would be included in the total equipment costs. Springs has a population of 214 600 and Witbank 198 500 (Helders, 2004). Assuming each person produces 73 grams of sludge per day (National Research Council, 1996), Springs and Witbank would produce 16 738 kg day^{-1} and 15 483 kg day^{-1} of sludge respectively. The largest amount of primary sewage sludge required was by AMD site 3, at 5 110 kg day^{-1}. This shows that there is a sufficient population to provide for the AMD sites.

The cost of 32% hydrochloric acid was quoted at R161 for 25 litres from Merck (Jagels, 2004).

7.2.2.2 Electrical Costs

The major requirement for electricity is to power the compressor, pumps and agitator. To calculate the total work done by each pump, Equation 7-4 was used (Sinnott, 2000):

$$Power = \frac{\Delta P Q_p}{\eta_p} \tag{7-4}$$

where ΔP = pressure differential across the pump (N m^{-2})

Q_p = flow rate (m^3 s)

η_p = pump efficiency

The efficiency of the pump was taken to be 0.65 (Sinnott, 2000). The operating cost of the pump was then calculated as the product of the power required and cost of electricity. The cost of electricity is R0.19 kW h^{-1} (ESKOM, 2004).

For the agitator power consumption, Sinnott (2000) estimates the power requirements of agitated tanks for various applications. It is estimated that for mild reactions that a power requirement of 0.04 – 0.1 kW m^{-3} would be attained. Hence a value of 0.07 kW m^{-3} was assumed. From this value, the power cost can be calculated.

To calculate the work done by the compressor, Sinnott (2000) provides the following equation for an isothermal compressor:

$$-W = \frac{RT}{M} \ln\left(\frac{P_2}{P_1}\right) \tag{7-5}$$

where W = work done (J g^{-1})

R = Universal gas constant (8.314 J K^{-1} mol^{-1})

T = Temperature (K)

M = molecular mass of gas (g mol^{-1})

P_1 = initial pressure (bar)

P_2 = final pressure (bar)

The initial pressure was atmospheric pressure, approximately 1 bar. Air is usually distributed at 6 bar (Sinnott, 2000), hence the final pressure was set at 6 bar. The compressor was assumed to have an efficiency of 65% (Sinnott, 2000). The power cost was then calculated.

7.2.2.3 Disposal Costs

The cost of disposal of ten 210 litre drums into a landfill was quoted as being R2 877 (Ncube, 2004). The price included the cost of transporting the waste from the site to the landfill. The expected cake solids concentration of digested sludge is 20 % solids (Sincero and Sincero, 1996). This is the same concentration used by the Cape Flats Treatment Works (Toll, 2004). The mass of sludge produced per day from the settling tank is known from the mass balance. Using these values, the cost of waste disposal for the year can be calculated.

Sulphur sludge was initially not considered as a disposal cost as sulphur, which is relatively pure, could be sold. As stated in Section 6.4.3, this assumption was proved to be invalid for the process flowsheet used in this study and further work would be required to address this. For the purposes of this study, this assumption was retained.

7.3 Summary

This chapter detailed the methodology of the economic analysis. Included in this chapter is the methodology of obtaining the capital costs as well as the operating costs of the plant.

Chapter 8

RESULTS OF ECONOMIC EVALUATION

This chapter aims to compare the economic viability of the use of a range of potential carbon sources and electron donors to treat the required volumes of AMD at specific sites. The chapter starts with an example of the economic analysis performed on AMD site 2 using primary sewage sludge as the carbon source. Economic analyses on the three AMD sites using the three carbon substrates can be found in Appendix E. Furthermore, a comparison of the capital and operating costs is presented for each of the AMD sites using each of the carbon sources.

8.1 Treatment of AMD from AMD Site 2 Using Primary Sewage Sludge as Carbon Source: Detailed Example of the Economic Evaluation

A detailed example of the economic results of AMD site 2 using primary sewage sludge as the carbon substrate and type 5 concrete as the material of construction is presented. Prior to the costing of the equipment, the sizing of the units for this case study is presented.

8.1.1 Sizing of Equipment

Both reactors were sized at 10 000 m^3. The mixer was sized at 1 m^3. The hydrochloric acid holding tank was sized at 4.7 m^3 and the AMD holding tank as 6000 m^3 (Section 7.1.2.2).

The amount of substrate required for this plant was 4 070 kg day^{-1} primary sewage sludge (Section 6.1.6). The density of primary sewage sludge was assumed to have a density of 1.1 kg l^{-1}. The capacity of the holding tank for primary sewage sludge was set for three days; hence the volume of the tank was 11.1 m^3.

The volume of the settling tank was calculated according to Sincero and Sincero (1996), detailed in Section 7.1.2. The flowrate into the settling tank was 1 000 m^3 day^{-1}. The overflow velocity was assumed to be 1.5 m h^{-1} (Rossle and Pretorius, 2001), resulting in a settling area of 27.8 m^2 with a settling diameter of 5.95 m. The detention time was assumed to be 1.5 h (Sincero and Sincero, 1996). The settling zone, calculated by multiplying the flowrate by the detention time, was 62.5 m^3. The height of the settling zone, calculated by dividing the settling zone by the area of the settling zone, was 2.25 m.

The diameter of the settling tank of 10.45 m was equated to the diameter of the settling zone plus two times the height of the settling zone. The depth of the tank of 3.25 m is calculated as the height of the settling zone plus free board and sludge zone, which are both assumed to be 0.5 m. This then gives a volume of the settling tank as 278.6 m^3. The same calculations were applied to both settling tanks.

8.1.2 Capital Costs

The major equipment contributions to the capital costs of the plant were the two reactors, the two settlers, the mixer and the three holding tanks. The calculation of the cost of the anaerobic reactor is presented as an example. The results of the rest of the major equipment are presented in Table 8-1, based on similar calculations.

Ball and Schroeder (2000) costed an anaerobic reactor with a volume of 1 094 m^3 at R186 000 in 2000. The volume of reactor used in this study was 10 000 m^3. To scale the cost according to the size, Equation 7-1 is used.

$$\frac{C_a}{C_b} = \left(\frac{A_a}{A_b}\right)^n \qquad (7\text{-}1)$$

where A = Equipment attribute
C = Purchased cost
n = Cost exponent

subscripts a = equipment with required attribute
b = equipment with base attribute

The cost exponent of the reactor is 0.6 (Turton *et al.*, 1998). Solving this equation

$$C_a = C_b\left(\frac{A_a}{A_b}\right)^n = R186000\left(\frac{10000}{1094}\right)^{0.6} = R702000$$

The cost of a reactor with a volume of 10 000 m^3 was approximated as R702 000 in 2000. The effect of inflation between the years 2000 and 2004 was taken into account using Equation 7-2. The Marshall & Swift equipment cost indices were used. The Marshall and Swift index value for 2000 is given as 1086 and for 2004 as 1140 (Cost Indices, 2004).

$$C_2 = C_1\left(\frac{I_2}{I_1}\right) = R\ 702000 \quad \left(\frac{1140}{1086}\right) = R\ 735000 \qquad (7\text{-}2)$$

The cost of the reactor was estimated as R735 000 in 2004. The results of the capital costs for all major equipment is presented in Table 8-1.

Table 8-1: Results of using the factorial method for the sizing and costing of the major units.

	Volume used by Ball and Schroeder (m^3)	Cost quoted by Ball and Schroeder (R)	Volume used in this study (m^3)	Cost in this study (R)
Anaerobic reactor	1 093.5	186 022	10 000	735 000
Aerobic reactor	1 093.5	186 022	10 000	735 000
Mixer	1 093.5	186 022	1	2 925
Settling tank	729	171 757	278	101 000
Sulphur settling tank	729	171 757	278	101 000
AMD holding tank	237.4	68 722	6 000	459 000
Substrate holding tank	237.4	68 722	12.2	10 500
HCl holding tank	237.4	68 722	4.7	6 280

8.1.3 Ancillary Equipment

8.1.3.1 Pumps

The pump costs were calculated in the same manner as that of the major equipment. Ball and Schroeder quote the price of a pump as being R10 000. Using the same method of calculation as for the major equipment, the cost of a pump is R10 500. There were three pumps hence the cost of the pumps is R31 500.

8.1.3.2 Compressor

The factorial method was used to calculate the capital cost of the compressor (Equation 7-3). The cost constant, the index value as well as the size range applicable was provided by Sinnott (2000). These values are provided in Table 8-2.

Table 8-2: Cost factors used in Equation 7-3 (Sinnott, 2000).

Equipment	**Size range** (S, driver power, kW)	**Cost constant** (C, £)	**Index** (n)
Centrifugal compressor	20-500	580	0.8

Using the Equation 7-3,

$$Cost = C_e = CS^n = 580(223.0)^{0.8} = £43\ 900. \quad (7\text{-}3)$$

The calculation for the driver power is shown in Section 8.1.4.2c. Taking inflation into account, the cost now becomes £47 100. Assuming a conversion rate of R12.50/£, the cost of the compressor is estimated at R589 000.

A summary of the capital costs and estimate using the factorial method for the complete capital cost of the plant, described in Chapter 7, is presented in Table 8-3.

8.1.4 Operating Costs

8.1.4.1 Fixed Operating Costs

Included in the fixed operating costs are the costs of insurance, patents and royalties, maintenance and repairs and labour costs. It was assumed that eight operators working between four shifts (two per shift) would be needed and each would be paid a salary of R53 750 a year. It was also assumed that four supervisors would be needed and each would be paid a salary of R88 000 a year. This gives a total labour cost of

Table 8-3: *Results of the capital cost calculations for AMD site 2 using primary sewage sludge as the carbon substrate.*

Description	Factor	Cost (Rands)
Major equipment		
Substrate tank		10 500
AMD tank		459 000
HCl tank		6 280
Anaerobic reactor		735 000
Aerobic reactor		735 000
Settler 1		101 000
Settler 2		101 000
Mixer		2 930
Major equipment total		2 150 000
Ancillary Equipment		
Pump 1		10 500
Pump 2		10 500
Pump 3		10 500
Compressor		589 000
Ancillary Equipment Total		621 000
Total Equipment Cost	TEC	2 770 000
Erection, foundation and minor structural work	0.45TEC	1 250 000
Piping and fittings	0.5TEC	1 360 000
Instrumentation, local and control room	0.15TEC	416 000
Electrical, power and lighting	0.1TEC	277 000
Site development	0.1TEC	277 000
Process buildings	0.1TEC	277 000
Total Physical Plant Cost	PPC	6 650 000
Design and engineering	0.25PPC	1 660 000
Contractors fee	0.05PPC	332 000
Contingency	0.2PPC	1 330 000
Total fixed Capital Required	FCR	9 970 000
Cost of land	0.06FCR	598 000
Total Capital	FCI	10 600 000

Table 8-4. *Results of the fixed operating costs for AMD site 2 using primary sewage sludge as the substrate.*

Factor	Multiplying factor used in economic evaluation	Amount (R/year)
Total Fixed Capital Investment	FCI	10 600 000
Insurance[2]	0.03FCI	317 000
Patents and royalties[1]	0.02FCI	211 000
Maintenance and repairs[1]	0.01FCI	106 000
Labour	COL	782 000
Total Fixed Operating Costs (FOC)	0.06FCI+COL	1 420 000

1 – Sinnott (2000)
2 – Turton *et al.* (1998)

R782 000 a year. Other fixed operating costs were calculated based on the factorial method described in Section 7.2.1. The results are presented in Table 8-4.

8.1.4.2 Variable Operating Costs

The three variable operating costs considered were electricity, disposal costs, and raw material costs. With primary sewage sludge, it was assumed that there would be no cost for the substrate. The potential negative substrate cost due to burden reduction is discussed in Section 8.4.

8.1.4.2a) *Electricity Needed for Stirrers*

Sinnott (2000) stated that for mild mixing, the power requirement would range between 0.04 and 0.10 kW m^{-3}. A power requirement of 0.07 kW m^{-3} was assumed. Using a volume of 10 000 m^3, a power requirement of 700 kW was needed. Electricity was costed at R0.19 per kWhr (Eskom, 2004). Hence, the cost of electricity to drive each agitator was R1 170 000 per year. This calculation applied to both stirrers, hence the total cost of electricity to power the stirrers was R2 340 000 per year.

8.1.4.2b) *Electricity Needed for Pumps*

The power required by the pump was calculated using Equation 7-4.

$$Power = \frac{\Delta P Q_p}{\eta_p} \tag{7-4}$$

The pressure difference across the pump was assumed to be 100 000 N m^{-2} (Sinnott, 2000). The efficiency of the pump was assumed to be 65% (Sinnott, 2000). The flowrate into the pump was 1 000 m^3. Hence, solving Equation 7-4, the power requirement of the pump is 1 780 J s^{-1}. This results in an annual electricity cost of R2 960 per pump. Considering three pumps, the total cost of electricity for the pumps is R8 890.

8.1.4.2c) *Electricity Needed for Compressor*

The work done by the compressor was calculated using Equation 7-5.

$$-W = \frac{RT}{M} \ln\left(\frac{P_2}{P_1}\right) \tag{7-5}$$

The final pressure was assumed to be 6 bar and the initial pressure 1 bar. The work done by the compressor was found to be 154 kJ kg^{-1}. As the compressor was assumed to be 65% efficient, the total work done was 237 kJ kg^{-1}. From the mass balance, the flow into the compressor is 81 400 kg day^{-1}. Thus the work done is calculated to be 223 kJ s^{-1}. This is the value used for the compressor sizing in Section 8.1.3.2. Using the same method as with the pumps (Section 8.1.4.2a), the electrical cost of the compressor amounts to R372 000 per year.

8.1.4.2d) *Disposal Costs*

The mass of sludge that was sent for disposal was 9 480 kg day^{-1}. The density of the sludge stream was calculated based on a weighted average of 20% solids and 80% water. Arnold (1995), who studied solid waste management in Addis Ababa, reported a solids density of 230 kg m^{-3}. Using this value, a sludge density of 846 kg m^{-3} was obtained. This value falls within the range given by Sincero and Sincero (1996) for landfill densities, which range from 297 to 891 kg m^{-3}. Using this value, the volume of sludge produced was 11 200 l day^{-1}. The volume of a disposal drum is 210 l (Ncube, 2004). Hence, in one year, 19 500 drums would be needed. The disposal of a drum was costed at R288 (Ncube, 2004). Thus the cost of disposal was R5 600 000 per year. The potential negative substrate cost due to burden reduction is discussed in Section 8.4.

8.1.4.2e) *Cost of Hydrochloric Acid*

The amount of HCl required was 173 kg day^{-1}. The density of HCl is 1 193 kg m^{-3} (Sinnott, 2000). The cost of HCl as given by Merck was R161 for 25 litres with a

concentration of 32% (Jagels, 2004). Thus taking the concentration into account, the cost of HCl for the year is R1 070 000.

A summary of the operating costs for this example is presented in Table 8-5. The results showed that the variable operating costs and fixed operating costs accounted for 87.4% and 12.6% of the total operating costs respectively. The biggest contributor to the operating cost was the disposal costs, which accounted for 54.1% of the total operating costs. Labour only accounted for 7.5% of the total operating costs. Detailed economic results of the three AMD sites using the three carbon substrates are presented in Appendix E. Economic comparisons across the three AMD sites using the three carbon sources are presented in Section 8.3.

Table 8-5: *Results of the operating cost for AMD site 2 using primary sewage sludge as the carbon substrate.*

Description		
Variable operating costs	**Factor**	**Annual cost** (R/yr)
Electricity		2 710 000
Disposal costs		5 600 000
Hydrochloric cost		1 070 000
Total variable operating costs		9 380 000
Fixed operating costs		
Cost of labour		
Total operators per shift		2
Number of shifts		4
Salary per annum		53 750
Total cost of operating labour		430 000
Total supervisors per shift		1
No. of shifts		4
Salary per annum		88 000
Total cost of supervisory labour		352 000
Total Cost Of Labour Per Annum	COL	782 000
Maintenance and repairs	0.01FCI	106 000
Insurance	0.03FCI	317 000
Patents and royalties	0.02FCI	211 000
Total fixed operating costs		1 420 000
Total Cost of operation	CO	10 800 000

8.2 Comparison Between Materials of Construction

Two types of material of construction were considered:

1) Stainless steel
2) Type 5 concrete with appropriate lining.

Table 8-6 compares the capital costs using the two materials of construction for AMD site 2 where molasses was used as the carbon substrate. Cost estimates for the equipment made from stainless steel were sourced from Ball and Schroeder (2000) and the capital costs estimates were obtained in a similar manner to that shown in the above example (Section 8.1.2).

The results show that using stainless steel was sixteen fold more expensive than using reinforced concrete. It is clear that stainless steel is prohibitively expensive and other solutions such as concrete should be sought. Hence, all other economic comparisons were performed using reinforced concrete as the material of construction.

***Table 8-6**: Comparison of capital cost using different materials of construction.*

Description	Factor	Stainless Steel	Reinforced concrete
Major equipment			
Substrate tank		426 000	19 100
AMD tank		6 770 000	459 000
HCL tank		92 700	6 280
Anaerobic reactor		14 000 000	735 000
Aerobic reactor		14 000 000	735 000
Settler 1		4 940 000	101 000
Settler 2		4 940 000	101 000
Mixer		55 700	2 930
Major equipment total		45 200 000	2 160 000
Ancillary Equipment			
Pump 1		10 500	10 500
Pump 2		10 500	10 500
Pump 3		10 500	10 500
Compressor		571 000	571 000
Ancillary Equipment Total		603 000	603 000
Total Equipment cost	TEC	45 800 000	2 760 000

8.3 Economic Comparison of AMD Sites Using each of the Substrates

To assess the economic effects of the three carbon substrates, a cost comparison between using the different substrates was needed. A comparison of the capital costs of each of the plants is shown Table 8-7.

Table 8-7: *Comparison of the total capital cost for each of the AMD sites using each of the substrates.*

AMD sites	Ethanol	Molasses	Primary sewage sludge
Site 1	10 300 000	10 500 000	10 300 000
Site 2	10 800 000	10 500 000	10 600 000
Site 3	11 100 000	10 500 000	10 800 000

Table 8-7 showed that the capital costs for each of the plants were relatively similar. This was expected as all of the major equipment pieces were sized equally for each of the plants with the substrate holding tank being the exception. Table 8-3 showed that the substrate holding tank accounted for 0.1% of the total capital when using primary sewage sludge as the carbon substrate for AMD site 2. Hence, changes in the cost of the substrate holding tank will not have a significant effect over the total operating cost of the system. Because of the similarity in capital costs in the analysis methodology used, the choice of carbon feedstock is a function of the operating costs only.

Figure 8-1 showed the operating costs at each of the AMD sites using the three carbon substrates. The positive slope of the lines indicated that, according to expectation, the higher the influent concentration of sulphate was, the higher the operating costs were. With the methodology used, primary sewage sludge was estimated to be much more expensive than either molasses or ethanol and that molasses is slightly more expensive than ethanol. To understand these results, a comparison of the components of the operating costs needed to be considered.

Since the fixed operating costs for all three plants were the same, only the variable operating costs will be considered. The three main variable operating costs considered are the hydrochloric acid, substrate and disposal costs. Due to the nature of the electrical calculations, the electrical costs of the plants were approximated to be the same and will not be compared.

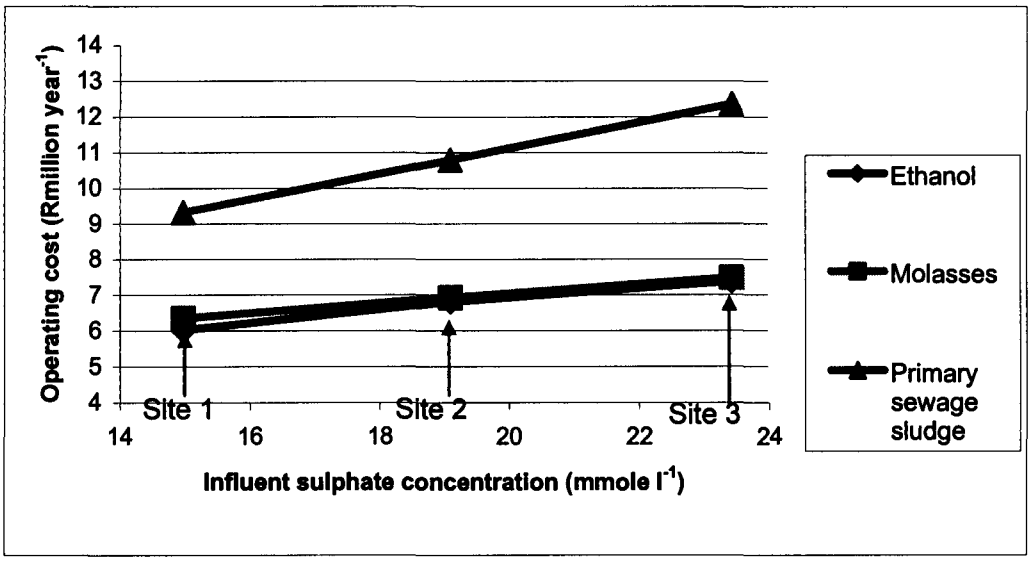

Figure 8-1: *Comparison of operating costs for each of the AMD sites, in the absence of burden reduction.*

8.3.1 Hydrochloric Acid Costs

The cost of the hydrochloric acid for each case study is shown in Table 8-8. The results showed that, as expected the cost of hydrochloric acid increased as the influent sulphate concentration increased. Fluctuations of the cost of hydrochloric acid by using different carbon sources can be attributed to the amounts of HS^- and bicarbonate formed in the liquid. As shown in Table 6-2, these are the two ions most affected by the change in pH. Hence, variations in the concentration of these ions have a direct effect on the cost of the hydrochloric acid.

Table 8-8: Cost of hydrochloric acid.

AMD sites	Ethanol	Molasses	Primary sewage sludge
Site 1	846 000	961 000	969 000
Site 2	1 120 000	1 040 000	1 070 000
Site 3	1 220 000	1 110 000	1 140 000

8.3.2 Substrate Costs

Primary sewage sludge was assumed to have zero cost associated to it and is not included in this analysis. The cost of the pipeline for primary sewage sludge was assumed to be included in the construction costs. The negative cost of using primary sewage sludge is considered in Section 8.4. Figure 8-2 gives a comparison of the substrate costs at the three AMD sites for two carbon substrates. The positive slope of the lines in Figure 8-2 corresponds to more carbon substrate being used to treat a higher concentration of sulphate. Figure 8-2 shows that the substrate cost of molasses is less than ethanol.

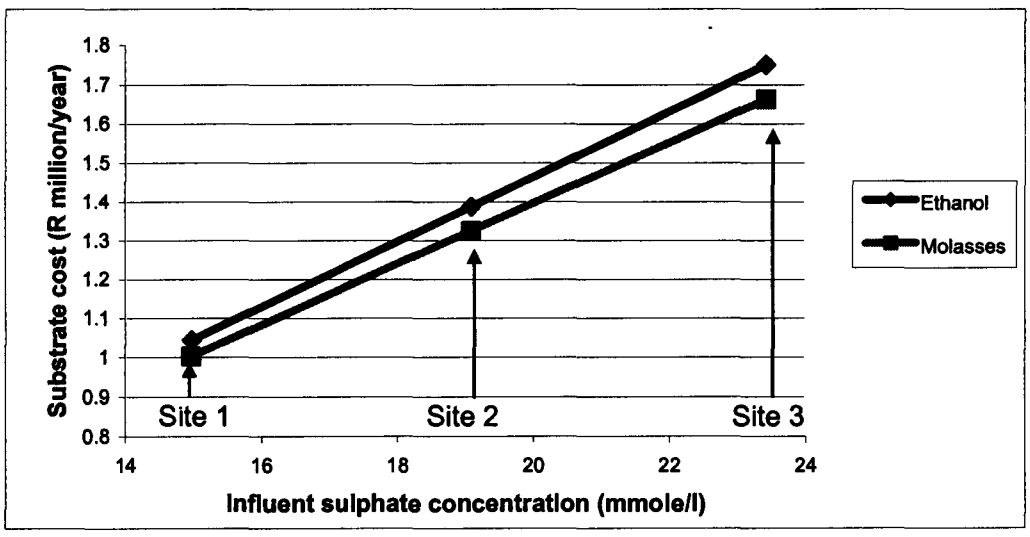

Figure 8-2: Substrate costs at each of the AMD sites.

8.3.3 Disposal costs

Figure 8-3 shows that the disposal costs for biomass sludge from the first settler is much higher when primary sewage sludge was used as the substrate than with other substrates. The more complex the carbon source is, the higher the disposal costs. The disposal cost of primary sewage sludge is about 50 times more expensive than ethanol and 12.5 times more expensive than molasses. The benefit of the negative cost and burden reduction of using primary sewage sludge is considered in Section 8.4.

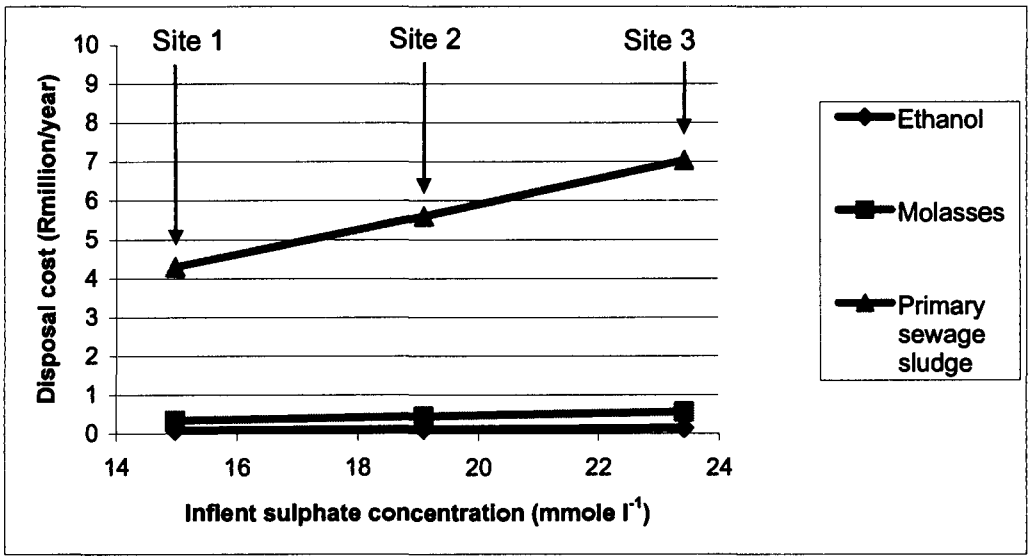

Figure 8-3: Disposal costs for each of the AMD sites.

8.4 Burden Reduction Potential on Using Primary Sewage Sludge as a Carbon Substrate

Figure 8.1 showed that using primary sewage sludge was the most expensive carbon substrate with this process configuration. Figure 8-3 shows that this can be attributed to the high disposal costs. However, this cost should be offset against the reduction in cost of not having to dispose of the primary sewage sludge in a wastewater treatment works. In other words, the primary sewage sludge would have to be disposed of in any case, hence using it as the carbon substrate in AMD treatment would reduce the amount that would be sent to a wastewater treatment works. The cost saved by not disposing of the fraction of primary sewage sludge that was degraded should be subtracted from the disposal costs and the operating costs as it is a savings.

Table 8-9 shows the true operating cost of using primary sewage sludge for the three AMD sites. The cost saved was calculated by the difference between the cost of disposing all primary sewage sludge that enters the AMD treatment system and the cost of disposal in this process. The true operating cost was then the difference between the operating cost found in Figure 8-1 and the cost saved.

***Table 8-9**: The true operating cost of using primary sewage sludge for each of the AMD sites.*

AMD site	Cost of landfilling all primary sewage sludge entering system (R year^{-1})	Cost saved by not disposing of fraction that degraded (R year^{-1})	True operating cost (R year^{-1})
Site 1	9 270 000	4 960 000	4 360 000
Site 2	12 000 000	6 440 000	4 360 000
Site 3	15 100 000	8 070 000	4 290 000

Figure 8-4 presents the operating costs taking the savings owing to burden reduction using primary sewage sludge into account. The results showed that, the operating cost attributed to primary sewage sludge proved to be the most economically viable option of the three carbon sources analysed. Further it shows that on use of a complex particulate carbon source of zero cost, where disposal of the particulate carbon source is not legislated but may for example be used as compost, bears the highest operating cost, assuming the need to dispose of the biomass sludge from the AMD treatment process to landfill. This assumption was made owing to the presence of sulphide and metal components in the sludge.

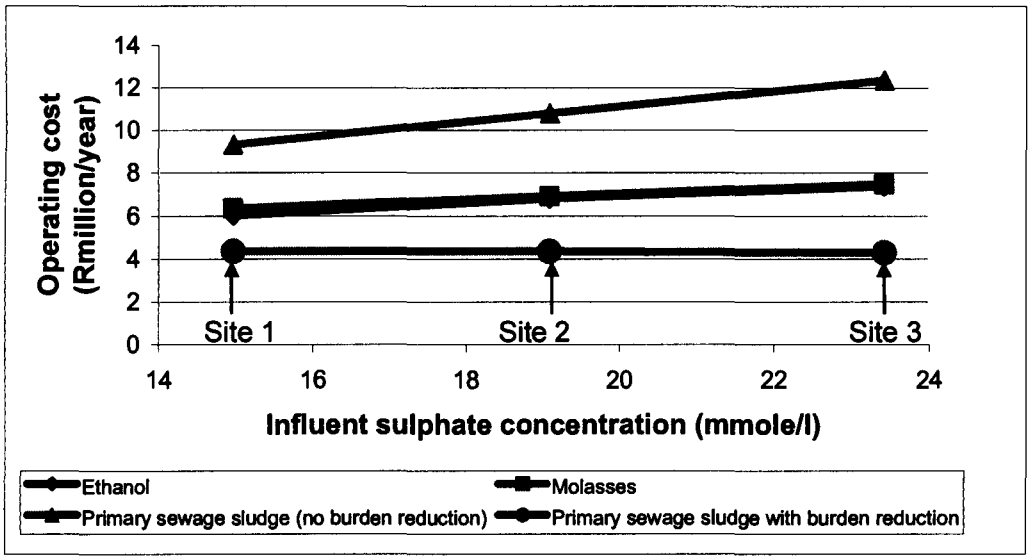

***Figure 8-4**: Operating costs of the three AMD sites using the three carbon sources and the true operating cost of using primary sewage sludge.*

8.5 Summary

The results showed that using stainless steel as the material of construction was economically unviable as it was found to be sixteen fold more expensive than using reinforced concrete.

Due to the nature of the calculations, the capital costs of the three AMD sites with each of the carbon substrates were similar. Differences arose due to the sizing of the substrate tank. However, the substrate tank accounted for 0.1% of the total capital costs.

Each of the factors governing the variable operating costs was dependent on the choice of carbon source. Figure 8-2 showed that a more complex carbon source (molasses) was cheaper than a simple carbon source (ethanol) and Figure 8-3 showed that the disposal cost of a complex carbon source was higher than for a simple carbon source. Figure 8-1 shows that in this study, ethanol was the most economically viable choice when the cost saving of not sending the primary sewage sludge for treatment was not taken into account. However, when the savings of not sending the primary sewage sludge from wastewater treatment for disposal was included (i.e. the negative

cost of the primary sewage sludge appreciated), primary sewage sludge proved to be the most economically viable option. This was an important finding as it showed that there was a high burden reduction on the wastewater treatment works and hence, primary sewage sludge should be strongly recommended for use in the treatment of acid mine drainage.

Chapter 9

AVAILABILITY OF CARBON SOURCE AND ITS IMPACT ON EFFLUENT QUALITY

This chapter deals with the impact of the carbon source on the effluent quality and the effect of the availability of the carbon source.

9.1 Impact of the Carbon Source: Effluent Quality

The effect of the substrate used can be considered on the following criteria:

- Amount and quality of biomass sludge produced
- Amount and quality of sulphur product formed
- Water quality

9.1.1 Biomass Sludge

Table 9-1 summarises the amount of biomass sludge produced as a function of the AMD site and carbon source. Further the COD content of the biomass sludge is compared. The bacteria present in the sludge and sulphate reporting to the sludge is compared across the three carbon sources for the case of AMD site 2 in Table 9-2.

The use of complex carbon sources, which are not fully biodegradable, is seen to increase the quantity of biomass sludge for disposal. Further the COD content of this

sludge is increased, as illustrated by the increase from a 28% COD content based on the ethanol process to a 35% COD content based on the primary sewage sludge (PSS) process. The sulphate removed with the biomass sludge product is seen to increase with the complexity of the carbon source.

Table 9-1: *Quality and quantity of biomass sludge produced on treating 1 000 m^3 per day AMD.*

Carbon source used ($kg\ day^{-1}$)	AMD site 1		AMD site 2		AMD site 3	
	Sludge produced ($kg\ day^{-1}$)	COD in sludge ($kg\ day^{-1}$)	Sludge produced ($kg\ day^{-1}$)	COD in sludge ($kg\ day^{-1}$)	Sludge produced ($kg\ day^{-1}$)	COD in sludge ($kg\ day^{-1}$)
Ethanol	141	40	187	53	236	67
Molasses	568	161	752	213	945	267
PSS	7 290	2 580	9 480	3 350	11 900	4 210

Table 9-2: *Quality of the biomass sludge on treatment of AMD from AMD site 2.*

Carbon source	Ethanol	Molasses	PSS
Bacteria ($kg\ day^{-1}$)	32	62	303
SO_4 ($kg\ day^{-1}$)	0.04	0.15	1.9

9.1.2 Sulphur Product

The excess sulphide formed is partially oxidised to elemental sulphur and recovered in its solid form (Fig. 3-1, Stream 15). The solid sulphur product reclaimed is described in Table 9-3. The residual COD material in the water stream treated, following use of complex carbohydrate as carbon source and electron donor, results in a much reduced sulphur content of this sulphur product while approximately the same mass of elemental sulphur is recovered. Hence, in order to form a sulphur product with monetary value, improved solid-liquid separation is required upstream of the oxidation reactor. This may be achieved using an anaerobic reactor with biomass retention or an improved solid-liquid unit operation.

Table 9-3: Quality of the elemental sulphur product formed.

Carbon source	AMD site 1 (kg day^{-1})			AMD site 2 (kg day^{-1})			AMD site 3 (kg day^{-1})		
	Total	**COD**	**S^0**	**Total**	**COD**	**S^0**	**Total**	**COD**	**S^0**
Ethanol	190	54	20	260	76	29	320	93	34
Molasses	420	121	19	550	157	24	680	194	27
PSS	2 660	935	20	3 460	1220	25	4 340	1 520	29

9.1.3 Effluent Water Quality

The use of a complex carbon source, while providing cost benefit, results in the increased level of residual COD in the treated effluent (Table 9-4). In order to meet the water quality standards, consideration of the addition of the carbon substrate to sulphate containing stream has to be made. Further, the need for subsequent treatment of the water released from the AMD treatment system must be considered. A possible solution for the reduction of the COD concentration is to employ a reactor that can separate the solids residence time from the hydraulic residence time and retain a high solids residence time.

Table 9-4: Quality of the treated effluent water.

Carbon source	AMD site 1 (kg day^{-1})		AMD site 2 (kg day^{-1})		AMD site 3 (kgday^{-1})	
	Total	COD	Total	COD	Total	COD
Ethanol	1 000 000	66	1 000 000	73	1 000 000	79
Molasses	1 000 000	267	1 000 000	336	1 000 000	408
PSS	989 000	649	991 000	803	994 000	972

9.2 Availability of the Carbon Source

The availability of the carbon source is a major factor in the choice of a carbon feedstock for the biological treatment of acid mine drainage. In this feasibility study, the potential use of industrial grade ethanol, molasses and primary sewage sludge were considered. The choice of carbon feedstock had to be based on its availability. The availability of the carbon source directly affected the cost of the substrate. This

impacted the operating costs of the treatment system. Examples explaining the effect of the availability of the carbon source are presented.

Ethanol was sourced from Triangle Solvents, Germiston, at a cost of R4.95 per litre (Triangle Solvents, 2004), which included the delivery costs to both the Far East Rand and Witbank. An alternative source for the ethanol is NCP Alcohols, located in Durban. Ethanol from NCP Alcohols was quoted at R8 per litre including transport to either location. The effects of this difference on the day-to-day running costs of the three AMD sites are shown in Table 9-5. The difference in price shows the importance of the location of the carbon feedstock. If ethanol were to become unavailable from Triangle Solvents, hence needing to be purchased from NCP Alcohols, the cost of the substrate would increase and this would cause the operating costs to increase accordingly.

***Table 9-5**: The cost of ethanol per day for each AMD site.*

Source of ethanol	Cost of Ethanol delivered to site (R per litre)	AMD site 1 (R per day)	AMD site 2 (R per day)	AMD site 3 (R per day)
NCP Alcohols	8.00	6 150	8 200	10 300
Triangle Solvents	4.95	3 990	5 310	6 710

A similar argument could be raised for molasses as that of ethanol. For this study molasses was sourced from Voermol Feeds (du Plessis, 2004), who quoted the cost of animal grade molasses at R1367.8 per ton. Molasses is a by-product from the sugar manufacturing industry and one of its major uses is as animal feed. Hence, the price of this product is subject to market related fluctuations. Were the demand for molasses to increase, the price of the molasses would increase accordingly.

For primary sewage sludge to be a sustainable option there needs to be minimum population in the surrounding area. Table 9-6 showed the amount of primary sewage sludge required for treatment of AMD at each of the AMD sites and the population required to generate this sludge requirement. The population required was based on an

average production of 73 g of primary sewage sludge per person per day (National Research Council, 1996).

***Table 9-6**: The amount of sludge and population required for each AMD site.*

	AMD site 1	AMD site 2	AMD site 3
Sludge required (kg/day)	3 130	4 070	5 100
Population required (people)	42 900	55 800	69 900

Currently the population of Witbank is 198 500 and towns in the Far East Rand have populations that are significantly more than the required populations e.g. Brakpan, Springs and Tembisa have populations of 228 700, 214 600 and 376 600 (Helders, 2004) respectively. With this current population, primary sewage sludge would be a viable option for these areas. However, if there were to be a sudden drop in the population owing to mine closures or the like, or a sudden increase in sulphate concentration or the volume of AMD requiring treatment, sewage sludge may become inadequate as a carbon source to treat AMD. It should be noted that, based on the South African communities located in the AMD generation regions, this would require at least a threefold change. Importing primary sewage sludge from other treatment plants could be considered, however the associated transport cost would increase the operating costs.

An alternative carbon source that has not been considered quantitatively in this project is vinasse. Vinasse is a residual substance from sugar alcohol distillation. Currently a sugar manufacturing company is dumping large volumes of vinasse from their refinery into the ocean (Von Blottnitz, 2004). This vinasse has a high COD value and could be used as a carbon source for the biological treatment of acid mine drainage. There has been very little research done into the composition of vinasse; however research done by Cortez and Perez (1997) found an approximate formula for it as $C_{0.0331}H_{0.0860}N_{0.0012}O_{0.0194}$. If this is used as the choice of carbon feedstock, the only substrate cost incurred would be its transportation from Durban to the AMD sites. It would be assumed that the company would supply the substrate at no cost, as it is a waste product. Further, its use would result in relief of an effluent disposal burden.

In each of the above examples, the importance of the availability of the carbon source is shown. Since the treatment of AMD will be ongoing for many years, the supply of the carbon source and electron donor will also have to be continuous for this duration. The option of buying ethanol if it can be attained at a low cost may seem to be a viable choice considering the low amounts of waste produced by using this carbon substrate. However, market related fluctuations in the cost of this substrate over time may cause it to be an uneconomical option.

Of the examples presented above, the most sustainable option of carbon source would be primary sewage sludge. Primary sewage sludge would always be available as long as there is a population in the area. The only requirement would be to have a population level above a certain threshold. The potential burden relief of using primary sewage sludge increases the attractiveness of this carbon source. The option of using waste from a company may be limited due to transport cost to the AMD sites. Additionally, if the companies were to be able to find an alternate use for their waste products, then alternate carbon and electron donors would need to be found.

9.3 Summary

The impact of the carbon source on the effluent quality was considered. The COD content in the biomass sludge product increased from a 28% COD content based on the ethanol process to a 35% COD content based on the primary sewage sludge process. The solid sulphur product reclaimed was approximately the same for all cases. The use of a complex carbon source, while providing a cost benefit, resulted in the increased level of residual COD in the final treated effluent. Subsequent treatment of the water released from the AMD treatment system must be considered.

The effects of the availability of the carbon sources were considered. Primary sewage sludge was considered as the most sustainable carbon source due to the accessibility of it. Considering the duration of time required for the treatment of AMD, a carbon source that has to be bought becomes unattractive. Hence, readily available waste products would potentially be the best option. However, complex carbon sources would generally produce a higher disposal cost.

Chapter 10

RESULTS OF SENSITIVITY ANALYSIS

Four critical factors considered to affect the overall feasibility of the treatment plants were the substrate costs, disposal costs, sulphate loading as well as the hydraulic residence time. For analysis of sensitivity to these factors, the AMD site 2 was selected as the basis of analysis. To take into account the capital costs as well as the operating costs of the system, a 10 year cost analysis was performed. A straight line depreciation method was used to calculate the depreciation of the major equipment pieces. A salvage value of 10% of the major equipment was assumed. Comparisons were made between the original cost and the cost by altering one of these factors. Effluent quality is another major factor that should be analysed however, in this sensitivity analysis the sulphate concentrations were lowered to below EPA levels and the sulphide levels were lowered to 8.5 mg l^{-1}. Hence, by default these water quality standards were met and will not be further analysed in this section. Only the COD concentration in the final effluent was addressed. The sensitivity analysis takes into account the burden reduction of using primary sewage sludge.

10.1 Hydraulic Residence Time

To test sensitivity to hydraulic residence time, the system was analysed at lower hydraulic residence times of 9, 8 and 5 days by lowering the volume of the anaerobic reactor to 9 000 m^3, 8 000 m^3 and 5 000 m^3 respectively while the flow into the system remained at 1 000 m^3 of AMD per day. The primary factors considered in the analysis are the amount of substrate required, amount of sludge disposed, final COD

concentration in the effluent, capital costs and the operating costs of the system. Sulphates were reduced to the below acceptable EPA concentration of 250 mg l^{-1} and sulphides were reduced to below 10 mg l^{-1}.

Table 10-1 showed the amount of each carbon substrate required at the various hydraulic residence times. The amount of ethanol needed as the hydraulic residence time decreased, remained constant. Ethanol is a simple carbon compound and does not need the 10 day residence time used in the base study. The maximum rate of consumption was reached at a hydraulic residence time of less than 5 days. The amount of molasses needed, remained relatively constant as the hydraulic residence time was lowered to 8 days. However, further lowering of the hydraulic residence time to five days led to an increase in the amount of substrate required. This showed that the maximum rate of consumption for molasses lay between a hydraulic residence time of 5 and 8 days. At 5 days residence time, the consumption rate was lower than the feed rate, hence the fermentable substrate was not fully converted. To compensate for this more substrate was added. The amount of primary sewage sludge required increased as the hydraulic residence time decreased. Because of the slow rates of reaction for hydrolysis and the high fraction of non-degradable matter (33.45%), the majority of the primary sewage sludge remained undegraded and thus a larger amount of sludge was required.

***Table 10-1**: Amount of substrate required at various residence times at AMD site 2 (all results are in kg day^{-1}).*

Hydraulic residence time	Ethanol	Molasses	Primary sewage sludge
10	557	2 800	4 070
9	557	2 810	4 280
8	557	2 810	4 540
5	557	3 060	6 040

Because of the impact of the amount of sludge being sent to landfill and its effect on the operating cost of the system, this criterion needs to be considered. Table 10-2 showed the amount of sludge disposed to landfill at the various hydraulic residence times. Similar trends were found to those in Table 10-1. Disposal costs for the system using ethanol as the carbon substrate were unaffected by the changes to the residence time. Disposal costs for molasses increased and primary sewage sludge increased at a high rate. These trends are complementary to the substrate usage. For ethanol, the rate of consumption was not affected because the amount of sludge produced remained unaffected. For molasses, the amount of sludge disposed of remained relatively constant between residence times of 8 to 10 days, but increased appreciably when the residence time was lowered to 5 days owing to partial conversion of molasses. This resulted in extra sludge being formed and disposed of, hence the increase in sludge that was disposed of for molasses at a residence time of 5 days. Similarly, the amount of sludge disposed of when primary sewage sludge was used as a substrate increased, as seen in Table 10-2.

***Table 10-2**: Amount of sludge that is disposed of at various residence times at AMD site 2 (all results in kg day^{-1}).*

Hydraulic residence time	Ethanol	Molasses	Primary sewage sludge
10	187	752	9 480
9	187	754	10 100
8	187	756	10 900
5	187	811	15 200

Table 10-3 showed the COD concentration in the final effluent. As expected, the COD concentration in the effluent leaving the system to the river increased as the hydraulic residence time decreased for all the carbon substrates. The more complex carbon sources produced a higher concentration of COD and were more affected by the decrease in hydraulic residence time. All of the systems had failed to meet the COD water quality standards of 75 mg l^{-1} (DWAF, 1996 and Finn, 2004) with the

current process configuration. Lowering of the hydraulic residence time further added to the COD in the effluent.

Table 10-3: *Effects of decreasing hydraulic residence time on COD concentration in the final effluent at AMD site 2 (results in kg day^{-1}).*

Hydraulic residence time	Ethanol	Molasses	Primary sewage sludge
10	139	393	851
9	139	399	900
8	139	407	978
5	142	526	1 450

Only the capital cost of two pieces of major equipment were affected by the changes to the hydraulic residence time. By lowering the size of the anaerobic reactor, the capital cost for this major piece of equipment was lowered. The other unit that was affected was the substrate holding tank. This unit is dependent on the amount of substrate needed. A general decrease in capital costs for all substrates was seen as the hydraulic residence time decreased (Table 10-4). Table 10-1 showed that the amount of substrate required either increased or remained the same; hence the cost of the substrate tank would either increase or remain the same. At any residence time, the capital costs for all three systems were relatively similar and varied by less than 12%.

Table 10-4: *Capital costs of systems at various hydraulic residence times at AMD site 2 (Units in Rands).*

Hydraulic residence time	Ethanol	Molasses	Primary sewage sludge
10	10 800 000	10 500 000	10 600 000
9	10 600 000	10 400 000	10 400 000
8	10 400 000	10 200 000	10 200 000
5	9 840 000	9 580 000	9 600 000

Due to the changes in the amount of substrate required and the amount of sludge that needed to be disposed of to landfill, it is useful to consider the operating costs at various hydraulic residence times. Table 10-5 showed that the operating cost of the ethanol system decreased as the hydraulic residence time decreased. Since the substrate required and the amount of sludge produced remained the same, this decrease had to be attributed to some of the fixed operating costs of the system that are determined by the total capital costs i.e. the insurance, maintenance and repairs and patents and royalty costs. Molasses initially decreased in cost for the same reason as ethanol. However, the increase in cost from lowering the hydraulic residence time to 5 days was due to the increase in sludge disposal as well as substrate utilised. Similarly, the operating cost of primary sewage sludge systems increased as the hydraulic residence time decreased due to the increase in sludge disposal required both in the absence of accounting for burden reduction and when burden reduction was considered.

Table 10-5: *Operating costs of systems at various hydraulic residence times at AMD site 2 (units in Rands year^{-1}).*

Hydraulic residence time	Ethanol	Molasses	Primary sewage sludge (no burden reduction)	Primary sewage sludge (with burden reduction)
10	6 810 000	6 920 000	10 800 000	4 360 000
9	6 780 000	6 670 000	11 100 000	4 450 000
8	6 760 000	6 900 000	11 600 000	4 570 000
5	6 730 000	7 020 000	14 100 000	5 230 000

To take into account the effects to both the capital costs and the operating costs of the system, a 10 year cost analysis was performed. A straight line depreciation method was used to calculate the depreciation of major equipment. A salvage value of 10% of the major equipment was assumed. Comparisons were made between the cost of the base case system operated at a hydraulic residence time of 10 days and the cost of the altered system. Table 10-6 shows the change in the total cost on altering the hydraulic residence time. As the hydraulic residence time decreased, the cost of the systems using ethanol as the carbon substrate decreased. Negative values showed a lowering

in cost. The effect of using an anaerobic reactor of half the volume with ethanol as substrate only resulted in a 1.07% savings. For molasses, a 0.4% savings could be made by using a residence time of 8 days, however using a residence time of 5 days increased the cost by 1.71% over a residence time of 10 days. Using primary sewage sludge at a residence time of 5 days, the cost increased by 31.1% in comparison with a residence time of 10 days when burden reduction was not taken into account. This increase was only 7.44% when the burden reduction was taken into account, greatly decreasing the sensitivity of systems that utilise primary sewage sludge as the carbon source. However, primary sewage sludge systems still proved to be the most sensitive of the three carbon sources to changes in the hydraulic residence time even when the burden reduction potential of primary sewage sludge was taken into account. These results showed that the more complex the carbon source was, the higher the hydraulic residence time required. Further, choosing the correct residence time resulted in minimal costs.

***Table 10-6**: Percentage change in cost by reducing the hydraulic residence time (results in percentage).*

Hydraulic residence time	Ethanol	Molasses	Primary sewage sludge (no burden reduction)	Primary sewage sludge (with burden reduction)
9	-0.511	-3.63	3.33	0.830
8	-0.647	-0.352	7.40	2.06
5	-1.07	1.57	31.1	7.44

10.2 Substrate Costs

To test the sensitivity of the systems to the cost of the substrate, the cost of ethanol and molasses was increased by 10, 20 and 50%. A 10 year cost analysis was performed and compared to the original 10 year cost of the system. Primary sewage sludge was not included, as it does not have a direct cost associated with it in this approach to the analysis. Table 10-7 shows that molasses was slightly less sensitive

than ethanol to changes in substrate costs, although a greater mass of molasses was needed than ethanol. The significant differences between the sensitivity of the two carbon sources indicated that the more expensive the simple carbon source used, the more sensitive the system was to price changes.

***Table 10-7**: Percentage change in cost by increasing the substrate cost.*

Substrate Increases	Ethanol	Molasses
10%	2.23	2.07
20%	4.46	4.14
50%	11.1	10.4

10.3 Disposal Costs

To test the sensitivity of the system to changes in the disposal costs, the cost of disposal was increased by 10, 20 and 50%. A 10 year cost analysis was performed and compared to the 10 year cost of the base-case system. The results of this analysis are shown in Table 10-8. Primary sewage sludge was the most sensitive to an increase in disposal costs when burden reduction was not considered and ethanol the least. However, when the burden reduction was taken into account, the cost of using primary sewage sludge as the carbon substrate decreased by 36% when the disposal costs was increased by 50%. The primary sewage sludge that was utilised in the reduction process was a negative cost to the system due to the burden reduction potential of it. Since more than half the carbon substrate that entered the system was utilised, an increase in the disposal cost would result in an increase in the burden reduction potential (a higher negative value). Primary sewage sludge still remained the most sensitive to changes to the disposal cost. In this case, it was shown that the system became more sensitive to disposal costs as the carbon source became more complex.

Table 10-8: Percentage change in cost by increasing the disposal costs.

Disposal Increases	Ethanol	Molasses	Primary sewage sludge (no burden reduction)	Primary sewage sludge (with burden reduction)
10%	0.165	0.653	5.25	-7.21
20%	0.331	1.31	10.5	-14.4
50%	0.827	3.27	26.1	-36.0

Comparing Tables 10-7 and 10-8, ethanol was much more sensitive to changes in substrate cost than to disposal costs. Ethanol showed an 11.1% increase in cost when the substrate cost was increased by 50%, but only 0.8% increase in cost when the disposal costs were increased by 50%. Similarly, molasses was more sensitive to changes in substrate cost than to changes in disposal cost.

10.4 Sulphate Loading

To test the sensitivity of sulphate loading on the system, the sulphate concentration was increased by 10%, 20% and 50%. A 10 year cost analysis was performed and compared to the 10 year cost of the base-case system. The results of this analysis are presented in Table 10-9. The effects of increasing the sulphate concentration resulted in an increase in the amount of substrate needed and an increase in the amount of sludge disposal required. Primary sewage sludge, without the burden reduction being taken into account, proved the most sensitive substrate with molasses and ethanol showing similar sensitivities to changes in sulphate loading. When the burden reduction of primary sewage sludge was taken into account, a reduction of 29% in the cost was found. The primary sewage sludge that was utilised in the reduction process was a negative cost to the system, due to the burden reduction potential of it. Since more than half the carbon substrate was utilised, an increase in the amount of carbon substrate utilised would result in an increase in the burden reduction potential (a higher negative value). Primary sewage sludge experienced the highest sensitivity to influent sulphate concentration when the burden reduction was taken into account.

Table 10-9: Percentage change in cost by increasing the sulphate loading.

Sulphate increases	Ethanol	Molasses	Primary sewage sludge (no burden reduction)	Primary sewage sludge (with burden reduction)
10%	3.81	3.73	6.18	-5.44
20%	7.49	7.45	12.4	-11.2
50%	17.9	18.1	30.3	-29.0

Changes to the sulphate loading also affected the COD concentration of the final effluent that exited the system. Table 10-10 shows the COD concentration of the final effluent when the influent sulphate concentration was increased. As expected, the COD concentration in the final effluent increased as the influent sulphate concentration increased and this was most marked with complex carbon sources. When more sulphate was added to the system more substrate was needed, hence there was a higher COD concentration entering the system, which resulted in a higher COD concentration exiting the system. This could be addressed by the introduction of a reactor with high biomass retention and the separate hydraulic and solids residence times.

Table 10-10: Effect of sulphate loading on COD concentration of the final effluent.

Sulphate increases	Ethanol	Molasses	Primary sewage sludge
0%	139	393	851
10%	147	426	911
20%	153	461	985
50%	180	564	1190

10.5 Summary

The results showed that by lowering the hydraulic residence time, the amount of substrate required for systems using ethanol as the carbon substrate remained constant, whereas the amount of primary sewage sludge required increased i.e. substrate requirements increased with increasing complexity of the carbon source. The COD concentration in the final effluent remained relatively constant for ethanol and increased for systems using molasses and primary sewage sludge. The operating costs of primary sewage sludge systems proved to be the most sensitive with a decrease in hydraulic residence time, even when the burden reduction potential was taken into account. This finding was confirmed by a 10 year cost analysis across systems with hydraulic residence times of 10, 9, 8 and 5 days. Primary sewage sludge showed a 31.1% increase in cost over 10 years when the hydraulic residence time was decreased from 10 to 5 days when the burden reduction was not accounted for and a 7.44% increase in cost when the burden reduction was considered. Primary sewage sludge was the most sensitive to changes to the hydraulic residence time.

As expected, ethanol was more sensitive to changes in substrate cost than molasses due to ethanol being more expensive than molasses.

Primary sewage sludge was found to be the most sensitive to changes to disposal cost even when the burden reduction was taken into account. An increase in disposal cost lead to a decrease in the cost of primary sewage sludge systems when the burden reduction was taken into account. This was due to the substrate that was utilised having a negative cost to the system.

The primary sewage sludge system showed the greatest sensitivity to changes in sulphate loading when the burden reduction was not considered. When the burden reduction was accounted for, primary sewage sludge was found to be the least sensitive of the three carbon sources. All of the systems had failed to meet the COD concentration criteria of 75 mg l^{-1}, with the current process configuration.

The results show that primary sewage sludge systems were the most sensitive to process changes. Scenarios considered in the sensitivity analysis have clearly illustrated the benefits of the burden reduction when using primary sewage sludge, and hence the benefits of primary sewage sludge as a carbon source in AMD treatment.

Chapter 11

CONCLUSIONS AND RECOMMENDATIONS

11.1 Approach to Study

This thesis aimed to evaluate three different substrates as sources of carbon and electron donor capacity in terms of their availability and their impact on both final water quality and process economics to determine if the carbon substrate was the limiting factor in terms of process economics.

The hypotheses proposed for this study were:

- The carbon source as well as its availability were the limiting factors in the process of sulphate reduction in terms of economics
- The applicability of the carbon source may be tested using a kinetic model of the process

An example flowsheet for the treatment of acid mine drainage was developed. The flowsheet consisted of:

- An anaerobic reactor, to convert sulphate to sulphide,
- A mixer to lower the pH to optimal conditions for the aerobic reactor,
- A settler to remove the sludge formed in the anaerobic reactor,
- An aerobic reactor to convert sulphide to elemental sulphur, and
- A sulphur settler to remove the sulphur formed in the aerobic reactor as well as some of the biomass formed in the aerobic reactor and the some of the residual biomass from the anaerobic reactor.

A mathematical model describing the anaerobic treatment of sulphate was developed using MATLAB based on the work of Knobel (2002). The model was subsequently verified using a statistical approach based on the chi-squared statistic. The substrates verified were:

- Propionate for sulphate reduction in the absence of methane production,
- Acetate for sulphate reduction in the absence of methane production,
- Anaerobic digestion of glucose for without sulphate reduction, and
- Anaerobic digestion of a mixer of acetate, propionate and sucrose without sulphate reduction

The model of the anaerobic reactor was shown to predict the experimental data obtained at the 90% confidence limit.

Three substrates serving as carbon source and electron donor were analysed using sulphate concentrations based on AMD from three mining sites located in South Africa. The three substrates analysed were:

- ethanol,
- molasses, and
- primary sewage sludge.

The sulphate concentrations at the three AMD sites assessed were:

- 1 447 mg l^{-1} for AMD site 1,
- 1 833 mg l^{-1} for AMD site 2, and
- 2 248 mg l^{-1} for AMD site 3.

11.2 Conclusions Drawn

The mass balance showed that AMD site 3, which treated the highest sulphate concentration of the three AMD sites, required the highest concentration of substrate and produced the most amount of by-products. The amount of sludge produced and residual COD in the final effluent stream increased as the complexity of the carbon source increased. This was seen by primary sewage sludge producing the highest amount of sludge and residual COD in the final effluent stream. Subsequent treatment of the water released from the AMD treatment system must be considered. With the current system setup, ethanol was the only carbon substrate that was able to meet the EPA standards. Both the molasses and primary sewage sludge failed in this respect. A possible solution to this problem would be to use a reactor that could separate the solids residence time from the hydraulic residence time and have a high retention of solids e.g. the UASB reactor.

The economic analysis showed that using reinforced concrete was a much more viable economic option than stainless steel. The capital cost of using stainless steel was 16 times more expensive than the capital cost of using reinforced concrete as the material of construction.

Ethanol proved to be the most economically viable option when the burden reduction of primary sewage sludge was not taken into account. When the burden reduction was taken into account, the economic results showed that primary sewage sludge was the most economically viable option. Further primary sewage sludge is the most readily available.

With regards to the availability of the carbon source, primary sewage sludge was considered as the most sustainable carbon source due to the accessibility of it. Considering the duration of time required for the treatment of AMD, a carbon source that has to be bought becomes unattractive. Hence readily available waste products would potentially be the best option. However, complex carbon sources would generally produce a higher disposal cost.

It was initially hypothesised that the more complex the carbon source, the less expensive it would be and hence the less expensive the treatment process would be. When the burden reduction potential of primary sewage sludge was not taken into account, the opposite was found to hold. The least complex carbon source provided the least expensive process. This resulted because of the increased waste production associated with the complex carbon sources as well as high disposal costs. The undegradable fractions of the complex carbon sources were a major contributor to the sludge for disposal. Primary sewage sludge consisted of 33.45% undegradable matter whereas molasses consisted of 4.2% unfermentable sugars and 6.3% undegradable proteins.

The sensitivity analysis showed that systems using primary sewage sludge as the carbon substrate were the most sensitive to changes in operating conditions. Ethanol was found to be most sensitive to changes in substrate cost whereas molasses was more sensitive to changes in disposal cost. The sensitivity analysis verified that ethanol was the most economically viable option when the burden reduction potential of primary sewage sludge was not taken into account. Although systems using ethanol as a substrate were the most sensitive to changes in substrate cost, ethanol proved to be the least sensitive in all other cases. However, where the burden reduction of using primary sewage sludge was taken into account, based on the results presented in Section 8.4, primary sewage sludge was found to be the most economically viable option of the three carbon sources analysed.

11.3 Recommendations for Future Work

The current investigation has identified several areas that require further investigation. The identification of these areas consequently shows the limitations of the current study.

11.3.1 Other Carbon Compounds

In this study, industrial waste products were identified as possible carbon sources for the treatment of acid mine drainage. Vinasse is one such compound, another as

byproducts of the paper industry. Further studies into these wastes would be beneficial, both to assess process performance and substrate availability.

11.3.2 Reactor Choice

In this study, a CSTR was used. Although this reactor setup is not able to uncouple the hydraulic residence time from the solids residence time, it does provide a platform for comparing the various substrates. The operating conditions under which this reactor reduced the COD concentration of the effluent water stream to the acceptable concentration of 75 mg l^{-1} were very limited. Extension of the analytic framework to consider a reactor in which the solids residence time could be uncoupled from the hydraulic residence time is proposed. Further, a reactor design providing a high retention of solids should be considered e.g. USAB.

11.3.3 Model Development

Continuous improvements to the model should be made as research into this area improves. In this study, ethanol was added to the model and sucrose was modelled as two moles of glucose. Refinement with respect to the fermentation of sucrose and amino acids is recommended. Chapter 5 showed that certain values used in the model were assumed e.g. H_2S inhibition on lactate fermentation. Since the simulation model was based on work previously done by Knobel (1999) and no addition literature was found for these assumed values, that author's assumed values were retained. This illustrates the need for expanded kinetic studies to consider H_2S, VFA inhibition and amino acid degradation.

11.3.4 Inclusion of the Precipitation Unit

For this study, it was assumed that the AMD stream went through a precipitation unit to remove incoming metals as metal sulphide such that the concentration of metal ions entering the anaerobic reactor was negligible. For a more complete study of the AMD, the precipitation unit should be included in the simulation model.

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Appendix A

STOICHIOMETRY AND RATE CONSTANTS USED IN THE MODEL

Table A-1: Stoichiometry of Hydrolysis reactions used in the model.

$C_5H_7O_2N + H_2O$	→	$C_5H_9O_3N$
$C_6H_{10}O_5 + H_20$	→	$C_6H_{12}O_6$
$C_{51}H_{98}O_6 + 3H_20$	→	$CH_2OHCHOHCH_2OH + 3CH_3(CH_2)_{14}COOH$

Table A-2: Stoichiometry of Fermentation reactions used in the model.

Amino Acid Fermentation

$C_5H_9O_3N + 3H_2O$	→	$2CH_3COOH + CO_2 + H_2 + NH_3$
$C_5H_9O_3N + 3H_2O$	→	$CH_3CH_2COOH + 2CO_2 + 3H_2 + NH_3$
$C_5H_9O_3N$	→	$aFER + H_2O$

Glucose Fermentation

$C_6H_{12}O_6$	→	$0.67CH_3COOH + 0.67CH_3CHOHCOOH + 0.33CH_3CH_2CH_2COOH + 2H_2 + 1.33CO_2$
$C_6H_{12}O_6 + NH_3$	→	gFER

Table A-2 cont.: Stoichiometry of Fermentation reactions used in the model.

Lactate Fermentation

$CH_3CHOHCOOH \rightarrow 0.5CH_3COOH + 0.5CH_3CH_2COOH + 1.33CO_2$

$CH_3CHOHCOOH + NH_3 \rightarrow$ lFER

Glycerol Fermentation

$CH_2OHCHOHCH_2OH + H_2O \rightarrow CH_3COOH + 3H_2$

$CH_2OHCHOHCH_2OH + NH_3 \rightarrow$ glyFER $+ CO_2 + H_2O + 4H_2$

Table A-3: Stoichiometry of Long Chain Fatty Acid Beta Oxidation used in the model.

$CH_3(CH_2)_{14}COOH + 14H_2O \rightarrow 8CH_3COOH + 14H_2$

$5CH_3(CH_2)_{14}COOH + 16NH_3 + 22H_2O \rightarrow$ 16 BOB $+ 14H_2$

Table A-4: Stoichiometry of Acetogenesis reactions used in the model.

Butyrate Acetogenesis

$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$

$5CH_3CH_2CH_2COOH + 4NH_3 \rightarrow$ 4bACE $+ 2H_2O + 10H_2$

Propionate Acetogenesis

$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$

$5CH_3CH_2COOH + 3NH_3 \rightarrow$ 3pACE $+ 4H_2O + 5H_2$

Table A-5: Stoichiometry of methanogenesis reactions used in the model.

H_2 Methanogenesis

$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$

$5CO_2 + 10H_2 + NH_3 \rightarrow hMPB + 8H_2O$

AcH Methanogenesis

$CH_3COOH \rightarrow CH_4 + CO_2$

$5CH_3COOH + 2NH_3 \rightarrow 2aMPB + 6H_2O$

Table A-6: Stoichiometry of Sulphate reduction equations used in the model.

H_2 sulphate reduction

$4H_2 + H_2SO_4 \rightarrow H_2S + 4H_2O$

$5CO_2 + 10H_2 + NH_3 \rightarrow hSRB + 8H_2O$

AcH Sulphate Reduction

$CH_3COOH + H_2SO_4 \rightarrow H_2S + CO_2$

$CH_3COOH + 2NH_3 \rightarrow 2aSRB + 6H_2O$

LaH Sulphate Reduction

$2CH_3CHOHCOOH + H_2SO_4 \rightarrow 2CH_3COOH + H_2S + 2CO_2 + 2H_2O$

$5CH_3CHOHCOOH + 3NH_3 \rightarrow 3lSRB + 9H_2O$

PrH Sulphate Reduction

$CH_3CH_2COOH + 0.75H_2SO_4 \rightarrow 0.75H_2S + CH_3COOH + CO_2 + H_2O$

$CH_3CH_2COOH + 3NH_3 \rightarrow 3pSRB + 4H_2O + 5H_2$

Table A-6 cont.: Stoichiometry of Sulphate reduction equations used in the model.

BuH Sulphate Reduction

$2CH_3CH_2CH_2COOH + H_2SO_4 \rightarrow 4CH_3COOH + H_2S$

$5CH_3CH_2CH_2COOH + 3NH_3 \rightarrow 4bSRB + 2H_2O + 2.5H_2$

EtOH Sulphate reduction

$C_2H_5OH + H_2SO_4 \rightarrow H_2S + 2CO_2 + 2H_2$

$5C_2H_5OH + 2NH_3 \rightarrow 2eSRB + 5H_2$

Table A-7: Stoichiometry of acid base reactions.

Sulphide

$$HS^- + H^+ \Leftrightarrow H_2S$$

$$S^{2-} + H^+ \Leftrightarrow HS^-$$

Carbon dioxide

$$H_2CO_3 \Leftrightarrow CO_2 + H_2O$$

$$HCO_3^- + H^+ \Leftrightarrow H_2CO_3$$

$$CO_3^{2-} + H^+ \Leftrightarrow HCO_3^-$$

Sulphate

$$HSO_4^- + H^+ \Leftrightarrow H_2SO_4$$

$$SO_4^{2-} + H^+ \Leftrightarrow HSO_4^-$$

Ammonia

$$NH_3 + H^+ \Leftrightarrow NH_4^+$$

Acetate

$$CH_3COO^- + H^+ \Leftrightarrow CH_3COOH$$

Table A-7 cont.: Stoichiometry of acid base reactions.

Propionate

$$CH_3CH_2COO^- + H^+ \Leftrightarrow CH_3CH_2COOH$$

Butyrate

$$CH_3CH_2CH_2COO^- + H^+ \Leftrightarrow CH_3CH_2CH_2COOH$$

Lactate

$$CH_3CHOHCOO^- + H^+ \Leftrightarrow CH_3CHOHCOOH$$

Table A-8: Rate constants used for hydrolysis reactions.

Rate constant	Value	Unit	Source
$k_{protein}$	0.09	d^{-1}	Rouke, 1968
$k_{carbohydrates}$	0.29	d^{-1}	Rouke, 1968
k_{lipids}	0.09	d^{-1}	Rouke, 1968

Table A-9: Rate constants used for amino acid fermentation.

Rate constant	Value	Unit	Source
μ_{max}	1.5	d^{-1}	no data available
Y	0.57	mmole/mmole	no data available
K_s	0.153	mmole/l	no data available
K_{IH2S}	3.12	mmole/l	no data available
K_{IVFA}	10	mmole/l	no data available

Table A-10: Rate constants for Glucose fermentation.

Rate constant	Value	Unit	Source
μ_{max}	5.124	d^{-1}	Stamatelatou *et al.* (2003)
Y	0.112	mmole/mmole	Stamatelatou *et al.* (2003)
K_s	0.082	mmole/l	Stamatelatou *et al.* (2003)
K_{IVFA}	10	mmole/l	Costello, 1991
K_{IH2S}	17.19	mmole/l	no data available

Table A-11: rate constants for Lactate fermentation.

Rate constant	Value	Unit	Source
μ_{max}	2.552	d^{-1}	Stamatelatou *et al.* (2003)
Y	0.1	mmole/mmole	Stamatelatou *et al.* (2003)
K_s	1.11	mmole/l	Stamatelatou *et al.* (2003)
K_{IVFA}	10	mmole/l	Costello, 1991
K_{IH2S}	3.12	mmole/l	no data available

Table A-12: Rate constants for glycerol fermentation.

Rate constant	Value	Unit	Source
μ_{max}	10	d^{-1}	Costello, 1991
Y	0.4	mmole/mmole	Costello, 1991
K_s	0.25	mmole/l	Costello, 1991
K_{IVFA}	10	mmole/l	Costello, 1991
K_{IH2S}	3.12	mmole/l	no data available

Table A-13: Rate constants for long chain fatty acid beta oxidation.

Rate constant	Value	Unit	Source
μ_{max}	1.2	d^{-1}	Gujer and Zehnder, 1983
Y	0.674	mmole/mmole	Gujer and Zehnder, 1984
K_s	0.19	mmole/l	Gujer and Zehnder, 1985
K_{IVFA}	10	mmole/l	no data available
K_{IH2S}	3.12	mmole/l	no data available

Table A-14: Rate constants for butyrate acetogenesis.

Rate constant	Value	Unit	Source
μ_{max}	0.264	d^{-1}	Kalyuzhnyi (1997)
Y	0.04	mmole/mmole	Kalyuzhnyi (1997)
K_s	1.1	mmole/l	Kalyuzhnyi (1997)
K_{IVFA}	3	mmole/l	Costello, 1991
K_{IH2S}	0.811	mmole/l	no data available

Table A-15: Rate constants for propionate acetogenesis.

Rate constant	Value	Unit	Source
μ_{max}	0.36	d^{-1}	Lawrence and McCarty (1969)
Y	0.03	mmole/mmole	Lawrence and McCarty (1969)
K_s	0.5	mmole/l	Lawrence and McCarty (1969)
K_{IVFA}	30	mmole/l	Costello, 1991
K_{IH2S}	0.83	mmole/l	Maillacheruvu and Parkin (1996)

Table A-16: Rate constants for sulphate reduction using hydrogen as a substrate.

Rate constant	Value	Unit	Source
μ_{max}	5	d^{-1}	Kalyuzhnyi and Fedorovich (1998)
Y	0.021	mmole/mmole	Kalyuzhnyi and Fedorovich (1998)
K_s	0.0015	mmole/l	Kalyuzhnyi and Fedorovich (1998)
K_{IVFA}	100	mmole/l	no data available
K_{IH2S}	4.65	mmole/l	Maillacheruvu and Parkin (1996)
K_{SO4}	0.0093	mmole/l	Kalyuzhnyi and Fedorovich (1998)

Table A-17: Rate constants for sulphate reduction using acetate as a substrate.

Rate constant	Value	Unit	Source
μ_{max}	0.51	d^{-1}	Kalyuzhnyi and Fedorovich (1998)
Y	0.023	mmole/mmole	Kalyuzhnyi and Fedorovich (1998)
K_s	0.375	mmole/l	Kalyuzhnyi and Fedorovich (1998)
K_{IVFA}	10	mmole/l	no data available
K_{IH2S}	4.75	mmole/l	Kalyuzhnyi and Fedorovich (1998)
K_{SO4}	0.2	mmole/l	Kalyuzhnyi and Fedorovich (1998)

Table A-18: Rate constants for sulphate reduction using lactate as a substrate.

Rate constant	Value	Unit	Source
μ_{max}	2.5	d^{-1}	Traore (1982)
Y	0.02	mmole/mmole	Traore (1982)
K_s	0.0488	mmole/l	Traore (1982)
K_{IVFA}	10	mmole/l	no data available
K_{IH2S}	7.83	mmole/l	Okabe 1995
K_{SO4}	0.00877	mmole/l	Traore (1982)

Table A-19: Rate constants for sulphate reduction using propionate as a substrate.

Rate constant	Value	Unit	Source
μ_{max}	0.81	d^{-1}	Kalyuzhnyi and Fedorovich (1998)
Y	0.03	mmole/mmole	Kalyuzhnyi and Fedorovich (1998)
K_s	2.56	mmole/l	Kalyuzhnyi and Fedorovich (1998)
K_{IVFA}	10	mmole/l	no data available
K_{IH2S}	8.89	mmole/l	Kalyuzhnyi and Fedorovich (1998)
K_{SO4}	0.077	mmole/l	Kalyuzhnyi and Fedorovich (1998)

Table A-20: Rate constants for sulphate reduction using butyrate as a substrate.

Rate constant	Value	Unit	Source
μ_{max}	0.41	d^{-1}	Schauder, 1986
Y	0.04	mmole/mmole	Maillacheruvu and Parkin (1996)
K_s	0.309	mmole/l	Maillacheruvu and Parkin (1996)
K_{IVFA}	10	mmole/l	no data available
K_{IH2S}	15.6	mmole/l	no data available
K_{SO4}	0.17	mmole/l	no data available

Table A-21: Rate constants for sulphate reduction using ethanol as a substrate.

Rate constant	Value	Unit	Source
μ_{max}	0.8	d^{-1}	Erasmus (2000)
Y	0.02	mmole/mmole	Erasmus (2000)
K_s	0.124	mmole/l	Erasmus (2000)
K_{IVFA}	10	mmole/l	Erasmus (2000)
K_{IH2S}	5.6	mmole/l	no data available
K_{SO4}	0.124	mmole/l	Erasmus (2000)

Table A-22: Rate constants for methanogenesis using hydrogen as a substrate.

Rate constant	Value	Unit	Source
μ_{max}	1	d^{-1}	Kalyuzhnyi and Fedorovich (1998)
Y	0.002	mmole/mmole	Kalyuzhnyi and Fedorovich (1998)
K_s	0.008125	mmole/l	Kalyuzhnyi and Fedorovich (1998)
K_{IVFA}	3	mmole/l	Costello, 1991
K_{IH2S}	20.71	mmole/l	Maillacheruvu and Parkin (1996)

Table A-23: Rate constants for methanogenesis using acetate as a substrate.

Rate constant	Value	Unit	Source
μ_{max}	0.36	d^{-1}	Kalyuzhnyi (1997)
Y	0.0127	mmole/mmole	Lawrence and McCarty (1969)
K_s	0.875	mmole/l	Kalyuzhnyi and Fedorovich (1998)
K_{IVFA}	10	mmole/l	Costello, 1991
K_{IH2S}	3.65	mmole/l	Maillacheruvu and Parkin (1996)

Appendix B:

CONSTANTS USED IN THE MODEL

$R = 8.206 \times 10^{-6}$ atm.l/(mmole.K)
T = 25+298.15 Kelvin

Table B-1:Henry's Law constants.

Compound	Henry's Law Constant (atm/(mmole/l))	Source
H_2	1.336	Ebbing (1996)
H_2S	1.218e-2	Ebbing (1996)
CO_2	0.0376	Ebbing (1996)
CH_4	0.8755	Ebbing (1996)

Table B-2: Mass Transfer coefficients at 25°C.

Compound	k_La (d^{-1})	Source
H_2	2000	No data found, values taken from Knobel (1999)
H_2S	4320	No data found, values taken from Knobel (1999)
CO_2	500	No data found, values taken from Knobel (1999)
CH_4	1e5	No data found, values taken from Knobel (1999)

Table B-3: Constants used for pH inhibition for sulphate reduction.

a_{LL}	10	No data found, values taken from Knobel (1999)
a_{UL}	10	No data found, values taken from Knobel (1999)
pH_{LL}	6.3	No data found, values taken from Knobel (1999)
pH_{UL}	8.4	No data found, values taken from Knobel (1999)

Table B-4: Constants used for pH inhibition for methanogens.

a_{LL}	20	No data found, values taken from Knobel (1999)
a_{UL}	20	No data found, values taken from Knobel (1999)
pH_{LL}	7.1	No data found, values taken from Knobel (1999)
pH_{UL}	8.0	No data found, values taken from Knobel (1999)

Table B-5: Constants used for pH inhibition for fermenters and beta oxidizers.

a_{LL}	4	No data found, values taken from Knobel (1999)
a_{UL}	0.5	No data found, values taken from Knobel (1999)
pH_{LL}	5.0	No data found, values taken from Knobel (1999)
pH_{UL}	10.5	No data found, values taken from Knobel (1999)

Appendix C

PROGRAM SCRIPT

Appendix C-1: start.m

```
% Steps to follow when using this program:
% 1) Set the values that are initially in the tank, units are set at
%    mmoles per litre. Usually you will only have your bacteria in the tank at this %
     point. Note that C(34)-C(37) are pressures and are in atm.
% 2) Select the options that you wish to use
% 3) Select the time span use wish to evaluate
% 4) Open the equations m-file and follow the instructions there
% 5) Once you are satisfied with the equations m-file click the start and run icon
above.

clear
clc

tic

clear equations
global minim
minim = [];

%initially in the tank
CO(1) = 0; % proteins (C5H7O2N)
CO(2) = 0; % Carbohydrates (C6H10O5)
CO(3) = 0; % Lipids (C51H98O6)
CO(4) = 0; % Amino Acid (C5H9O3N)
CO(5) = 0; % Glucose (C6H12O6)
CO(6) = 0; % Glycerol (CH2OHCHOHCH2OH)
CO(7) = 0; % palmitic Acid (CH3(CH2)14COOH)
CO(8)=0.00; % H2
CO(9)=0;  % AcH
CO(10)=0.0; % LaH
CO(11)=0;  % PrH
CO(12)=0.0; % BuH
CO(13)=0;  % SO4
CO(14)=0.0; % H2S
CO(15)=0.0; % CO2
```

```
CO(16)=0.0; %  CH4
CO(17)=0;  %  NH3
CO(18)=0;  %  EOH
CO(19)=0;  %   biomass aFER (amino acid)
CO(20)=0;  %   biomass gFER (glucose)
CO(21)=0;  %  biomass lFER (lactate)
CO(22)=0;  %   biomass glyFER (glycerol)
CO(23)=0;  %   biomass BOB (beta oxidation)
CO(24)=0;  %  biomass bACE
CO(25)=0;  %  biomass pACE
CO(26)=10;  %  biomass hSRB
CO(27)=0;  %  biomass aSRB
CO(28)=0;  %  biomass lSRB
CO(29)=0;  %  biomass pSRB
CO(30)=0;  %  biomass bSRB
CO(31)=10;  %  biomass eSRB
CO(32)=10.0;%  biomass hMPB
CO(33)=0;  %  biomass aMPB$
CO(34)= 0.000;     %  pressures  H2
CO(35)=0.000;      %             H2S
CO(36)= 0.000;     %             CO2
CO(37)=0.000;      %             CH4

%varPassedOut=0;
options =
odeset('AbsTol',0.001,'RelTol',0.001,'MaxOrder',3,'initialstep',0.00001,'MaxStep',500,
'BDF','on');
%


[t,C]=ode15s(@equations, [0 500], CO,options);
%varPassedOut=varPassedOut(2:end);
res=[t,C];
plot(t,C);
h=legend('proteins', 'Carbohydrates', 'Lipids','Amino
Acid','Glucose','Glycerol','palmitic Acid',...

'H2','AcH','LaH','PrH','BuH','SO4','H2S','CO2','CH4','NH3','EOH','aFER','gFER','lFER
', 'glyFER',...

'BOB','bACE','pACE','hSX','aSX','lSX','pSX','bSX','eSX','hMX','aMX','PH2','PH2S','P
CO2','PCH4');

  toc
```

Appendix C-2: equations.m

```
%  Please follow the instructions on the start m-file before starting this
%  section
```

```
% 1) select which reactions you wish to use
% 2) Go to the test vector and switch on the reaction that you want to
%    use. (0-off and 1-on)
% 3) Next, select the volume of the reactor (for both the ga and liquid
%    sections) and the flowrates (gas and liquid) into the reactor.
% 4) The parameters for the various bacteria is for pH inhibition (refer
%    to thesis for more information on this)
% 5) Insert the values of compounds that are continuously added to the
%    reactor. Note that this can be altered so that compounds can be
%    added periodically or added in for a much shorter time than what
%    the program is running for to the reactor (refer to MATLAB help notes).
% 6) Seo is the initial values that will be sent to the equilib m-file.
%    If you wish to change the values in this program do so now.
% 7) Constants as well as the data for each reaction is given. This data
%    can be changed if you wish
% 8) Return to the start m-file


%% Explanation on extracting more information from MATLAB during execution

% MATLAB will only pass out the variables t and C. If you wish to extract
% say the concentration of all the ions ie. the Se variable, then set
minim(n,2:22)=Se.
% values for Se will now be found in the workspace. Remember that
% minim(n,1)=t. Also the length of x in minim(n,2:x) must equal all the
% variables to be extrated -1 (because the first variable has been set as t already).
% eg if you are to extract Se as well as pH, then minim(n,2:23)=[Se,pH]

% The list below shows the different compounds used in the program and
% their corresponding numbers. 34-37 refer to the gas pressures of these
% compounds

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% 1  proteins (C5H7O2N)
% 2  Carbohydrates (C6H10O5)
% 3  Lipids (C51H98O6)
% 4  Amino Acid (C5H9O3N)
% 5  Glucose (C6H12O6)
% 6  Glycerol (CH2OHCHOHCH2OH)
% 7  palmitic Acid (CH3(CH2)14COOH)
% 8  H2
% 9  AcH
% 10 LaH
% 11 PrH
% 12 BuH
% 13 SO4
% 14 H2S
% 15 CO2
```

```
%  16 CH4
%  17 NH3
%  18 EOH
%  19 biomass aFER (amino acid)
%  20 biomass gFER (glucose)
%  21 biomass lFER (lactate)
%  22 biomass glyFER (glycerol)
%  23 biomass BOB (beta oxidation)
%  24 biomass bACE
%  25 biomass pACE
%  26 biomass hSRB
%  27 biomass aSRB
%  28 biomass lSRB
%  29 biomass pSRB
%  30 biomass bSRB
%  31 biomass eSRB
%  32 biomass hMPB
%  33 biomass aMPB
%  pressure 34  H2
  %       35  H2S
  %       36  CO2
  %       37  CH4
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

function [dC] = equations(t,C)

global minim
persistent n

if isempty(n)
   n = 1;
else
   n = n+1;
end



dC = zeros(37,1);
format bank
for i=1:37;
if C(i)<=0
   C(i)=0;
else C(i)=C(i);
end
end

Test=zeros(1,17);
Test(1)=0;     %  protein hydrolysis
Test(2)=0;     %  carbohydrate hydrolysis
```

```
Test(3)=0;    %  Lipid hydrolysis
Test(4)=0;    %  Amino acid fermentation
Test(5)=0;    %  Glucose fermentation
Test(6)=0;    %  Lactate fermentation
Test(7)=0;    %  Glycerol Fermentation
Test(8)=0;    %  Long chain fatty acid beta oxidation
Test(9)=0;    %  BuH Acetogenesis
Test(10)=0;%1;   %  PrH Acetogenesis
Test(11)=1;%1;   %  H2 sulphate reduction
Test(12)=0;   %  AcH sulphate reduction
Test(13)=0;   %  LaH sulphate reduction
Test(14)=0;%1;   %  PrH sulphate reduction
Test(15)=0;   %  BuH sulphate reduction
Test(16)=1;   %  EOH sulphate reduction
Test(17)=1;%1;   %  H2 methanogenesis
Test(18)=0;%1;   %  AcH methanogenesis
Test(19)=1;   %  Cell death

%constants

%th=20;          %  USAB          %  day    (hydraulic residence time)
%ts=10;          %  USAB          %  day    (solids residence time)

Qgin=0;            %  l/day      %%%%% flowrate of gas into reactor
Vv=1000000;          %  l        %%%%% vapour volume of reactor %
%Vl=Qgout*th;        %  l        %%%%% liquid volume of reactor %%%%%use this
for USAB
Vl=10000000;         %  l        %%%%% use this for CSTR
Ql=1000000;        %  l/day      %%%%% volume of liquid flowing into system
%%%%% CSTR
%Qgout = 100;        %  l/day      %%%%% gas flow rate out of reactor
                 %%%%%%%% hydraulic residence time = vol/flowrate
%%%%%%%%  pH inhibition parameters %%%%%%%

%  sulphate parameters

aLLS=10;
aULS=10;
pHLLS=6.3;
pHULS=8.4;

%  methanogens parameters

aLLM=20;
aULM=20;
pHLLM=7.1;
pHULM=8.0;

%  Fermenters and beta oxidizers
```

```
aLLF = 4;
aULF = 0.5;
pHLLF =5;
pHULF = 10.5;


% continuously added to tank
Csludgein=0;          % mmoles/l

if  Csludgein==0;
   CProteinin = 0;                % (mmoles/l)
   CCarbohydratein = 0;
   CLipidin = 0;
   CAcHin = 0;
else
   CProteinin = 0.52*Csludgein;
   CCarbohydratein = 0.254*Csludgein;
   CLipidin = 0.054*Csludgein;
   CAcHin = 0.172*Csludgein;
end
CAminoAcidin = 0;
CGlucosein = 0;
CGlycerolin = 0;
CPalmiticin = 0;
CH2in = 0;
CLaHin=0;
CPrHin=0;
CBuHin=0;
CSO4in=19.09*1.5;
CH2Sin=0;
CCO2in=0;
CCH4in=0;
CNH3in=10;
CEOHin=19.10;
Cafxin=0;
Cgfxin=0;
Clfxin=0;
Cglyfxin=0;
Cbobxin=0;
Chsxin=0;
Casxin=0;
Clsxin=0;
Cpsxin=0;
Cbsxin=0;
Cesxin=0;
Cesxin=0;
Chmxin=0;
Camxin=0;
Cbaxin=0;
Cpaxin=0;
```

```
PinH2=0;
PinH2S=0;
PinCO2=0;
PinCH4=0;

Se0=zeros(1,22);

Se0 = [3.9811e-005, (0.85*C(14)), (0.1*C(14)), (0.05*C(14)), (0.85*C(13)),
(0.1*C(13)), (0.05*C(13)),...
    (0.6*C(17)), (0.4*C(17)), (0.9*C(15)), (0.06*C(15)), (0.03*C(15)),(0.01*C(15)),
(0.6*C(9)),...
    (0.4*C(9)), (0.6*C(11)), (0.4*C(11)), (0.6*C(12)), (0.6*C(12)), (0.5*C(10)),
(0.5*C(10))];

options=optimset('maxfunevals',10000,'tolfun',100,'tolX',100,'MaxIter',1000000);

Se=fminsearch(@equilib,Se0,options,C);
H=abs(Se(1));                          % mmole/l
H2Sl=abs(Se(3)+Se(4));
VFA=abs(Se(14)+Se(16)+Se(18)+Se(20));
pH=-log10(H/1000);
disp([t]);
disp([pH]);
disp('H2Sl VFA')
disp([H2Sl VFA]);

%%%%  Constants  %%%%

HH2=1.336;          %  (atm/(mmole/l))
HH2S=1.218e-2;      %  (atm/(mmole/l))
HCO2=0.0376;        %  (atm/(mmole/l))
HCH4=0.8755;        %  (atm/(mmole/l))

klaH2=2000;       %  (d-1)
klaH2S=4320;      %  (d-1)
klaCO2=500;       %  (d-1)
klaCH4=1e5;       %  (d-1)

R = 8.206e-6;       %  atm.l/(mmole.K)
T = 25+273.15;      %  kelvin

KIH2=1500/HH2;

%  reactions
nr= 0;

%  1  Protein Hydrolysis
%  C5H7O2N + H2O ---> C5H9O3N

nr=nr+1;
```

```
if Test(1)==1 & C(1)>0;
k=0.09;                          % day^-1
rProtein(nr) = -k*C(1);          % mmoles/l/day
rAminoAcid(nr) = -rProtein(nr);
else
k=0;
rProtein(nr) = 0;                % mmoles/l/day
rAminoAcid(nr) = 0;
end


%  2  Carbohydrate Hydrolysis
%  C6H10O5 + H2O ---> C6H12O6

nr = nr+1;

if Test(2)==1 & C(2)>0;
k =0.29;                         % day^-1
rCarbohydrate(nr) = -k*C(2);
rGlucose(nr) = -rCarbohydrate(nr);
else
   k =0;                         % day^-1
rCarbohydrate(nr) = 0;
rGlucose(nr) = 0;
end

%  3  Lipid Hydrolysis
%  C51H98O6 +3H2O ---> CH2OHCHOHCH2OH + 3CH3(CH2)14COOH

nr=nr+1;
if Test(3)==1 & C(3)>0;
k=0.09;                          % day^-1
rLipid(nr) = -k*C(3);
rGlycerol(nr) = -rLipid(nr);
rPalmitic(nr) = -3*rLipid(nr);
else
   k=0;                          % day^-1
rLipid(nr) = 0;
rGlycerol(nr) = 0;
rPalmitic(nr) = 0;
end

%  4  Amino Acid Fermentation
%  C5H9O3N + 3H2O ---> 2CH3COOH + CO2 +H2 +NH3
%  C5H9O3N + 3H2O ---> CH3CH2COOH +2CO2 +3H2 + NH3
%  C5H9O3N ---> aFER + H2O

nr=nr+1;
```

```
umax = 1.5;
Y = 0.57;                    % mmole/mmole
Ks = 0.153;                  % mmole/l
AcPrRatio = 0.64;
KIH2S = 3.12;                % mmole/l
KIVFA=10;                    % mmole/l

if Test(4)==1 & real(C(4))>0  & real(C(19))>0  & real(C(17))>=0 ;
rx(nr) =
umax*C(19)*ESU(C(4),Ks)*EpH(pH,aLLF,aULF,pHLLF,pHULF)*ENI(H2Sl,KIH2
S)*ENI(VFA,KIVFA);
else
   rx(nr)=0;
end

if rx(nr)>0 & Test(4)==1;

rAminoAcid(nr)=-rx(nr)/Y;
AAcell=-rx(nr);
AAAcPr=rAminoAcid(nr)-AAcell;
rAcH(nr)=-AcPrRatio*AAAcPr;
rPrH(nr)=-(1-AcPrRatio)*AAAcPr;
rCO2(nr)=(rAcH(nr)/2) +(rPrH(nr)*2);
rH2(nr)=rAcH(nr)/2 +rPrH(nr)*3;
rNH3(nr) = -AAAcPr;
else
  rAminoAcid(nr)=0;
AAcell=0;
AAAcPr=0;
rAcH(nr)=0;
rPrH(nr)=0;
rCO2(nr)=0;
rH2(nr)=0;
rNH3(nr) = 0;
end

%  5  Glucose Fermentation
% C6H12O6 --->
0.67CH3COOH+0.67CH3CHOHCOOH+0.33CH3CH2CH2COOH+2H2+1.33CO2
% C6H12O6 + NH3 ---> gFER

nr = nr+1;

Y = 0.112;                   %     mM/mM
umax = 5.124;                %     day
Ks =   0.082;%170/180.26     ;   %     mmole/l ((mg/l)/(mg/mmole))
KIVFA=10;
KIH2S=17.19;

if Test(5)==1 & real(C(5))>0 & real(C(20))>0 & real(C(17))>0   ;
```

```
rx(nr)=umax*C(20)*C(5)/(Ks+C(5))*EpH(pH,aLLF,aULF,pHLLF,pHULF)*ENI(VF
A,KIVFA)*ENI(C(34),KIH2)*ENI(H2Sl,KIH2S);
%
else
   rx(nr)=0;
end

if rx(nr) >0 & Test(5)==1;

rGlucose(nr)=(-rx(nr))/Y;
rGlucosecellprod(nr)=(-rx(nr))*5/6;
rGlucoseprod(nr)=rGlucose(nr)-rGlucosecellprod(nr);

rAcH(nr)=-0.67*rGlucoseprod(nr);
rLaH(nr)=-0.67*rGlucoseprod(nr);
rBuH(nr)=-0.33*rGlucoseprod(nr);
rH2(nr)=-2*rGlucoseprod(nr);
rCO2(nr)=-1.33*rGlucoseprod(nr);
rNH3(nr)=-rGlucosecellprod(nr);

else

rGlucose(nr)=0;
rGlucosecellprod(nr)=0;
rGlucoseprod(nr)=0;

rAcH(nr)=0;
rPrH(nr)=0;
rBuH(nr)=0;
rH2(nr)=0;
rCO2(nr)=0;
rNH3(nr)=0;
end


%  6  Lactate Fermentation
%  CH3CHOHCOOH ---> 0.5CH3COOH + 0.5CH3CH2COOH + 1.33CO2
%  CH3CHOHCOOH + NH3 ---> lFER

nr=nr+1;
Y =    0.1;            %   mM/mM
umax =        2.552;          %   day
Ks =   100/90.08;          %   mmole/l
KIVFA = 10;
KIH2S=100/32.06;

if  Test(6)==1 & real(C(21))>0 & real(C(17))>0 & real(C(10))>0 ; %
rx(nr)=umax*C(21)*C(10)/(Ks+C(10))*EpH(pH,aLLF,aULF,pHLLF,pHULF)*ENI(
C(34),KIH2)*ENI(VFA,KIVFA);
```

```
else
   rx(nr)=0;
end

if rx(nr)>0 & Test(6)==1;
rLaH(nr)=(-rx(nr))/Y;
rLaHcellprod(nr)=(-rx(nr))*5/3;
rLaHprod(nr)=rLaH(nr)-rLaHcellprod(nr);

rAcH(nr)=-0.5*rLaHprod(nr);
rPrH(nr)=-0.5*rLaHprod(nr);
rCO2(nr)=-1.33*rLaHprod(nr);
rNH3(nr)=rLaHcellprod(nr);

else
   rLaH(nr)=0;
   rLaHcellprod(nr)=0;
   rLaHprod(nr)=0;

   rAcH(nr)=0;
   rPrH(nr)=0;
   rCO2(nr)=0;
   rNH3(nr)=0;
end

%  7  Glycerol Fermentation
%CH2OHCHOHCH2OH + H2O ---> CH3COOH + 3H2
% CH2OHCHOHCH2OH + NH3 ---> glyFER + CO2 + H2O +4H2
nr=nr+1;

umax=10;
Ks=23/92.09;
Y=0.4;
KIVFA=10;
KIH2S = 100/32.06;

if Test(7)==1 & real(C(6))>0 & real(C(22))>0 & real(C(17))>0 ;
rx(nr) =
umax*C(22)*ESU(C(6),Ks)*ENI(H2Sl,KIH2S)*ENI(VFA,KIVFA)*EpH(pH,aLLF,a
ULF,pHLLF,pHULF);
else
   rx(nr)=0;
end

if   rx(nr)>0 & Test(7)==1;
rGlycerol(nr) = -rx(nr)/Y;
Glycell = -rx(nr)*2;
GlyAcH = rGlycerol(nr)-Glycell;
rAcH(nr) = -GlyAcH;
rH2(nr) = -Glycell*2-GlyAcH*3;
```

```
rCO2(nr) = rx(nr);
rNH3(nr) = -rx(nr);
else
 rGlycerol(nr) = 0;
Glycell = 0;
GlyAcH = 0;
rAcH(nr) = 0;
rH2(nr) = 0;
rCO2(nr) = 0;
rNH3(nr) = 0;
end

%  8  Long Chain Fatty Acid Beta Oxidation
% CH3(CH2)14COOH +14H2O ---> 8CH3COOH + 14H2
% 5CH3(CH2)14COOH + 16NH3 + 22H2O ---> 16 BOB +14H2
nr=nr+1;

umax=0.12;
Ks=49.8/256.43;
Y=0.3*(256.43/114.12);
KIVFA=10;
KIH2S=100/32.06;
if Test(8)==1 & real(C(7))>0 & real(C(23))>0 & real(C(17))>0;
rx(nr) =
umax*C(23)*ESU(C(7),Ks)*ENI(H2Sl,KIH2S)*ENI(VFA,KIVFA)*EpH(pH,aLLF,a
ULF,pHLLF,pHULF)*ENI(C(34),KIH2);
else
   rx(nr)=0;
end

if rx(nr)>0 & Test(8)==1;
rPalmitic(nr) = -rx(nr)/Y;
Palcell = -rx(nr)*5/16;
PalAcH = rPalmitic(nr)-Palcell;
rAcH(nr) = -Palcell*8;
rNH3(nr) = -rx(nr);
rH2(nr) = (rAcH(nr)*14/8) + (rx(nr)*75/16);
else
   rPalmitic(nr) = 0;
Palcell = 0;
PalAcH = 0;
rAcH(nr) = 0;
rNH3(nr) = 0;
rH2(nr) = 0;
end


%  9  BuH Acetogenesis
% CH3CH2CH2COOH + 2H2O ---> 2CH3COOH + 2H2
% 5CH3CH2CH2COOH + 4NH3 ----> 4bACE +2H2O + 10H2
```

```
nr=nr+1;

umax = 0.264;%0.326;
Ks = 1.1;
Y = 0.04;%0.052*(88.11/114.12);
KIVFA = 3;%30
KIH2S = 26/32.06;

if Test(9)==1 & (C(12))>0 & (C(24))>0 & (C(17))>0;

rx(nr)=umax*C(24)*C(12)/(Ks+C(12))*EpH(pH,aLLM,aULM,pHLLM,pHULM)*E
NI(VFA,KIVFA)*ENI(H2Sl,KIH2S)*ENI(C(34),KIH2);
else
   rx(nr)=0;
end
if rx(nr)>0 & Test(9)==1;
rBuH(nr)=-rx(nr)/Y;
rBuHcell(nr)=-rx(nr)*5/4;
rBuHAcH(nr)=rBuH(nr)-rBuHcell(nr);
rAcH(nr)=-rBuHAcH(nr)*2;
rH2(nr)=-rBuHcell(nr)*2-rBuHAcH(nr)*2;
rNH3(nr)=-rx(nr);
else
   rBuH(nr)=0;
rBuHcell(nr)=0;
rBuHAcH(nr)=0;
rAcH(nr)=0;
rH2(nr)=0;
rNH3(nr)=0;
end


%  10  PrH Acetogenesis
%  CH3CH2COOH + 2H2O ---> CH3COOH + CO2 + 3H2
%  5CH3CH2COOH + 3NH3 ---> 3pACE + 4H2o + 5H2

nr=nr+1;

umax=0.36;%1.5;%0.16;
Ks=0.5;%2.205;
Y = 0.03;%0.015;
KIVFA=30;
KIH2S=26.6/32.06;%6.72;

if  Test(10)==1 & real(C(11))>0 & real(C(25))>0 & real(C(17))>0;

rx(nr)=umax*C(25)*C(11)/(Ks*(1+(VFA/KIVFA))+C(11)*(1+(H2Sl/KIH2S)))*EpH
(pH,aLLM,aULM,pHLLM,pHULM)*ENI(C(34),KIH2);
else
```

```
  rx(nr)=0;
end
if rx(nr)>0 & Test(10)==1;
rPrH(nr)=-rx(nr)/Y;
rPrHcell(nr)=-rx(nr)*5/3;
rPrHAcH(nr)=rPrH(nr)-rPrHcell(nr);
rAcH(nr)=-rPrHAcH(nr);
rH2(nr)=-rPrHcell(nr)-rPrHAcH(nr)*3;
rCO2(nr)=-rPrHAcH(nr);
rNH3(nr)=-rx(nr);
else
   rPrH(nr)=0;
rPrHcell(nr)=0;
rPrHAcH(nr)=0;
rAcH(nr)=0;
rH2(nr)=0;
rCO2(nr)=0;
rNH3(nr)=0;
end


%  11  H2 sulphate reduction
%  4H2 + H2SO4 ---> H2S + $h2O
%  5CO2 + 10H2 + NH3 ---> hSRB + 8H2O

nr=nr+1;

Y=0.021;%%1.232*(2.02/114.12);%0.0094;%0.7*(2.02/114.12);
umax = 5;
Ks=0.0015;%(4.88e-3)/2.02;
KSO4 = 0.0093;
KIH2S=4.65;%422/32.06;%17.19;
KIVFA=100;

if Test(11)==1 & real(C(8))>0 & real(C(15))>0 & real(C(13))>0 & real(C(26))>0 &
real(C(17))>0 & real(C(34))>0;
rx(nr)=umax*C(26)*EUI(C(8),Ks,H2Sl,KIH2S)*EpH(pH,aLLS,aULS,pHLLS,pHUL
S)*ENI(VFA,KIVFA);
else
   rx(nr)=0;
end
if rx(nr)>0 & Test(11)==1;
rH2(nr)=(-rx(nr))/Y;
rH2cellprod(nr)=(-rx(nr))*10;
rH2H2Sprod(nr)=rH2(nr)-rH2cellprod(nr);
rSO4(nr)=(rH2H2Sprod(nr))/4;
rH2S(nr)=(-rH2H2Sprod(nr))/4;
rCO2(nr)=(-rx(nr))*5;
rNH3(nr)=(-rx(nr));
else
```

```
   rH2(nr)=0;
rH2cellprod(nr)=0;
rH2H2Sprod(nr)=0;
rSO4(nr)=0;
rH2S(nr)=0;
rCO2(nr)=0;
rNH3(nr)=0;
end

%  12  AcH Sulphate Reduction
% CH3COOH + H2SO4 ---> H2S + CO2
% CH3COOH + 2NH3 ---> 2aSRB + 6H2O
nr=nr+1;

umax = 0.51;
Ks = 0.375;
Y = 0.023; %0.072*(60.05/114.12);
KIVFA=10;
KIH2S=4.75;%8.91;
KsSO4=0.2;

if Test(12)==1 & (C(13))>0 & (C(9))>0 & (C(27))>0 & (C(17))>0 ;

rx(nr)=umax*C(27)*EUI(C(9),Ks,H2Sl,KIH2S)*ESU(C(13),KsSO4)*EpH(pH,aLLS,
aULS,pHLLS,pHULS)*ENI(VFA,KIVFA);
else
 rx(nr)=0;
end

  if rx(nr)>0 & Test(12)==1;
rAcH(nr) = (-rx(nr))/Y;
rAcHcellprod(nr)=-rx(nr)*5/2;
rAcHH2S(nr)=rAcH(nr)-rAcHcellprod(nr);
rSO4(nr)=rAcHH2S(nr);
rH2S(nr)=-rAcHH2S(nr);
rCO2(nr)=-rAcHH2S(nr)*2;
rNH3(nr)=-rx(nr);
else
   rAcH(nr) = 0;
rAcHcellprod(nr)=0;
rAcHH2S(nr)=0;
rSO4(nr)=0;
rH2S(nr)=0;
rCO2(nr)=0;
rNH3(nr)=0;
end

%  13  LaH Sulphate Reduction
% 2CH3CHOHCOOH + H2SO4 ---> 2CH3COOH + H2S + 2CO2+ 2H2O
%  5CH3CHOHCOOH + 3MH3 ---> 3lSRB + 9H2O
```

```
nr=nr+1;

umax = 2.50;
Ks = 0.0488;
Y = 0.02;%0.046*(90.08/114.12);
KIVFA=10;
KIH2S=251/32.06;
KsSO4=1/114.12;

if Test(13)==1 & real(C(13))>0 & real(C(10))>0 & real(C(28))>0 & real(C(17))>0 ;
rx(nr)=umax*C(28)*ESU(C(10),Ks)*ESU(C(13),KsSO4)*EpH(pH,aLLS,aULS,pHL
LS,pHULS)*ENI(H2Sl,KIH2S)*ENI(VFA,KIVFA);
else
   rx(nr)=0;
end
if rx(nr)>0 & Test(13)==1;
rLaH(nr) = -rx(nr)/Y;
rLaHcellprod(nr)=-rx(nr)*2/3;
rLaHH2S(nr)=rLaH(nr)-rLaHcellprod(nr);
rSO4(nr)=rLaHH2S(nr)/2;
rH2S(nr)=-rLaHH2S(nr)/2;
rCO2(nr)=-rLaHH2S(nr);
rAcH(nr)=-rLaHH2S(nr);
rNH3(nr)=-rx(nr);
else
   rLaH(nr) = 0;
rLaHcellprod(nr)=0;
rLaHH2S(nr)=0;
rSO4(nr)=0;
rH2S(nr)=0;
rCO2(nr)=0;
rAcH(nr)=0;
rNH3(nr)=0;
end

%  14  PrH Sulphate Reduction
%CH3CH2COOH + 0.75H2SO4 ---> 0.75H2S + CH3COOH + CO2 + H2O
%CH3CH2COOH + 3NH3 ---> 3pSRB + 4H2O +5H2

nr=nr+1;

umax = 0.81;
Ks = 2.56;
Y = 0.03;
KIVFA=10;
KIH2S=8.89;
KsSO4=0.077;

if  Test(14)==1 & real(C(13))>0 & real(C(11))>0 & real(C(29))>0 & real(C(17))>0;
```

```
rx(nr)=umax*C(29)*EUI(C(11),Ks,H2Sl,KIH2S)*ESU(C(13),KsSO4)*EpH(pH,aLL
S,aULS,pHLLS,pHULS)*ENI(VFA,KIVFA);
else
   rx(nr)=0;
end

if rx(nr)>0 & Test(14)==1;
rPrH(nr) = -rx(nr)/Y;
rPrHcellprod(nr)=-rx(nr)*5/3;
rPrHH2S(nr)=rPrH(nr)-rPrHcellprod(nr);
rSO4(nr)=rPrHH2S(nr)*0.75;
rH2S(nr)=-rPrHH2S(nr)*0.75;
rAcH(nr)=-rPrHH2S(nr);
rCO2(nr)=-rPrHH2S(nr);
rH2(nr)=-rPrHcellprod(nr);
rNH3(nr)=-rx(nr);
else
   rPrH(nr) = 0;
rPrHcellprod(nr)=0;
rPrHH2S(nr)=0;
rSO4(nr)=0;
rH2S(nr)=0;
rAcH(nr)=0;
rCO2(nr)=0;
rH2(nr)=0;
rNH3(nr)=0;
end

%  15  BuH Sulphate Reduction
%  2CH3CH2CH2COOH + H2SO4 ---> 4CH3COOH +H2S
%  5CH3CH2CH2COOH + 3NH3 ---> 4bSRB + 2H2O + 2.5H2
nr=nr+1;

umax = 0.41;
Ks = 0.309;
Y = 0.04*(88.11/114.12);
KIVFA=10;
KIH2S=15.6;
KsSO4=0.17;

 if  Test(15)==1 & C(13)>0 & C(12)>0 & C(30)>0 & C(17)>0;

rx(nr)=umax*C(30)*EUI(C(12),Ks,H2Sl,KIH2S)*ESU(C(13),KsSO4)*EpH(pH,aLL
S,aULS,pHLLS,pHULS)*ENI(VFA,KIVFA);

else
   rx(nr)=0;
end
```

```
if rx(nr)>0 & Test(15)==1;
rBuH(nr) = -rx(nr)/Y;
rBuHcellprod(nr)=-rx(nr)*5/4;
rBuHH2S(nr)=rBuH(nr)-rBuHcellprod(nr);
rSO4(nr)=rBuHH2S(nr)/2;
rH2S(nr)=-rBuHH2S(nr)/2;
rAcH(nr)=-rBuHH2S(nr)*2;
rH2(nr)=-rBuHcellprod(nr)/2;
rNH3(nr)=-rx(nr);
else
   rBuH(nr) = 0;
rBuHcellprod(nr)=0;
rBuHH2S(nr)=0;
rSO4(nr)=0;
rH2S(nr)=0;
rAcH(nr)=0;
rH2(nr)=0;
rNH3(nr)=0;
end

%  16  EOH Sulphate reduction

%   C2H5OH + H2SO4 ====> H2S + 2CO2 + 2H2
%  5C2H5OH + 2NH3 =====> 2EOH + 5H2

nr=nr+1;

umax = 0.8;%0.35;
Ks = 0.124;%0.8;
Y = 0.02;%0.0002;
KIVFA=10;
KIH2S=5.6;%20;
KsSO4=0.124;%0.3;

 if  Test(16)==1 & C(13)>0 & C(18)>0 & C(31)>0 & C(17)>0;

rx(nr)=umax*C(31)*EUI(C(18),Ks,H2Sl,KIH2S)*ESU(C(13),KsSO4)*EpH(pH,aLL
S,aULS,pHLLS,pHULS)*ENI(VFA,KIVFA);
%rx(nr)=umax*C(31)*ESU(C(18),Ks)*ESU(C(13),KsSO4);
else
   rx(nr)=0;
end

if rx(nr)>0 & Test(16)==1;
rEOH(nr) = -rx(nr)/Y;
rEOHcellprod(nr)=-rx(nr)*5/4;
rEOHH2S(nr)=rEOH(nr)-rEOHcellprod(nr);
rSO4(nr)=rEOHH2S(nr);
rH2S(nr)=-rEOHH2S(nr);
rCO2(nr)=-rEOHH2S(nr)*2;
```

```
rH2(nr)=-rEOHcellprod(nr)-rEOHH2S(nr)*2;
rNH3(nr)=-rx(nr);
else
   rEOH(nr) = 0;
rEOHcellprod(nr)=0;
rEOHH2S(nr)=0;
rSO4(nr)=0;
rH2S(nr)=0;
rCO2(nr)=0;
rH2(nr)=0;
rNH3(nr)=0;
end

%  17  H2 Methanogenesis
% 4H2 + CO2---> CH4 + 2H2O
%  5CO2 + 10H2+NH3 ---> hMPB + 8H2O

nr = nr+1;

umax = 1;
Ks = 0.008125;%30e-3/2.02;
Y = 0.002;%0.39*(2.02/114.12);
KIVFA = 10;
KIH2S = 664/32.06;

if Test(17)==1 & real(C(8))>0 & real(C(15))>0 & real(C(32))>0 & real(C(17))>0;
rx(nr)=umax*C(32)*EUI(C(8),Ks,H2Sl,KIH2S)*EpH(pH,aLLM,aULM,pHLLM,pH
ULM)*ENI(VFA,KIVFA);
else
   rx(nr)=0;
end

if rx(nr)>0 & Test(17)==1;
rH2(nr)=(-rx(nr))/Y;
rH2cellprod(nr)=(-rx(nr))*10;
rH2CH4prod(nr)=rH2(nr)-rH2cellprod(nr);
rCH4(nr)=-rH2CH4prod(nr)/4;
rCO2(nr)=(rH2CH4prod(nr)/4)+(rH2cellprod(nr)/2);
rNH3(nr)=(-rx(nr));
else
   rH2(nr)=0;
rH2cellprod(nr)=0;
rH2CH4prod(nr)=0;
rCH4(nr)=0;
rCO2(nr)=0;
rNH3(nr)=0;
end

%  18  AcH Methanogenesis
%  CH3COOH ---> CH4+CO2
```

```
%  5CH3COOH +2NH3---> 2aMPB + 6H2O
nr=nr+1;

umax=0.36;%0.24;
Ks=0.875;
Y=0.0127;%0.04*(60.05/114.12);
KIVFA=10;
KIH2S=117/32.06;

if Test(18)==1 & real(C(9))>0 & real(C(33))>0 & real(C(17))>0;
rx(nr)=umax*C(33)*EUI(C(9),Ks,H2Sl,KIH2S)*EpH(pH,aLLM,aULM,pHLLM,pH
ULM)*ENI(VFA,KIVFA);
else
   rx(nr)=0;
end

if rx(nr)>0 & Test(18)==1;
rAcH(nr) = (-rx(nr))/Y;
rAcHcellprod(nr)=-rx(nr)*5/2;
rAcHCH4(nr)=rAcH(nr)-rAcHcellprod(nr);
rCH4(nr)=-rAcHCH4(nr);
rCO2(nr)=-rAcHCH4(nr);
rNH3(nr)=-rx(nr);
else
   rAcH(nr) = 0;
rAcHcellprod(nr)=0;
rAcHCH4(nr)=0;
rCH4(nr)=0;
rCO2(nr)=0;
rNH3(nr)=0;
end

%  End of Reactions

kaH2S=(8.9e-8)*1e3;            %  (mmole/l)/atm
kaH2CO3=(4.3e-7)*1e3;


N(1)=-klaH2*((C(34)/HH2)-C(8));          %  (mmole/(l.d))
N(2)=-klaH2S*((C(35)/HH2S)-C(14));       %  (mmole/(l.d))
N(3)=-klaCO2*((C(36)/HCO2)-C(15));       %  (mmole/(l.d))
N(4)=-klaCH4*((C(37)/HCH4)-C(16));       %  (mmole/(l.d))

if N(1)>0; N(1)=N(1);else
   N(1)=0;end
if N(2)>0; N(2)=N(2);else
   N(2)=0;end
if N(3)>0; N(3)=N(3);else
   N(3)=0;end
if N(4)>0; N(4)=N(4);else
```

```
   N(4)=0;end


if Test(19)==1
kd=[0.02 0.02 0.02 0.02 0.01 0.027 0.01 0.013 0.013 0.02 0.021 0.02 0.02 0.013
0.013];
else
   kd=[0 0 0 0 0 0 0 0 0 0 0 0 0 0 0];
end

asum=kd(1)*C(19)+kd(2)*C(20)+kd(3)*C(21)+kd(4)*C(22)+kd(5)*C(23)+kd(6)*C(
24)+kd(7)*C(25)...

+kd(8)*C(26)+kd(9)*C(27)+kd(10)*C(28)+kd(11)*C(29)+kd(12)*C(30)+kd(13)*C(3
1)+kd(14)*C(32)+kd(15)*C(33);


M=(C(34)+C(35)+C(36)+C(37));
if M<=0;
   M=1;
else M=M;
end

Qgout=Vl*R*T*(N(1)+N(2)+N(3)+N(4));          % l/day
%Qgout=Vl*(N(1)*HH2+N(2)*HH2S+N(3)*HCO2+N(4)*HCH4);

%  Summing rates

rProtein=rProtein + asum;                    %  (mmole/l/d)
rCarbohydrate=(sum(rCarbohydrate(1:2)));
rLipid=(sum(rLipid(1:3)));
rAminoAcid=(sum(rAminoAcid(1:4)));
rGlucose=(sum(rGlucose(1:5)));
rGlycerol=(sum(rGlycerol(1:7)));
rPalmitic=(sum(rPalmitic(1:8)));
rH2=(sum(rH2(1:17)));
rAcH=(sum(rAcH(1:18)));
rLaH=(sum(rLaH(1:13)));
rPrH=(sum(rPrH(1:14)));
rBuH=(sum(rBuH(1:15)));
rSO4=(sum(rSO4(1:16)));
rH2S=(sum(rH2S(1:16)));
rCO2=(sum(rCO2(1:18)));
rNH3=(sum(rNH3(1:18)));
rCH4=(sum(rCH4(1:18)));
rEOH=(sum(rEOH(1:16)));


%%% differential equations  %%%%%
```

```
%%% For CSTR


%for i=1:35;
%if C(i)<=0
%   C(i)=0;
%else C(i)=C(i);
%end
%end

dC(1)=((CProteinin-C(1))*Ql/Vl)+(rProtein);              %  (mmole/l/day)
dC(2)=((CCarbohydratein-C(2))*Ql/Vl)+(rCarbohydrate);
dC(3)=((CLipidin-C(3))*Ql/Vl)+(rLipid);
dC(4)=((CAminoAcidin-C(4))*Ql/Vl)+(rAminoAcid);
dC(5)=((CGlucosein-C(5))*Ql/Vl)+(rGlucose);
dC(6)=((CGlycerolin-C(6))*Ql/Vl)+(rGlycerol);
dC(7)=((CPalmiticin-C(7))*Ql/Vl)+(rPalmitic);
dC(8)=((CH2in-C(8))*Ql/Vl)+(rH2)-N(1);
dC(9)=((CAcHin-C(9))*Ql/Vl)+(rAcH);
dC(10)=((CLaHin-C(10))*Ql/Vl)+(rLaH);
dC(11)=((CPrHin-C(11))*Ql/Vl)+(rPrH);
dC(12)=((CBuHin-C(12))*Ql/Vl)+(rBuH);
dC(13)=((CSO4in-C(13))*Ql/Vl)+(rSO4);
dC(14)=((CH2Sin-C(14))*Ql/Vl)+(rH2S)-N(2);
dC(15)=((CCO2in-C(15))*Ql/Vl)+(rCO2)-N(3);
dC(16)=((CCH4in-C(16))*Ql/Vl)+(rCH4)-N(4);
dC(17)=((CNH3in-C(17))*Ql/Vl)+(rNH3);
dC(18)=((CEOHin-C(18))*Ql/Vl)+(rEOH);

dC(19)=((Cafxin-C(19))*Ql/Vl)+rx(4)-kd(1)*C(19);
dC(20)=((Cgfxin-C(20))*Ql/Vl)+rx(5)-kd(2)*C(20);
dC(21)=((Clfxin-C(21))*Ql/Vl)+rx(6)-kd(3)*C(21);
dC(22)=((Cglyfxin-C(22))*Ql/Vl)+rx(7)-kd(4)*C(22);
dC(23)=((Cbobxin-C(23))*Ql/Vl)+rx(8)-kd(5)*C(23);
dC(24)=((Cbaxin-C(24))*Ql/Vl)+rx(9)-kd(6)*C(24);
dC(25)=((Cpaxin-C(25))*Ql/Vl)+rx(10)-kd(7)*C(25);
dC(26)=((Chsxin-C(26))*Ql/Vl)+rx(11)-kd(8)*C(26);
dC(27)=((Casxin-C(27))*Ql/Vl)+rx(12)-kd(9)*C(27);
dC(28)=((Clsxin-C(28))*Ql/Vl)+rx(13)-kd(10)*C(28);
dC(29)=((Cpsxin-C(29))*Ql/Vl)+rx(14)-kd(11)*C(29);
dC(30)=((Cbsxin-C(30))*Ql/Vl)+rx(15)-kd(12)*C(30);
dC(31)=((Cesxin-C(31))*Ql/Vl)+rx(16)-kd(13)*C(31);
dC(32)=((Chmxin-C(32))*Ql/Vl)+rx(17)-kd(14)*C(32);
dC(33)=((Camxin-C(33))*Ql/Vl)+rx(18)-kd(15)*C(33);

dC(34)=((Qgin*PinH2-Qgout*C(34))/Vv)+(Vl/Vv*R*T*N(1));          %  atm/day
dC(35)=((Qgin*PinH2S-Qgout*C(35))/Vv)+(Vl/Vv*R*T*N(2));
dC(36)=((Qgin*PinCO2-Qgout*C(36))/Vv)+(Vl/Vv*R*T*N(3));
dC(37)=((Qgin*PinCH4-Qgout*C(37))/Vv)+(Vl/Vv*R*T*N(4));
```

```
minim(n,1) = t;
minim(n,2:22) = Se;

disp('N Qgout')
disp([N Qgout])
disp(C(34:37))
disp('SO4')
disp(C(13));
```

Appendix C-3: equilib.m

```
%  Se = equilibrium concentration
%  1  H+
%  2  H2S(l)
%  3  HS-
%  4  S--
%  5  H2SO4
%  6  HSO4-
%  7  SO4--
%  8  NH3
%  9  NH4+
%  10 CO2(l)
%  11 H2CO3
%  12 HCO3-
%  13 CO3--
%  14 AcH
%  15 Ac-
%  16 PrH
%  17 Pr-
%  18 BuH
%  19 Bu-
%  20 LaH
%  21 La-

function res = equilib(Se,C)

SeCH = [1 0 -1 -2 0 -1 -2 0 1 0 0 -1 -2 0 -1 0 -1 0 -1 0 -1];
T=298;

%  Constants
KH2S = 8.9e-8*1e3;
KHS = 1.2e-13*1e3;
KCO2 = 1.5e-4*1e3;
KH2CO3=4.3e-7*1e3;
KHCO3=4.8e-11*1e3;
KH2SO4=1e8*1e3;
KHSO4=1.1e-2*1e3;
KNH4=5.6e-19*1e3;
```

```
KH2O=1e-14*1e6;
KAcH=1.7e-5*1e3;
KPrH=1.3e-5*1e3;
KBuH=1.4e-5*1e3;
KLaH=1.3e-4*1e3;



%  charge balance

resi(1) = sum(Se(1:21).*SeCH(1:21));

%  Mass balance and equilibria
I = 0.5.*((Se(1:21)./1000).*(SeCH(1:21).^2));
A = 1.82e6*(78.3*T)^-1.5;
a = 10.^(-A.*(SeCH(1:21).^2).*((sqrt(I)./(sqrt(I)+1))-(0.3.*I)));
ASe = Se(1:21).*a;

%  H2S(l), HS-, S--, H2S(v)

resi(2)= (Se(2)+Se(3)+Se(4)-C(14));
resi(3)=KH2S*ASe(2)-ASe(3)*ASe(1);
resi(4)=KHS*ASe(3)-ASe(4)*ASe(1);


%  H2SO4, HSO4-, SO4--

resi(5)=(Se(5)+Se(6)+Se(7)-C(13));
%resi(6)=KH2SO4*ASe(5)-ASe(6)*ASe(1);
resi(6)=0;
resi(7)=KHSO4*ASe(6)-ASe(7)*ASe(1);

%  NH3, NH4+

resi(8)=(Se(8)+Se(9)-C(17));
%resi(9)=ASe(8)*ASe(1)-KNH4*ASe(9);
resi(9)=KNH4*ASe(9)-ASe(8)*ASe(1);

%  CO2, H2CO3, HCO3-,CO3--

resi(10)=(Se(10)+Se(11)+Se(12)+Se(13)-C(15));
resi(11)=KCO2*ASe(10)-ASe(11);
resi(12)=KH2CO3*ASe(11)-ASe(12)*ASe(1);
resi(13)=KHCO3*ASe(12)-ASe(13)*ASe(1);

%  AcH

resi(14)=(Se(14)+Se(15)-C(9));
resi(15)=KAcH*ASe(14)-ASe(15)*ASe(1);
```

```
% PrH

resi(16)=(Se(16)+Se(17)-C(11));
resi(17)=KPrH*ASe(16)-ASe(17)*ASe(1);

% BuH

resi(18)=(Se(18)+Se(19)-C(12));
resi(19)=KBuH*ASe(18)-ASe(19)*ASe(1);

% LaH

resi(20)=(Se(20)+Se(21)-C(10));
resi(21)=KLaH*ASe(20)-ASe(21)*ASe(1);

res=sum(sqrt(resi(1:21).^2));
%disp([Se]')
%disp('res');
%disp([res]);
```

Appendix C-4: EUI.m

```
% substrate utilistion with uncompetitive inhibition

function E = EUI(S,Ks,I,KI)
E=S/(Ks+S*(1+I/KI));
```

Appendix C-5 : ESU.m

```
% Monod substrate utilisation function

function E=ESU(S,Ks)
E=S/(Ks+S);
```

Appendix C-6 : EpH.m

```
% pH inhibition function

function E=EpH(pH,aLL,aUL,pHLL,pHUL)
E=1/((1+exp(-aLL*(pH-pHLL)))*(1+exp(aUL*(pH-pHUL))));
```

Appendix C-7: ENI.m

```
% Non competitive inhibition

function E=ENI(I,KI)
  E=KI/(KI+I);
```

Appendix D

RESULTS OF THE MOLE AND MASS BALANCE

Tables D-1 to D-6 are the mole and mass balances for the AMD sites using molasses as the substrate with a HRT of 10. Tables D-7 to D-12 are the mole and mass balances for the AMD sites using primary sewage sludge as the substrate with a HRT of 10. Tables D-13 to D-18 are the mole and mass balances for the AMD sites using ethanol as the substrate with a HRT of 10. Tables D-19 to D-36 are mole and mass balances for the various substrates at various hydraulic residence times. Tables D-37 to D-54 are the mole and mass balances for the various carbon substrates with increasing sulphate loading.

Table D-1: Results of the mole balance for AMD site 2 using molasses as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1800	0	1800	0	0	1800	0	1000	800	1000	0	0	500	400
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	7500	0	700	0	0	700	0	700	0	700	0	0	700	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	19100	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
Hydrogen sulphide	0	0	2500	14200	0	7000	7100	9600	7100	0	300	0	0	300	0
Carbon dioxide	0	0	12200	22000	0	0	22000	12200	22000	0	22000	0	0	22000	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	100	0	100	0	0	100	0	100	0	100	0	0	100	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4600	0	4600	0	4600	0	4600	0	0	4600	0
Sulphur	0	0	0	0	0	0	0	0	0	0	6900	0	0	700	6200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	6.E+05	6.E+05	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2.E+06	2.E+06	0	0
Bacteria	0	0	0	1200	0	0	1200	0	700	500	1000	0	0	600	500
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	33400	5.55E+07	0	0	5.55E+07	66500

Table D-2: Results of the mass balance for AMD site 2 using molasses as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	180	0	200	0	0	0	200	110	90	0	0	110	80	50
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	1350	0	120	0	0	0	120	120	0	0	0	120	120	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	10	0	0	0	10	10	0	0	0	10	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	10	0	0	0	10	10	0	0	0	10	10	0
sulphates	1830	0	0	250	0	0	0	250	250	0	0	0	250	250	0
hydrogen sulphide	0	0	90	480	0	450	530	40	40	0	0	0	10	10	0
Carbon dioxide	0	0	540	970	0	0	540	970	970	0	0	0	970	970	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	170	0	170	0	0	0	170	170	0	0	0	170	170	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	170	0	0	179	170	0	0	0	170	170	0
Oxygen	0	0	0	0	0	0	0	0	0	0	2170	2180	0	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	7180	7150	0	0	0
Sulphur	0	0	0	0	0	0	0	0	0	0	0	0	30	0	20
Bacteria	0	0	0	140	0	0	0	140	80	60	0	0	80	50	40
Water	1000000	0	0	1000000	0	0	0	1000000	999400	600	0	0	999400	999000	440

Table D-3: Results of the mole balance for AMD site 3 using molasses as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1900	0	2200	0	0	2200	0	1200	1000	1200	0	0	700	500
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	9400	0	800	0	0	800	0	800	0	800	0	0	800	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	23400	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	5300	15900	0	7900	8000	13300	8000	0	300	0	0	300	0
Carbon dioxide	0	0	22300	21400	0	0	21400	22300	21400	0	21400	0	0	21400	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	100	0	100	0	0	100	0	100	0	100	0	0	100	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4900	0	4900	0	4900	0	4900	0	0	4900	0
Sulphur	0	0	0	0	0	0	0	0	0	0	7700	0	0	800	7000
Oxygen	0	0	0	0	0	0	0	0	0	0	0	642700	638800	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2417600	2417600	0	0
Bacteria	0	0	0	1500	0	0	1500	0	900	700	1300	0	0	700	800
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	41900	5.55E+07	0	0	5.54E+07	77400

Table D-4: Results of the mass balance for AMD site 3 using molasses as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	220	0	250	0	0	0	250	140	110	0	0	140	80	80
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	1700	0	150	0	0	0	150	150	0	0	0	150	150	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	10	0	0	0	10	10	0	0	0	10	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	10	0	0	0	10	10	0	0	0	10	10	0
sulphates	2250	0	0	250	0	0	0	250	250	0	0	0	250	250	0
hydrogen sulphide	0	0	180	540	0	500	680	40	40	0	0	0	10	10	0
Carbon dioxide	0	0	980	940	0	0	980	940	940	0	0	0	940	940	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	180	0	0	180	180	0	0	0	180	180	0
Sulphur	0	0	0	0	0	0	0	0	0	0	0	0	30	0	30
Oxygen	0	0	0	0	0	0	0	0	0	0	2520	2500	0	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	8300	8300	0	0	0
Bacteria	0	0	0	180	0	0	0	180	100	80	0	0	100	80	50
Water	1000000	0	0	1000000	0	0	0	1000000	9.99E+05	780	0	0	9.99E+05	9.99E+05	540

Table D-5: Results of the mole balance for AMD site 1 using molasses as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1200	0	1300	0	0	1300	0	700	600	700	0	0	400	300
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	5700	0	500	0	0	500	0	500	0	500	0	0	500	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	15000	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	400	12000	0	6000	6000	6400	6000	0	300	0	0	300	0
Carbon dioxide	0	0	2400	22700	0	0	22700	2400	22700	0	22700	0	0	22700	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	100	0	100	0	0	100	0	100	0	100	0	0	100	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4300	0	4300	0	4300	0	4300	0	0	4300	0
Sulphur	0	0	0	0	0	0	0	0	0	0	5800	0	0	600	5200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	479300	476400	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	1802900	1802900	0	0
Bacteria	0	0	0	900	0	0	900	0	500	400	800	0	0	500	400
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	25200	5.55E+07	0	0	5.55E+07	54300

Table D-6: Results of the mass balance for AMD site 1 using molasses as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	130	0	150	0	0	150	0	60	70	60	0	0	50	40
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	1020	0	90	0	0	90	0	90	0	90	0	0	90	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
sulphates	1440	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	10	410	0	200	210	220	210	0	10	0	0	10	0
Carbon dioxide	0	0	110	1000	0	0	1000	110	1000	0	1000	0	0	1000	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	160	0	160	0	160	0	160	0	0	160	0
Sulphur	0	0	0	0	0	0	0	0	0	0	160	0	0	20	170
Oxygen	0	0	0	0	0	0	0	0	0	0	0	15340	15240	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	50480	50480	0	0
Bacteria	0	0	0	110	0	0	110	0	60	50	90	0	0	50	40
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	1.00E+06	450	1.00E+06	0	0	9.99E+05	980

Table D-7: Results of the mole balance for AMD site 2 using primary sewage sludge as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	13700	0	9800	0	0	9800	0	3400	6300	3400	0	0	1200	2200
Carbohydrates	0	6700	0	3400	0	0	3400	0	1200	2200	1200	0	0	400	600
Lipids	0	1400	0	1000	0	0	1000	0	300	800	300	0	0	100	200
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	4500	0	100	0	0	100	0	100	0	100	0	0	100	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	500	0	0	500	0	500	0	500	0	0	500	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	19100	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	1900	14800	0	7400	7400	9200	7400	100	200	0	0	200	0
Carbon dioxide	0	0	8400	21600	0	0	21600	8400	21600	200	21600	0	0	21500	100
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	500	0	0	500	0	500	0	500	0	0	500	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4700	0	4700	0	4700	0	4700	0	0	4700	0
Sulphur	0	0	0	0	0	0	0	0	0	0	7100	0	0	700	6400
Oxygen	0	0	0	0	0	0	0	0	0	0	0	592800	589200	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2230000	2230000	0	0
Bacteria	0	0	0	4100	0	0	4100	0	1400	2700	1800	0	0	800	1200
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.51E+07	4.21E+05	5.51E+07	0	0	5.49E+07	1.99E+05

Table D-8: Results of the mass balance for AMD site 2 using primary sewage sludge as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1560	0	1110	0	0	1110	0	390	720	390	0	0	140	250
Carbohydrates	0	1090	0	550	0	0	550	0	190	360	190	0	0	70	120
Lipids	0	1150	0	790	0	0	790	0	280	510	280	0	0	100	180
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	270	0	10	0	0	10	0	10	0	10	0	0	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	40	0	0	40	0	40	0	40	0	0	40	0
Butyrate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
sulphates	1830	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	80	500	0	250	250	310	250	0	10	0	0	10	0
Carbon dioxide	0	0	370	960	0	0	960	370	950	10	950	0	0	950	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	170	0	170	0	170	0	170	0	0	170	0
Sulphur	0	0	0	0	0	0	0	0	0	0	230	0	0	20	210
Oxygen	0	0	0	0	0	0	0	0	0	0	0	18970	18850	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	62440	62440	0	0
Bacteria	0	0	0	470	0	0	470	0	160	300	200	0	0	70	130
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	9.92E+05	7580	9.92E+05	0	0	9.89E+05	3580

Table D-9: Results of the mole balance for AMD site 1 using primary sewage sludge as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	10500	0	7500	0	0	7500	0	2600	4900	2600	0	0	900	1700
Carbohydrates	0	5200	0	2600	0	0	2600	0	900	1700	900	0	0	300	600
Lipids	0	1100	0	800	0	0	800	0	300	500	300	0	0	100	200
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	3500	0	100	0	0	100	0	100	0	100	0	0	100	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	500	0	0	500	0	500	0	500	0	0	500	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	15000	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	0	12400	0	6200	6200	6200	6200	0	200	0	0	200	0
Carbon dioxide	0	0	0	22200	0	0	22200	0	22100	100	22100	0	0	22000	100
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	400	0	0	400	0	400	0	400	0	0	400	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4300	0	4300	0	4300	0	4300	0	0	4300	0
Sulphur	0	0	0	0	0	0	0	0	0	0	5900	0	0	600	5300
Oxygen	0	0	0	0	0	0	0	0	0	0	0	492900	489900	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	1854200	1854200	0	0
Bacteria	0	0	0	3100	0	0	3100	0	1100	2000	1400	0	0	500	900
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.52E+07	3.24E+06	5.52E+07	0	0	5.51E+07	1.56E+05

Table D-10: Results of the mass balance for AMD site 1 using primary sewage sludge as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1200	0	860	9	0	860	0	300	560	300	0	0	100	190
Carbohydrates	0	840	0	420	0	0	420	0	150	270	150	0	0	50	100
Lipids	0	880	0	610	0	0	819	0	210	390	210	0	0	70	140
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Hydrogen	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0
Acetate	0	210	0	10	0	0	10	0	10	9	10	0	0	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	40	0	0	40	0	40	0	40	0	0	40	0
Butyrate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
sulphates	1440	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	0	420	0	210	210	210	210	0	10	0	0	10	0
Carbon dioxide	0	0	0	980	0	0	980	0	970	10	970	0	0	970	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	160	0	160	0	180	0	160	0	0	160	0
Sulphur	0	0	0	0	0	0	0	0	0	0	190	0	0	20	170
Oxygen	0	0	0	0	0	0	9	0	0	0	0	15770	15680	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	51920	51920	0	0
Bacteria	0	0	0	360	0	0	360	0	120	230	180	0	0	80	100
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	9.94E+05	5.80E+03	9.94E+05	0	0	9.91E+05	2800

Table D-11: Results of the mole balance for AMD site 3 using primary sewage sludge as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	17200	0	12200	0	0	12200	0	4300	8000	4300	0	0	1500	2800
Carbohydrates	0	8400	0	4200	0	0	4200	0	1500	2600	1500	0	0	500	1000
Lipids	0	1600	0	1200	0	0	1200	0	400	800	400	0	0	100	300
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	5700	0	200	0	0	200	0	200	0	200	0	0	200	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	500	0	0	500	0	500	0	500	0	0	500	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	23400	0	0	2400	0	0	2400	0	2300	0	2300	0	0	2300	0
hydrogen sulphide	0	0	4600	16800	0	8400	8500	13000	8400	100	200	0	0	200	0
Carbon dioxide	0	0	17600	21100	0	0	21100	17600	20900	200	20900	0	0	20900	100
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	600	0	0	600	0	600	0	600	0	0	600	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	5100	0	5100	0	5000	0	5000	0	0	5000	0
Sulphur	0	0	0	0	0	0	0	0	0	0	8100	0	0	800	7300
Oxygen	0	0	0	0	0	0	0	0	0	0	0	674900	670800	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2538800	2538800	0	0
Bacteria	0	0	0	5100	0	0	5100	0	1600	3300	2200	0	0	800	1400
Water	55555600	0	0	55555600	0	0	55555600	0	55027300	528200	55027300	0	0	54783600	243800

Table D-12: Results of the mass balance for AMD site 3 using primary sewage sludge as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	2000	0	1400	0	0	1400	0	500	900	500	0	0	200	300
Carbohydrates	0	1400	0	700	0	0	700	0	200	400	200	0	0	100	200
Lipids	0	1400	0	1000	0	0	1000	0	300	600	300	0	0	100	200
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	300	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	2200	0	0	200	0	0	200	0	200	0	200	0	0	200	0
hydrogen sulphide	0	0	200	600	0	300	300	400	300	0	0	0	0	0	0
Carbon dioxide	0	0	800	900	0	0	900	800	900	0	900	0	0	900	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	200	0	200	0	200	0	200	0	0	200	0
Sulphur	0	0	0	0	0	0	0	0	0	0	300	0	0	0	200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	21600	21500	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	71100	71100	0	0
Bacteria	0	0	0	600	0	0	600	0	200	400	300	0	0	100	200
Water	1000000	0	0	1000000	0	0	1000000	0	990500	9500	990500	0	0	986100	4400

Table D-13: Results of the mole balance for AMD site 2 using ethanol as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	100	0	0	100	0	100	0	100	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	19100	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	0	16500	0	8200	8300	8200	8300	0	300	0	0	300	0
Carbon dioxide	0	0	0	21100	0	0	21100	0	21100	0	21100	0	0	21000	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	1000	0	300	0	0	300	0	300	0	300	0	0	300	0
Ethanol	0	12100	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	5000	0	5000	0	5000	0	5000	0	0	5000	0
Sulphur	0	0	0	0	0	0	0	0	0	0	8000	0	0	800	7200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	667900	663900	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2512700	2512700	0	0
Bacteria	0	0	0	800	0	0	800	0	400	300	800	0	0	400	300
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	8320	5.55E+07	0	0	5.55E+07	6.07E+04

Table D-14: Results of the mass balance for AMD site 2 using ethanol as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	10	0	0	10	0	10	0	10	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	1830	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	0	560	0	280	280	280	280	0	10	0	0	10	0
Carbon dioxide	0	0	0	930	0	0	930	0	930	0	930	0	0	930	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	560	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	160	0	160	0	160	0	160	0	0	160	0
Sulphur	0	0	0	0	0	0	0	0	0	0	260	0	0	30	230
Oxygen	0	0	0	0	0	0	0	0	0	0	0	21370	21250	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	70360	70360	0	0
Bacteria	0	0	0	70	0	0	70	0	40	30	90	0	0	50	40
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	1.00E+06	150	1.00E+06	0	0	9.99E+05	1090

Table D-15: Results of the mole balance for AMD site 3 using ethanol as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
proteins	0	0	0	100	0	0	100	0	100	100	100	0	0	0	0
carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
palmitic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AcH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LaH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PrH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BuH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SO4	23400	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
H2S	0	0	2100	19000	0	9400	9500	11500	9500	0	300	0	0	300	0
CO2	0	0	6800	20400	0	0	20400	6800	20400	0	20400	0	0	20400	0
CH4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NH3	0	1000	0	100	0	0	100	0	100	0	100	0	0	100	0
EOH	0	15300	0	0	0	0	0	0	0	0	0	0	0	0	0
HCL	0	0	0	0	5400	0	5400	0	5400	0	5400	0	0	5400	0
sulphur	0	0	0	0	0	0	0	0	0	0	9300	0	0	900	8400
bacteria	0	0	0	0	0	0	0	0	0	0	0	771200	768800	0	0
water	0	0	0	0	0	0	0	0	0	0	0	2901200	2901200	0	0
O2	0	0	0	800	0	0	800	0	500	400	900	0	0	500	400
N2	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	10500	5.55E+07	0	0	5.55E+07	70600

Table D-16: Results of the mass balance for AMD site 3 using ethanol as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
proteins	0	0	0	10	0	0	10	0	10	10	10	0	0	0	0
carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
palmitic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AcH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LaH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PrH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BuH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SO4	2250	0	0	250	0	0	250	0	250	0	250	0	0	250	0
H2S	0	0	70	650	0	320	330	390	320	0	10	0	0	10	0
CO2	0	0	300	900	0	0	900	300	900	0	900	0	0	900	0
CH4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NH3	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
EOH	0	700	0	0	0	0	0	0	0	0	0	0	0	0	0
HCL	0	0	0	0	200	0	200	0	200	0	200	0	0	200	0
sulphur	0	0	0	0	0	0	0	0	0	0	300	0	0	30	270
bacteria	0	0	0	0	0	0	0	0	0	0	0	24680	24530	0	0
water	0	0	0	0	0	0	0	0	0	0	0	81230	81230	0	0
O2	0	0	0	90	0	0	90	0	50	40	110	0	0	60	50
N2	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	1.00E+06	190	1.00E+06	0	0	9.99E+05	1270

Table D-17: Results of the mole balance for AMD site 1 using ethanol as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	100	0	0	100	0	0	0	0	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	15000	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	0	12400	0	6200	6200	6200	6200	0	300	0	0	300	0
Carbon dioxide	0	0	0	15900	0	0	15900	0	15800	0	15800	0	0	15800	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	1000	0	400	0	0	400	0	400	0	400	0	0	400	0
Ethanol	0	9100	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	3800	0	3800	0	3800	0	3800	0	0	3800	0
Sulphur	0	0	0	0	0	0	0	0	0	0	6000	0	0	600	5400
Oxygen	0	0	0	0	0	0	0	0	0	0	0	497200	494200	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	1870400	1870400	0	0
Bacteria	0	0	0	500	0	0	500	0	300	200	600	0	0	300	300
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	6300	5.55E+07	0	0	5.55E+07	45200

Table D-18: Results of the mass balance for AMD site 1 using ethanol as the substrate with a HRT of 10.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	10	0	0	10	0	0	0	0	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	1440	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	0	420	0	210	210	210	210	0	10	0	0	10	0
Carbon dioxide	0	0	0	700	0	0	700	0	700	0	700	0	0	700	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	20	0	10	0	0	10	0	10	0	10	0	0	10	0
Ethanol	0	420	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	140	0	140	0	140	0	140	0	0	140	0
Sulphur	0	0	0	0	0	0	0	0	0	0	190	0	0	20	170
Oxygen	0	0	0	0	0	0	0	0	0	0	0	15910	15610	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	52370	52370	0	0
Bacteria	0	0	0	60	0	0	60	0	30	20	70	0	0	40	30
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	1.00E+06	110	1.00E+06	0	0	9.99E+05	810

Table D-19: Results of the mole balance for AMD site 2 using molasses as the substrate with a HRT of 9.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1600	0	1700	0	0	1700	0	1000	800	1000	0	0	500	400
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	7500	0	700	0	0	700	0	700	0	700	0	0	700	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	19100	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	2800	14100	0	7000	7100	9800	7100	0	300	0	0	300	0
Carbon dioxide	0	0	13700	21800	0	0	21800	13700	21800	0	21800	0	0	21700	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	100	0	100	0	0	100	0	100	0	100	0	0	100	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4600	0	4600	0	4600	0	4600	0	0	4600	0
Sulphur	0	0	0	0	0	0	0	0	0	0	6800	0	0	700	6100
Oxygen	0	0	0	0	0	0	0	0	0	0	0	586300	582900	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2130600	2130600	0	0
Bacteria	0	0	0	1200	0	0	1200	0	700	500	1000	0	0	600	500
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	33500	5.55E+07	0	0	5.55E+07	66300

Table D-20: Results of the mass balance for AMD site 2 using molasses as the substrate with a HRT of 9.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	180	0	200	0	0	200	0	110	90	110	0	0	90	50
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	1360	0	120	0	0	120	0	120	0	120	0	0	120	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	20	0	0	20	0	20	0	20	0	0	20	0
sulphates	1830	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	100	480	0	240	240	340	240	0	10	0	0	10	0
Carbon dioxide	0	0	600	960	0	0	960	600	960	0	960	0	0	960	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	170	0	170	0	170	0	170	0	0	170	0
Sulphur	0	0	0	0	0	0	0	0	0	0	220	0	0	20	200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	18120	19010	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	59660	59660	0	0
Bacteria	0	0	0	140	0	0	140	0	80	90	120	0	0	70	50
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	9.99E+05	600	9.99E+05	0	0	9.98E+05	1190

Table D-21: Results of the mole balance for AMD site 2 using molasses as the substrate with a HRT of 8.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1600	0	1700	0	0	1700	0	1000	800	1000	0	0	500	400
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	7500	0	700	0	0	700	0	700	0	700	0	0	700	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	19100	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	3300	14100	0	7000	7100	10300	7100	0	300	0	0	300	0
Carbon dioxide	0	0	15700	21800	0	0	21800	15700	21800	0	21800	0	0	21800	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	100	0	100	0	0	100	0	100	0	100	0	0	100	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4600	0	4600	0	4600	0	4600	0	0	4600	0
Sulphur	0	0	0	0	0	0	0	0	0	0	6800	0	0	700	6100
Oxygen	0	0	0	0	0	0	0	0	0	0	0	565300	561900	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2126600	2126600	0	0
Bacteria	0	0	0	1300	0	0	1300	0	700	600	1100	0	0	600	500
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	33500	5.55E+07	0	0	5.55E+07	66200

Table D-22: Results of the mass balance for AMD site 2 using molasses as the substrate with a HRT of 8.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	180	0	200	0	0	200	0	110	90	110	0	0	90	50
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	1360	0	120	0	0	120	0	120	0	120	0	0	120	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	20	0	0	20	0	20	0	20	0	0	20	0
sulphates	1830	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	110	480	0	240	240	350	240	0	10	0	0	10	0
Carbon dioxide	0	0	690	960	0	0	960	690	960	0	960	0	0	960	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	170	0	170	0	170	0	170	0	0	170	0
Sulphur	0	0	0	0	0	0	0	0	0	0	220	0	0	20	200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	18090	17960	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	59550	59550	0	0
Bacteria	0	0	0	150	0	0	150	0	80	60	120	0	0	70	50
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	9.99E+05	800	9.99E+05	0	0	9.98E+05	1190

Table D-23: Results of the mole balance for AMD site 2 using molasses as the substrate with a HRT of 5.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1700	0	1800	0	0	1800	0	1000	800	1000	0	0	800	400
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	8200	0	700	0	0	700	0	700	0	700	0	0	700	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	100	0	0	0	0	100	0	0	0	0	0	0	0
Acetate	0	0	0	800	0	0	800	0	600	0	800	0	0	600	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	600	0	0	600	0	600	0	600	0	0	600	0
sulphates	19100	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	5200	14100	0	7000	7100	12200	7100	0	300	0	0	300	0
Carbon dioxide	0	0	25400	22000	0	0	22000	25400	22000	0	22000	0	0	22000	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	100	0	100	0	0	100	0	100	0	100	0	0	100	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4600	0	4600	0	4600	0	4600	0	0	4600	0
Sulphur	0	0	0	0	0	0	0	0	0	0	6800	0	0	700	6100
Oxygen	0	0	0	0	0	0	0	0	0	0	0	566700	563300	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2131900	2131900	0	0
Bacteria	0	0	0	1400	0	0	1400	0	800	600	1100	0	0	800	500
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	36000	5.55E+07	0	0	5.55E+07	67700

Table D-24: Results of the mass balance for AMD site 2 using molasses as the substrate with a HRT of 5.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	190	0	210	0	0	210	0	120	90	120	0	0	60	50
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	1480	0	130	0	0	130	0	130	0	130	0	0	130	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	40	0	0	40	0	40	0	40	0	0	40	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	60	0	0	60	0	60	0	60	0	0	60	0
sulphates	1630	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	180	480	0	240	240	420	240	0	10	0	0	10	0
Carbon dioxide	0	0	1120	970	0	0	970	1120	970	0	970	0	0	970	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	170	0	170	0	170	0	170	0	0	170	0
Sulphur	0	0	0	0	0	0	0	0	0	0	220	0	0	20	200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	18130	18030	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	59690	59690	0	0
Bacteria	0	0	0	160	0	0	160	0	90	70	130	0	0	70	60
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	9.99E+05	650	9.99E+05	0	0	9.98E+05	1220

Table D-25: Results of the mole balance for AMD site 2 using ethanol as the substrate with a HRT of 9.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	100	0	0	100	0	0	0	0	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	19100	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
Hydrogen sulphide	0	0	200	16300	0	8100	8200	8300	8200	0	300	0	0	300	0
Carbon dioxide	0	0	700	20500	0	0	20500	700	20500	0	20500	0	0	20400	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	1000	0	300	0	0	300	0	300	0	300	0	0	300	0
Ethanol	0	12100	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4900	0	4900	0	4900	0	4900	0	0	4900	0
Sulphur	0	0	0	0	0	0	0	0	0	0	8000	0	0	600	7200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	661000	657000	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2486500	2486500	0	0
Bacteria	0	0	0	700	0	0	700	0	400	300	800	0	0	400	300
Water	5.56E+07	0	0	5.56E+07	0	0	#######	0	5.55E+07	8300	5.55E+07	0	0	5.55E+07	60100

Table D-26: Results of the mass balance for AMD site 2 using ethanol as the substrate with a HRT of 9.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	10	0	0	10	0	10	0	10	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	1830	0	0	250	0	0	250	0	250	0	250	0	0	250	0
Hydrogen sulphide	0	0	10	560	0	280	280	280	280	0	10	0	0	10	0
Carbon dioxide	0	0	30	900	0	0	900	30	900	0	900	0	0	900	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	560	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	180	0	180	0	180	0	180	0	0	180	0
Sulphur	0	0	0	0	0	0	0	0	0	0	250	0	0	30	230
Oxygen	0	0	0	0	0	0	0	0	0	0	0	21150	21020	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	69620	69620	0	0
Bacteria	0	0	0	70	0	0	70	0	40	30	90	0	0	50	40
Water	1.00E+06	0	0	1.00E+06	0	0	######	0	1.00E+06	150	1.00E+06	0	0	9.99E+05	1080

Table D-27: Results of the mole balance for AMD site 2 using ethanol as the substrate with a HRT of 8.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	100	0	0	100	0	0	0	0	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	19100	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	200	16300	0	8100	8200	8300	8200	0	300	0	0	300	0
Carbon dioxide	0	0	800	20500	0	0	20500	800	20500	0	20500	0	0	20400	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	1000	0	300	0	0	300	0	300	0	300	0	0	300	0
Ethanol	0	12100	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4900	0	4900	0	4900	0	4900	0	0	4900	0
Sulphur	0	0	0	0	0	0	0	0	0	0	8000	0	0	800	7200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	660800	656900	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2486000	2486000	0	0
Bacteria	0	0	0	700	0	0	700	0	400	300	800	0	0	400	300
Water	5.56E+07	0	0	5.56E+07	0	0	######	0	5.55E+07	8300	5.55E+07	0	0	5.55E+07	60100

Table D-28: Results of the mass balance for AMD site 2 using ethanol as the substrate with a HRT of 8.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	10	0	0	10	0	10	0	10	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	1830	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	10	560	0	280	280	280	280	0	10	0	0	10	0
Carbon dioxide	0	0	30	900	0	0	900	30	900	0	900	0	0	900	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	560	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	180	0	180	0	180	0	180	0	0	180	0
Sulphur	0	0	0	0	0	0	0	0	0	0	250	0	0	30	230
Oxygen	0	0	0	0	0	0	0	0	0	0	0	21150	21020	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	69610	69610	0	0
Bacteria	0	0	0	80	0	0	80	0	40	30	90	0	0	50	40
Water	1.00E+06	0	0	1.00E+06	0	0	######	0	1.00E+06	150	1.00E+06	0	0	9.99E+05	1080

Table D-29: Results of the mole balance for AMD site 2 using ethanol as the substrate with a HRT of 5.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	100	0	0	100	0	0	0	0	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	19100	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	300	16400	0	8100	8200	8500	8200	0	300	0	0	300	0
Carbon dioxide	0	0	1300	20500	0	0	20500	1300	20500	0	20500	0	0	20400	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	1000	0	300	0	0	300	0	300	0	300	0	0	300	0
Ethanol	0	12100	0	100	0	0	100	0	100	0	100	0	0	100	0
Hydrochloric acid	0	0	0	0	4900	0	4900	0	4900	0	4900	0	0	4900	0
Sulphur	0	0	0	0	0	0	0	0	0	0	8000	0	0	800	7200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	661300	657300	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2487800	2487900	0	0
Bacteria	0	0	0	700	0	0	700	0	400	300	800	0	0	400	400
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	8300	5.55E+07	0	0	5.55E+07	60200

Table D-30: Results of the mass balance for AMD site 2 using ethanol as the substrate with a HRT of 5.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	10	0	0	10	0	0	0	0	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	1630	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	10	560	0	280	280	290	260	0	10	0	0	10	0
Carbon dioxide	0	0	60	900	0	0	900	60	900	0	900	0	0	900	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	560	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	160	0	160	0	160	0	160	0	0	160	0
Sulphur	0	0	0	0	0	0	0	0	0	0	250	0	0	30	230
Oxygen	0	0	0	0	0	0	0	0	0	0	0	21160	21030	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	69660	69660	0	0
Bacteria	0	0	0	60	0	0	60	0	40	30	90	0	0	50	40
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	1.00E+06	150	1.00E+06	0	0	9.99E+05	1080

Table D-31: Results of the mole balance for AMD site 2 using primary sewage sludge as the substrate with a HRT of 9.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	14400	0	10500	0	0	10500	0	3700	6800	3700	0	0	1300	2400
Carbohydrates	0	7000	0	3700	0	0	3700	0	1300	2400	1300	0	0	400	800
Lipids	0	1500	0	1100	0	0	1100	0	400	700	400	0	0	100	200
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	4800	0	200	0	0	200	0	200	0	200	0	0	200	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	800	0	0	600	0	800	0	600	0	0	800	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	19100	0	0	2600	0	0	2600	0	2500	0	2500	0	0	2500	0
hydrogen sulphide	0	0	2100	14800	0	7400	7400	9400	7400	100	200	0	0	200	0
Carbon dioxide	0	0	9600	21800	0	0	21800	9600	21800	200	21800	0	0	21500	100
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4700	0	4700	0	4700	0	4700	0	0	4700	0
Sulphur	0	0	0	0	0	0	0	0	0	0	7100	0	0	700	6400
Oxygen	0	0	0	0	0	0	0	0	0	0	0	592000	588400	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2227100	2227100	0	0
Bacteria	0	0	0	4100	0	0	4100	0	1400	2700	1800	0	0	600	1200
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.51E+07	448300	5.51E+07	0	0	5.49E+07	2.09E+05

Table D-32: Results of the mass balance for AMD site 2 using primary sewage sludge as the substrate with a HRT of 9.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1650	0	1190	0	0	1190	0	420	780	420	0	0	150	270
Carbohydrates	0	1140	0	590	0	0	590	0	210	390	210	0	0	70	130
Lipids	0	1210	0	850	0	0	850	0	300	550	300	0	0	100	190
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	290	0	10	0	0	10	0	10	0	10	0	0	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	40	0	0	40	0	40	0	40	0	0	40	0
Butyrate	0	0	0	20	0	0	20	0	20	0	20	0	0	20	0
sulphates	1830	0	0	250	0	0	250	0	240	0	240	0	0	240	0
hydrogen sulphide	0	0	70	500	0	250	250	320	250	0	10	0	0	10	0
Carbon dioxide	0	0	420	960	0	0	960	420	950	10	950	0	0	950	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	170	0	170	0	170	0	170	0	0	170	0
Sulphur	0	0	0	0	0	0	0	0	0	0	230	0	0	20	210
Oxygen	0	0	0	0	0	0	0	0	0	0	0	18940	18830	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	62360	62360	0	0
Bacteria	0	0	0	470	0	0	470	0	180	300	210	0	0	70	130
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	9.92E+05	8070	9.92E+05	0	0	9.88E+05	3750

Table D-33: Results of the mole balance for AMD site 2 using primary sewage sludge as the substrate with a HRT of 8.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	15300	0	11300	0	0	11300	0	4000	7400	4000	0	0	1400	2600
Carbohydrates	0	7500	0	4000	0	0	4000	0	1400	2600	1400	0	0	500	900
Lipids	0	1600	0	1100	0	0	1100	0	400	700	400	0	0	100	300
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	5100	0	200	0	0	200	0	200	0	200	0	0	200	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	700	0	0	700	0	700	0	700	0	0	700	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	19100	0	0	2600	0	0	2600	0	2500	0	2500	0	0	2500	0
hydrogen sulphide	0	0	2400	14800	0	7400	7400	9600	7400	100	200	0	0	200	0
Carbon dioxide	0	0	10900	21800	0	0	21800	10900	21600	200	21600	0	0	21500	100
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4700	0	4700	0	4700	0	4700	0	0	4700	0
Sulphur	0	0	0	0	0	0	0	0	0	0	7100	0	0	700	6400
Oxygen	0	0	0	0	0	0	0	0	0	0	0	590800	587200	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2222500	2222500	0	0
Bacteria	0	0	0	4100	0	0	4100	0	1400	2700	1800	0	0	800	1200
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.51E+07	4.82E+05	5.51E+07	0	0	5.49E+07	2.20E+05

Table D-34: Results of the mass balance for AMD site 2 using primary sewage sludge as the substrate with a HRT of 8.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1740	0	1290	0	0	1290	0	450	840	450	0	0	160	290
Carbohydrates	0	1210	0	650	0	0	850	0	230	420	230	0	0	80	150
Lipids	0	1280	0	920	0	0	920	0	320	600	320	0	0	110	210
Amino acids	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	20	0	0	20	0	20	0	20	0	0	20	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	300	0	10	0	0	10	0	10	0	10	0	0	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	50	0	0	50	0	50	0	50	0	0	50	0
Butyrate	0	0	0	20	0	0	20	0	20	0	20	0	0	20	0
sulphates	1830	0	0	250	0	0	250	0	240	0	240	0	0	240	0
hydrogen sulphide	0	0	80	500	0	250	250	330	250	0	10	0	0	10	0
Carbon dioxide	0	0	480	960	0	0	960	480	950	10	950	0	0	950	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	170	0	170	0	170	0	170	0	0	170	0
Sulphur	0	0	0	0	0	0	0	0	0	0	230	0	0	20	200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	18910	18790	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	62230	62230	0	0
Bacteria	0	0	0	470	0	0	470	0	160	300	210	0	0	70	130
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	9.91E+05	8670	9.91E+05	0	0	9.87E+05	3960

Table D-35: Results of the mole balance for AMD site 2 using primary sewage sludge as the substrate with a HRT of 5.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	20300	0	16400	0	0	16400	0	5700	10600	5700	0	0	2000	3700
Carbohydrates	0	9900	0	8000	0	0	8000	0	2100	3900	2100	0	0	700	1400
Lipids	0	2100	0	1700	0	0	1700	0	600	1100	600	0	0	200	400
Amino acids	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	6700	0	800	0	0	800	0	800	0	600	0	0	700	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	700	0	0	700	0	600	0	600	0	0	800	0
Butyrate	0	0	0	800	0	0	800	0	800	0	800	0	0	800	0
sulphates	19100	0	0	2600	0	0	2600	0	2500	0	2500	0	0	2500	0
hydrogen sulphide	0	0	4100	14800	0	7300	7400	11400	7300	100	200	0	0	200	0
Carbon dioxide	0	0	19100	21900	0	0	21900	19100	21600	300	21800	0	0	21500	100
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4700	0	4700	0	4700	100	4700	0	0	4600	0
Sulphur	0	0	0	0	0	0	0	0	0	0	7000	0	0	700	6300
Oxygen	0	0	0	0	0	0	0	0	0	0	0	583000	579500	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2193100	2193100	0	0
Bacteria	0	0	0	4200	0	0	4200	0	1500	2700	1800	0	0	800	1200
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.49E+07	6.75E+05	5.49E+07	0	0	5.46E+07	2.87E+05

Table D-36: Results of the mass balance for AMD site 2 using primary sewage sludge as the substrate with a HRT of 5.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	2320	0	1870	0	0	1870	0	650	1210	650	0	0	230	420
Carbohydrates	0	1810	0	980	0	0	980	0	340	630	340	0	0	120	220
Lipids	0	1700	0	1350	0	0	1350	0	470	880	470	0	0	170	310
Amino acids	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	30	0	0	30	0	30	0	30	0	0	30	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	400	0	50	0	0	50	0	50	0	50	0	0	40	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	50	0	0	50	0	50	0	50	0	0	50	0
Butyrate	0	0	0	70	0	0	70	0	70	0	70	0	0	70	0
sulphates	1830	0	0	250	0	0	250	0	240	0	240	0	0	240	0
hydrogen sulphide	0	0	140	500	0	250	250	390	250	0	10	0	0	10	0
Carbon dioxide	0	0	840	960	0	0	960	840	950	10	950	0	0	950	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	170	0	170	0	170	0	170	0	0	170	0
Sulphur	0	0	0	0	0	0	0	0	0	0	220	0	0	20	200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	18660	18540	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	61410	61410	0	0
Bacteria	0	0	0	480	0	0	480	0	170	310	210	0	0	70	130
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	8.88E+05	12140	9.88E+05	0	0	9.83E+05	5170

Table D-37: Results of the mole balance for AMD site 2 using molasses as the substrate with a HRT of 10 and with a 10% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1700	0	2000	0	0	2000	0	1100	900	1100	0	0	600	500
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	6300	0	700	0	0	700	0	700	0	700	0	0	700	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	21000	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	3700	15000	0	7500	7500	11200	7500	0	300	0	0	300	0
Carbon dioxide	0	0	16700	21700	0	0	21700	16700	21700	0	21700	0	0	21700	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4600	0	4600	0	4600	0	4600	0	0	4600	0
Sulphur	0	0	0	0	0	0	0	0	0	0	7300	0	0	700	6500
Oxygen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacteria	0	0	0	1400	0	0	1400	0	800	600	1100	604100	600400	600	500
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	37100	5.55E+07	2.27E+06	2.27E+06	5.54E+07	71500

Table D-38: Results of the mass balance for AMD site 2 using molasses as the substrate with a HRT of 10 and with a 10% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	200	0	220	0	0	220	0	120	100	120	0	0	70	50
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	1500	0	130	0	0	130	0	130	0	130	0	0	130	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
sulphates	2020	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	130	510	0	250	260	380	260	0	10	0	0	10	0
Carbon dioxide	0	0	730	960	0	0	960	730	960	0	960	0	0	960	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	170	0	170	0	170	0	170	0	0	170	0
Sulphur	0	0	0	0	0	0	0	0	0	0	230	0	0	20	210
Oxygen	0	0	0	0	0	0	0	0	0	0	0	19330	19210	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	63630	63630	0	0
Bacteria	0	0	0	160	0	0	160	0	90	70	130	0	0	70	60
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	9.99E+05	670	9.99E+05	0	0	9.98E+05	1290

Table D-39: Results of the mole balance for AMD site 2 using molasses as the substrate with a HRT of 10 and with a 20% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1900	0	2200	0	0	2200	0	1200	900	1200	0	0	700	500
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	9200	0	800	0	0	800	0	800	0	800	0	0	800	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	22900	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	5000	15700	0	7800	7900	12800	7900	0	300	0	0	300	0
Carbon dioxide	0	0	21200	21500	0	0	21500	21200	21500	0	21500	0	0	21500	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4900	0	4900	0	4900	0	4900	0	0	4900	0
Sulphur	0	0	0	0	0	0	0	0	0	0	7600	0	0	800	6900
Oxygen	0	0	0	0	0	0	0	0	0	0	0	635200	631300	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2389400	2389400	0	0
Bacteria	0	0	0	1500	0	0	1500	0	800	700	1200	0	0	700	500
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	40900	5.55E+07	0	0	5.54E+07	76200

Table D-40: Results of the mass balance for AMD site 2 using molasses as the substrate with a HRT of 10 and with a 20% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	220	0	250	0	0	250	0	140	110	140	0	0	80	60
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	1660	0	140	0	0	140	0	140	0	140	0	0	140	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
sulphates	2200	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	170	540	0	270	270	440	270	0	10	0	0	10	0
Carbon dioxide	0	0	930	950	0	0	950	930	950	0	950	0	0	940	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	180	0	180	0	100	0	180	0	0	180	0
Sulphur	0	0	0	0	0	0	0	0	0	0	240	0	0	20	220
Oxygen	0	0	0	0	0	0	0	0	0	0	0	20330	20200	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	66900	66900	0	0
Bacteria	0	0	0	170	0	0	170	0	100	80	140	0	0	80	60
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	9.99E+05	740	9.99E+05	0	0	9.98E+05	1370

Table D-41: Results of the mole balance for AMD site 2 using molasses as the substrate with a HRT of 10 and with a 50% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	2400	0	2700	0	0	2700	0	1500	1200	1500	0	0	900	700
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	11700	0	1000	0	0	1000	0	1000	0	1000	0	0	1000	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	28600	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	9300	17500	0	8700	8800	18000	8800	0	300	0	0	300	0
Carbon dioxide	0	0	34300	20900	0	0	20900	34300	20900	0	20900	0	0	20900	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	5200	0	5200	0	5200	0	5200	0	0	5200	0
Sulphur	0	0	0	0	0	0	0	0	0	0	8500	0	0	900	7700
Oxygen	0	0	0	0	0	0	0	0	0	0	0	709700	705400	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2669800	2669800	0	0
Bacteria	0	0	0	1900	0	0	1900	0	1100	800	1500	0	0	800	700
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	52300	5.55E+07	0	0	5.54E+07	88800

Table D-42: Results of the mass balance for AMD site 2 using molasses as the substrate with a HRT of 10 and with a 50% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	280	0	310	0	0	310	0	180	140	180	0	0	100	80
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	2110	0	180	0	0	180	0	180	0	180	0	0	180	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
sulphates	2750	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	320	800	0	300	300	610	300	0	10	0	0	10	0
Carbon dioxide	0	0	1510	920	0	0	920	1510	920	0	920	0	0	920	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	190	0	190	0	190	0	190	0	0	190	0
Sulphur	0	0	0	0	0	0	0	0	0	0	270	0	0	30	250
Oxygen	0	0	0	0	0	0	0	0	0	0	0	22710	22570	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	74750	74750	0	0
Bacteria	0	0	0	220	0	0	220	0	120	100	170	0	0	180	80
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	9.99E+05	940	9.99E+05	0	0	9.97E+05	1600

Table D-43: Results of the mole balance for AMD site 2 using ethanol as the substrate with a HRT of 10 and with a 10% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	100	0	0	100	0	180	0	100	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	21000	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	2400	17700	0	8800	8900	11200	8900	0	300	0	0	300	0
Carbon dioxide	0	0	8500	20800	0	0	20800	8500	20800	0	20880	0	0	20800	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	1000	0	200	0	0	200	0	200	0	200	0	0	200	0
Ethanol	0	13500	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	5200	0	5200	0	5280	0	5200	0	0	5200	0
Sulphur	0	0	0	0	0	0	0	0	0	0	8600	0	0	900	7800
Oxygen	0	0	0	0	0	0	0	0	0	0	0	716400	712180	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2695000	2695000	0	0
Bacteria	0	0	0	700	0	0	700	0	400	300	800	0	0	500	400
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	9300	5.55E+07	0	0	5.55E+07	65300

Table D-44: Results of the mass balance for AMD site 2 using as the substrate with a HRT of 10 and with a 10% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	10	0	0	10	0	10	10	10	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	2020	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	80	600	0	300	300	380	300	0	10	0	0	10	0
Carbon dioxide	0	0	370	920	0	0	920	370	920	0	920	0	0	910	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	620	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	190	0	190	0	190	0	190	0	0	190	0
Sulphur	0	0	0	0	0	0	0	0	0	0	280	0	0	30	250
Oxygen	0	0	0	0	0	0	0	0	0	0	0	22920	22790	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	75460	75460	0	0
Bacteria	0	0	0	80	0	0	80	0	50	40	100	0	0	50	40
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	1.00E+06	170	1.00E+06	0	0	9.99E+05	1180

Table D-45: Results of the mole balance for AMD site 2 using ethanol as the substrate with a HRT of 10 and with a 20% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	100	0	0	100	0	100	100	100	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	22900	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	1700	18700	0	9300	9400	11100	9400	0	300	0	0	300	0
Carbon dioxide	0	0	5900	20500	0	0	20500	5900	20500	0	20500	0	0	20500	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	1000	0	100	0	0	100	0	100	0	100	0	0	100	0
Ethanol	0	14900	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	5400	0	5400	0	5400	0	5400	0	0	5400	0
Sulphur	0	0	0	0	0	0	0	0	0	0	9200	0	0	900	8200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	760200	755600	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2859600	2859600	0	0
Bacteria	0	0	0	800	0	0	800	0	400	400	900	0	0	500	400
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	10200	5.55E+07	0	0	5.55E+07	69500

Table D-46: Results of the mass balance for AMD site 2 using ethanol as the substrate with a HRT of 10 and with a 20% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	10	0	0	10	0	10	10	10	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	2200	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	60	640	0	320	320	380	320	0	10	0	0	10	0
Carbon dioxide	0	0	260	900	0	0	900	260	900	0	900	0	0	900	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	690	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	200	0	200	0	200	0	200	0	0	200	0
Sulphur	0	0	0	0	0	0	0	0	0	0	290	0	0	30	260
Oxygen	0	0	0	0	0	0	0	0	0	0	0	24320	24160	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	80070	80070	0	0
Bacteria	0	0	0	90	0	0	90	0	50	40	100	0	0	60	50
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	1.00E+06	180	1.00E+06	0	0	9.99E+05	1250

Table D-47: Results of the mole balance for AMD site 2 using ethanol as the substrate with a HRT of 10 and with a 50% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	200	0	0	200	0	100	100	100	0	0	0	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	3300	0	0	0	0	3300	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	28600	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	5100	21300	0	10600	10700	15700	10700	0	300	0	0	300	0
Carbon dioxide	0	0	14700	19700	0	0	19700	14700	19700	0	19700	0	0	19700	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	1300	0	100	0	0	100	0	100	0	100	0	0	100	0
Ethanol	0	19100	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	5800	0	5800	0	5800	0	5800	0	0	5800	0
Sulphur	0	0	0	0	0	0	0	0	0	0	10400	0	0	1000	9400
Oxygen	0	0	0	0	0	0	0	0	0	0	0	867500	862300	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	3263600	3263600	0	0
Bacteria	0	0	0	1000	0	0	1000	0	600	400	1100	0	0	600	500
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.55E+07	13100	5.55E+07	0	0	5.55E+07	80200

Table D-48: Results of the mass balance for AMD site 2 using ethanol as the substrate with a HRT of 10 and with a 50% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	0	0	20	0	0	20	0	10	10	10	0	0	10	0
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lipids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen	0	0	10	0	0	0	0	10	0	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sulphates	2750	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	170	730	0	360	360	540	360	0	10	0	0	10	0
Carbon dioxide	0	0	850	870	0	0	870	650	870	0	870	0	0	870	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	880	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	210	0	210	0	210	0	210	0	0	210	0
Sulphur	0	0	0	0	0	0	0	0	0	0	330	0	0	30	300
Oxygen	0	0	0	0	0	0	0	0	0	0	0	27760	27590	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	91380	91380	0	0
Bacteria	0	0	0	120	0	0	120	0	60	50	130	0	0	70	60
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	1.00E+06	240	1.00E+06	0	0	9.98E+05	1440

Table D-49: Results of the mole balance for AMD site 2 using primary sewage sludge as the substrate with a HRT of 10 and with a 10% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	15200	0	10800	0	0	10800	0	3800	7000	3800	0	0	1300	2500
Carbohydrates	0	7400	0	3700	0	0	3700	0	1300	2400	1300	0	0	500	900
Lipids	0	1600	0	1100	0	0	1100	0	400	700	400	0	0	100	200
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	100	0	0	100	0	100	0	100	0	0	0	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	5000	0	200	0	0	200	0	200	0	200	0	0	100	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	500	0	0	500	0	500	0	500	0	0	500	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	21000	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	2100	15700	0	7800	7900	9900	7800	100	200	0	0	200	0
Carbon dioxide	0	0	8800	21500	0	0	21500	8800	21300	200	21300	0	0	21200	100
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	4900	0	4900	0	4800	0	4800	0	0	4800	0
Sulphur	0	0	0	0	0	0	0	0	0	0	7600	0	0	800	6800
Oxygen	0	0	0	0	0	0	0	0	0	0	0	629700	625900	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2368800	2368800	0	0
Bacteria	0	0	0	4500	0	0	4500	0	1800	2900	2000	0	0	700	1300
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.51E+07	4.66E+05	5.51E+07	0	0	5.49E+07	2.18E+05

Table D-50: Results of the mass balance for AMD site 2 using primary sewage sludge as the substrate with a HRT of 10 and with a 10% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1730	0	1230	0	0	1230	0	430	800	430	0	0	150	280
Carbohydrates	0	1200	0	810	0	0	810	0	210	390	210	0	0	70	140
Lipids	0	1270	0	870	0	0	870	0	300	570	300	0	0	110	200
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	300	0	10	0	0	10	0	10	0	10	0	0	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	40	0	0	40	0	40	0	40	0	0	40	0
Butyrate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
sulphates	2020	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	70	540	0	270	270	340	270	0	10	0	0	10	0
Carbon dioxide	0	0	390	950	0	0	950	300	940	10	940	0	0	930	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	180	0	180	0	180	0	180	0	0	180	0
Sulphur	0	0	0	0	0	0	0	0	0	0	240	0	0	20	220
Oxygen	0	0	0	0	0	0	0	0	0	0	0	20150	20030	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	66330	66330	0	0
Bacteria	0	0	0	520	0	0	520	0	180	340	220	0	0	80	150
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	9.92E+05	8380	9.92E+05	0	0	9.88E+05	3920

Table D-51: Results of the mole balance for AMD site 2 using primary sewage sludge as the substrate with a HRT of 10 and with a 20% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	16700	0	11800	0	0	11800	0	4100	7700	4100	0	0	1500	2700
Carbohydrates	0	8100	0	4100	0	0	4100	0	1400	2700	1400	0	0	500	900
Lipids	0	1700	0	1200	0	0	1200	0	400	800	400	0	0	100	300
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	5500	0	200	0	0	200	0	200	0	200	0	0	200	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	500	0	0	500	0	500	0	500	0	0	500	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	22900	0	0	2600	0	0	2600	0	2500	0	2500	0	0	2500	0
hydrogen sulphide	0	0	4100	16500	0	8200	8300	12400	8200	100	200	0	0	200	0
Carbon dioxide	0	0	16500	21200	0	0	21200	16500	21000	200	21000	0	0	20900	100
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	5000	0	5000	0	5000	0	5000	0	0	5000	0
Sulphur	0	0	0	0	0	0	0	0	0	0	8000	0	0	800	7200
Oxygen	0	0	0	0	0	0	0	0	0	0	0	663300	659300	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2495400	2495400	0	0
Bacteria	0	0	0	5000	0	0	5000	0	1700	3200	2100	0	0	800	1400
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.50E+07	5.12E+05	5.50E+07	0	0	5.48E+07	2.37E+05

Table D-52: Results of the mass balance for AMD site 2 using primary sewage sludge as the substrate with a HRT of 10 and with a 20% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	1900	0	1350	0	0	1350	0	470	880	470	0	0	170	310
Carbohydrates	0	1320	0	670	0	0	670	0	230	430	230	0	0	80	150
Lipids	0	1400	0	960	0	0	960	0	330	620	330	0	0	120	220
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	330	0	10	0	0	10	0	10	0	10	0	0	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	40	0	0	40	0	40	0	40	0	0	40	0
Butyrate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
sulphates	2200	0	0	250	0	0	250	0	240	0	240	0	0	240	0
hydrogen sulphide	0	0	140	560	0	280	280	420	280	0	10	0	0	10	0
Carbon dioxide	0	0	720	930	0	0	930	720	930	10	930	0	0	920	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	180	0	180	0	180	0	180	0	0	180	0
Sulphur	0	0	0	0	0	0	0	0	0	0	260	0	0	30	230
Oxygen	0	0	0	0	0	0	0	0	0	0	0	21230	21100	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	69870	69870	0	0
Bacteria	0	0	0	570	0	0	570	0	200	370	250	0	0	90	160
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	9.91E+05	9210	9.91E+05	0	0	9.87E+05	4260

Table D-53: Results of the mole balance for AMD site 2 using primary sewage sludge as the substrate with a HRT of 10 and with a 50% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	21000	0	14900	0	0	14900	0	5200	9700	5200	0	0	1800	3400
Carbohydrates	0	10200	0	5200	0	0	5200	0	1800	3400	1800	0	0	800	1200
Lipids	0	2200	0	1500	0	0	1500	0	500	1000	500	0	0	200	300
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	6900	0	200	0	0	200	0	200	0	200	0	0	200	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	500	0	0	500	0	500	0	500	0	0	500	0
Butyrate	0	0	0	200	0	0	200	0	200	0	200	0	0	200	0
sulphates	28600	0	0	2600	0	0	2600	0	2600	0	2600	0	0	2600	0
hydrogen sulphide	0	0	8200	18500	0	9200	9300	17400	9200	100	200	0	0	200	0
Carbon dioxide	0	0	28100	20600	0	0	20600	28100	20400	200	20400	0	0	20300	100
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	100	0	0	100	0	100	0	100	0	0	100	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	5300	0	5300	0	5300	100	5300	0	0	5300	0
Sulphur	0	0	0	0	0	0	0	0	0	0	8900	0	0	900	8100
Oxygen	0	0	0	0	0	0	0	0	0	0	0	743300	738800	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	2796100	2796100	0	0
Bacteria	0	0	0	6300	0	0	6300	0	2200	4100	2700	0	0	900	1700
Water	5.56E+07	0	0	5.56E+07	0	0	5.56E+07	0	5.49E+07	6.45E+05	5.49E+07	0	0	5.46E+07	2.91E+05

Table D-54: Results of the mass balance for AMD site 2 using primary sewage sludge as the substrate with a HRT of 10 and with a 50% increase in the influent sulphate concentration.

Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Proteins	0	2390	0	1700	0	0	1700	0	600	1110	600	0	0	210	390
Carbohydrates	0	1660	0	840	0	0	840	0	290	550	290	0	0	100	190
Lipids	0	1780	0	1200	0	0	1200	0	420	780	420	0	0	150	270
Amino acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palmitic acid	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
Hydrogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetate	0	420	0	10	0	0	10	0	10	0	10	0	0	10	0
Lactate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Propionate	0	0	0	40	0	0	40	0	40	0	40	0	0	40	0
Butyrate	0	0	0	10	0	0	10	0	10	0	10	0	0	10	0
sulphates	2750	0	0	250	0	0	250	0	250	0	250	0	0	250	0
hydrogen sulphide	0	0	280	630	0	310	320	590	310	0	10	0	0	10	0
Carbon dioxide	0	0	1240	910	0	0	910	1240	900	10	900	0	0	890	0
Methane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ammonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochloric acid	0	0	0	0	200	0	200	0	190	0	190	0	0	190	0
Sulphur	0	0	0	0	0	0	0	0	0	0	290	0	0	30	260
Oxygen	0	0	0	0	0	0	0	0	0	0	0	23780	23640	0	0
Nitrogen	0	0	0	0	0	0	0	0	0	0	0	78290	78290	0	0
Bacteria	0	0	0	720	0	0	720	0	250	470	300	0	0	110	200
Water	1.00E+06	0	0	1.00E+06	0	0	1.00E+06	0	9.88E+05	1.16E+04	9.88E+05	0	0	9.83E+05	5230

Appendix E

RESULTS FROM THE ECONOMIC ANALYSIS

Table E-1: Capital costs for various AMD sites using various substrates at a hydraulic residence time of 10 days.

Substrate		Ethanol			Primary sewage sludge			Molasses		
AMD site		2	1	3	2	1	3	2	1	3
Description	Factor	Rands	Rands	Rands	Rands	Rands	Rands	Rands	Rands	Rands
Major equipment										
substrate tank		14900	12500	17100	10500	8990	12100	19100	16100	21800
AMD tank		459000	459000	45900	45900	45900	45900	45900	45900	45900
HCl Tank		6280	6280	6280	6280	6280	6280	6280	6280	6280
Anaerobic reactor		735000	735000	735000	735000	735000	735000	735000	735000	735000
Aerobic reactor		735000	735000	735000	735000	735000	735000	735000	735000	735000
Settler 1		101000	101000	101000	101000	101000	101000	101000	101000	101000
Settler 2		101000	101000	101000	101000	101000	101000	101000	101000	101000
Mixer		2930	2930	2930	2930	2930	2930	2930	2930	2930
major equipment total		2150000	2150000	2160000	2150000	2150000	2150000	2160000	2160000	2160000
Ancillary Equipment										
pump 1		10500	10500	10500	10500	10500	10500	10500	10500	10500
pump 2		10500	10500	10500	10500	10500	10500	10500	10500	10500
pump 3		10500	10500	10500	10500	10500	10500	10500	10500	10500
compressor		648000	512000	727000	589000	508000	653000	571000	571000	571000
Ancillary Equipment Total		680000	543000	759000	620000	540000	685000	602000	802000	602000
Total Equipment cost	TEC	2830000	2700000	2920000	2770000	2690000	2840000	2760000	2760000	2760000
Erection, foundation and minor structural work	0.45TEC	1280000	1210000	1310000	1250000	1210000	1280000	1240000	1240000	1240000
Piping and fittings	0.5TEC	1420000	1350000	1460000	1385320	1340000	1420000	1380000	1380000	1380000
Instrumentation, local and control room	0.15TEC	425000	404000	437000	416000	403000	425000	414000	414000	415000
Electrical, power and lighting	0.1TEC	283000	270000	292000	277000	269000	284000	276000	276000.8	276000
Site Development	0.1TEC	283000	270000	292000	277000	269000	284000	276000	276000.8	276000
Process Buildings	0.1TEC	283000	270000	292000	277000	269000	284000	276000	276000.8	276000
Total Physical plant cost	PPC	6800000	6470000	7000000	6650000	6450000	6810000	6630000	6620000	6630000
Design and engineering	0.25PPC	1700000	1620000	1750000	1660000	1610000	1700000	1660000	1650000	1660000
Contractors fee	0.05PPC	340000	323000	350000	332000	323000	340000	331000	331000	332000
Contingency	0.2PPC	1360000	1290000	1400000	1330000	1290000	1360000	1330000	1320000	1330000
Total fixed capital required	FCR	10200000	9700000	10500000	9970000	9680000	10200000	9940000	9930000	9950000
Cost of land	0.06FCR	612000	582000	630000	598000	581000	613000	596000	596000	597000
Total capital	FCI	10800000	10300000	11100000	10600000	10300000	10800000	10500000	10500000	10500000

Table E-2: Operating costs for various AMD sites using various substrates at a hydraulic residence time of 10 days.

Substrate		Ethanol			Primary sewage sludge			Molasses		
AMD site		2	1	3	2	1	3	2	1	3
Description										
Variable operating costs	factor	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)
electricity(kWh)		2760000	2650000	2820000	2710000	2650000	2760000	2700000	2640000	2740000
Disposal Costs		111000	83000	140000	5603000	4309000	7033000	445000	336000	558000
Hydrochloric cost		1120000	850000	1220000	1070000	970000	1140000	1040000	960000	1110000
substrate cost		1390000	1040000	1750000	0	0	0	1330000	1000000	1660000
Total variable operating costs		5380000	4620000	5930000	9380000	7930000	10940000	5510000	4940000	6070000
Fixed operating costs										
cost of labour										
Total operators per shift		2	2	2	2	2	2	2	2	2
number of shifts		4	4	4	4	4	4	4	4	4
Salary per annum		53750	53750	53750	53750	53750	53750	53750	53750	53750
Total cost of operating labour		430000	430000	430000	430000	430000	430000	430000	430000	430000
Total supervisors per shift		1	1	1	1	1	1	1	1	1
No. of shifts		4	4	4	4	4	4	4	4	4
Salary per annum		88000	88000	88000	88000	88000	88000	88000	88000	88000
Total cost of supervisory labour		352000	352000	352000	352000	352000	352000	352000	352000	352000
Total cost of labour per annum	COL	782000	782000	782000	782000	782000	782000	782000	782000	782000
Maintenance and repairs	0.01FCI	103000	103000	111000	106000	103000	108000	105000	105000	105000
Insurance	0.03FCI	324000	309000	334000	317000	308000	325000	316000	316000	316000
Patents and Royalties	0.02FCI	216000	206000	222000	211000	205000	216000	211000	210000	211000
Total fixed operating costs		1430000	1400000	1450000	1420000	1400000	1430000	1410000	1410000	1410000
Total Cost of operation	CO	6810000	6020000	7380000	10800000	9320000	12370000	6920000	6350000	7480000

Table E-3: Capital costs for AMD site 2 using various substrates at various hydraulic residence times.

Substrate		Molasses			Ethanol			Primary sewage sludge		
Hydraulic residence time		9	8	5	9	8	5	9	8	5
Description	Factor	Rands	Rands	Rands	Rands	Rands	Rands	Rands	Rands	Rands
Major equipment										
substrate tank		19100	19100	20100	14900	14900	14900	10800	11200	13300
AMD tank		459000	459000	459000	459000	459000	459000	459000	459000	459000
HCl Tank		6280	6280	6280	6280	6280	6280	6280	6280	6280
Anaerobic reactor		690000	643000	485000	690000	643000	485000	690000	643000	485000
Aerobic reactor		735000	735000	735000	735000	735000	735000	735000	735000	735000
Settler 1		101000	101000	101000	101000	101000	101000	101000	101000	101000
Settler 2		101000	101000	101000	101000	101000	101000	101000	101000	101000
Mixer		2930	2930	2930	2930	2930	2930	2930	2930	2930
major equipment total		2110000	2070000	1910000	2110000	2060000	1900000	2110000	2060000	1900000
Ancillary Equipment										
pump 1		10500	10500	10500	10500	10500	10500	10500	10500	10500
pump 2		10500	10500	10500	10500	10500	10500	10500	10500	10500
pump 3		10500	10500	10500	10500	10500	10500	10500	10500	10500
compressor		568000	567000	568000	643000	643000	643000	588000	588000	581000
Ancillary Equipment Total		599000	599000	600000	674000	674000	674000	620000	619000	613000
Total Equipment cost	TEC	2710000	2670000	2510000	2780000	2740000	2580000	2730000	2680000	2520000
Erection, foundation and minor structural work	0.45TEC	1220000	1200000	1130000	1250000	1230000	1160000	1230000	1200000	1130000
Piping and fittings	0.5TEC	1360000	1330000	1250000	1390000	1370000	1290000	1360000	1340000	1260000
Instrumentation, local and control room	0.15TEC	407000	400000	376000	418000	410000	387000	409000	402000	377000
Electrical, power and lighting	0.1TEC	271000	271000	271000	271000	271000	271000	271000	271000	271000
Site Development	0.1TEC	271000	271000	271000	271000	271000	271000	271000	271000	271000
Process Buildings	0.1TEC	271000	271000	271000	271000	271000	271000	271000	271000	271000
Total Physical plant cost	PPC	6510000	6400000	6020000	6680000	6570000	6190000	6540000	6430000	6040000
Design and engineering	0.25PPC	1630000	1600000	1510000	1670000	1640000	1550000	1640000	1610000	1510000
Contractors fee	0.05PPC	326000	320000	301000	334000	328000	309000	327000	321000	302000
Contingency	0.2PPC	1300000	1280000	1200000	1340000	1310000	1240000	1310000	1290000	1210000
Total fixed capital required	FCR	9770000	9590000	9030000	10000000	9850000	9280000	9810000	9640000	9060000
Cost of land	0.06FCR	586000	576000	542000	601000	591000	557000	589000	578000	543000
Total capital	FCI	10400000	10200000	9580000	10600000	10400000	9840000	10400000	10200000	9600000

Table E-4: Operating costs AMD site 2 using various substrates at various hydraulic residence times.

Substrate		Molasses			Ethanol			Primary sewage sludge		
Hydraulic residence time		9	8	5	9	8	5	9	8	5
Description										
Variable operating costs	factor	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)
electricity(kWh)		2690000	2690000	2690000	2750000	2750000	2750000	2710000	2710000	2700000
Disposal Costs		301000	447000	479000	111000	111000	111000	5968000	6413000	8981000
Hydrochloric cost		1030000	1030000	1040000	1110000	1110000	1110000	1070000	1070000	1060000
substrate cost		1330000	1330000	1450000	1390000	1390000	1390000	0	0	0
Total variable operating costs		5360000	5500000	5660000	5360000	5360000	5360000	9740000	10190000	12750000
Fixed operating costs										
cost of labour										
Total operators per shift		2	2	2	2	2	2	2	2	2
number of shifts		4	4	4	4	4	4	4	4	4
Salary per annum		53750	53750	53750	53750	53750	53750	53750	53750	53750
Total cost of operating labour		430000	430000	430000	430000	430000	430000	430000	430000	430000
Total supervisors per shift		1	1	1	1	1	1	1	1	1
No. of shifts		4	4	4	4	4	4	4	4	4
Salary per annum		88000	88000	88000	88000	88000	88000	88000	88000	88000
Total cost of supervisory labour		352000	352000	352000	352000	352000	352000	352000	352000	352000
Total cost of labour per annum	COL	782000	782000	782000	782000	782000	782000	782000	782000	782000
Maintenance and repairs	0.01FCI	104000	102000	96000	106000	104000	98000	104000	102000	96000
Insurance	0.03FCI	311000	305000	287000	319000	313000	295000	312000	307000	288000
Patents and Royalties	0.02FCI	207000	203000	192000	212000	209000	197000	208000	204000	192000
Total fixed operating costs		1320000	1390000	1360000	1420000	1410000	1370000	1410000	1400000	1360000
Total Cost of operation	CO	6670000	6900000	7020000	6780000	6760000	6730000	11100000	11600000	14100000

Table E-5: Capital costs for AMD site 2 at increasing sulphate loading rates.

Substrate		Molasses			Ethanol			Primary sewage sludge		
Sulphate loading increase		10%	20%	50%	10%	20%	50%	10%	20%	50%
Description	Factor	Rands	Rands	Rands	Rands	Rands	Rands	Rands	Rands	Rands
Major equipment										
substrate tank		20300	21500	24900	15900	16900	19600	11200	11800	13600
AMD tank		459000	459000	459000	459000	459000	459000	459000	459000	459000
HCl Tank		6280	6280	6280	6280	6280	6280	6280	6280	6280
Anaerobic reactor		735000	735000	735000	735000	735000	735000	735000	735000	735000
Aerobic reactor		735000	735000	735000	735000	735000	735000	735000	735000	735000
Settler 1		101000	101000	101000	101000	101000	101000	101000	101000	101000
Settler 2		101000	101000	101000	101000	101000	101000	101000	101000	101000
Mixer		2930	2930	2930	2930	2930	2930	2930	2930	2930
major equipment total		2160000	2160000	2160000	2160000	2160000	2160000	2150000	2150000	2150000
Ancillary Equipment										
pump 1		10500	10500	10500	10500	10500	10500	10500	10500	10500
pump 2		10500	10500	10500	10500	10500	10500	10500	10500	10500
pump 3		10500	10500	10500	10500	10500	10500	10500	10500	10500
compressor		598000	623000	680000	685000	719000	799000	618000	645000	706000
Ancillary Equipment Total		629000	654000	712000	717000	750000	830000	650000	676000	737000
Total Equipment cost	TEC	2790000	2820000	2880000	2870000	2910000	2990000	2800000	2830000	2890000
Erection, foundation and minor structural work	0.45TEC	1260000	1270000	1290000	1290000	1310000	1350000	1260000	1270000	1300000
Piping and fittings	0.5TEC	1400000	1410000	1440000	1440000	1450000	1450000	1400000	1410000	1450000
Instrumentation, local and control room	0.15TEC	418000	422000	431000	431000	436000	448000	420000	424000	434000
Electrical, power and lighting	0.1TEC	279000	279000	279000	279000	279000	279000	279000	279000	279000
Site Development	0.1TEC	279000	279000	279000	279000	279000	279000	279000	279000	279000
Process Buildings	0.1TEC	279000	279000	279000	279000	279000	279000	279000	279000	279000
Total Physical plant cost	PPC	6890000	6760000	6900000	6890000	6980000	7170000	6720000	6790000	6940000
Design and engineering	0.25PPC	1670000	1690000	1730000	1720000	1740000	1790000	1680000	1700000	1730000
Contractors fee	0.05PPC	335000	338000	345000	345000	349000	359000	336000	339000	347000
Contingency	0.2PPC	1340000	1350000	1380000	1380000	1400000	1430000	1340000	1360000	1390000
Total fixed capital required	FCR	10000000	10100000	10400000	10300000	10500000	10800000	10100000	10200000	10400000
Cost of land	0.06FCR	602000	608000	621000	620000	628000	646000	605000	611000	624000
Total capital	FCI	10600000	10700000	11000000	11000000	11100000	11400000	10700000	10800000	11000000

Table E-6: Operating costs for AMD site 2 at increasing sulphate loading rates.

Substrate		Molasses			Ethanol			Primary sewage sludge		
Sulphate loading increase		10%	20%	50%	10%	20%	50%	10%	20%	50%
Description										
Variable operating costs	factor	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)
electricity(kWh)		2720000	2740000	2780000	2790000	2820000	2880000	2730000	2750000	2800000
Disposal Costs		494000	545000	696000	123000	136000	175000	8201000	6811000	8587000
Hydrochloric cost		1070000	1100000	1170000	1170000	1210000	1310000	1100000	1130000	1200000
substrate cost		1470000	1620000	2070000	1550000	1710000	2190000	0	0	0
Total variable operating costs		5760000	6010000	6720000	5630000	5870000	6550000	10030000	10700000	12600000
Fixed operating costs										
cost of labour										
Total operators per shift		2	2	2	2	2	2	2	2	2
number of shifts		4	4	4	4	4	4	4	4	4
Salary per annum		53750	53750	53750	53750	53750	53750	53750	53750	53750
Total cost of operating labour		430000	430000	430000	430000	430000	430000	430000	430000	430000
Total supervisors per shift		1	1	1	1	1	1	1	1	1
No. of shifts		4	4	4	4	4	4	4	4	4
Salary per annum		88000	88000	88000	88000	88000	88000	88000	88000	88000
Total cost of supervisory labour		352000	352000	352000	352000	352000	352000	352000	352000	352000
Total cost of labour per annum	COL	782000	782000	782000	782000	782000	782000	782000	782000	782000
Maintenance and repairs	0.01FCI	106000	107000	110000	110000	111000	114000	107000	108000	110000
Insurance	0.03FCI	319000	322000	329000	329000	333000	342000	321000	324000	331000
Patents and Royalties	0.02FCI	213000	215000	220000	219000	222000	228000	214000	216000	221000
Total fixed operating costs		1420000	1430000	1440000	1440000	1450000	1470000	1420000	1430000	1440000
Total Cost of operation	CO	7180000	7430000	8160000	7070000	7320000	8020000	11460000	12120000	14040000

Table E-7: Operating costs for AMD site 2 with increasing disposal costs.

Substrate		Molasses			Ethanol			Primary sewage sludge		
Disposal increase		10%	20%	50%	10%	20%	50%	10%	20%	50%
Description										
Variable operating costs	factor	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)
electricity(kWh)		2700000	2700000	2700000	2760000	2760000	2760000	2710000	2710000	2710000
Disposal Costs		489000	534000	667000	122000	133000	166000	6163000	6724000	8405000
Hydrochloric cost		1040000	1040000	1040000	1120000	1120000	1120000	1070000	1070000	1070000
substrate cost		1330000	1330000	1330000	1390000	1390000	1390000	0	0	0
Total variable operating costs		5550000	5600000	5730000	5390000	5400000	5440000	9940000	10500000	12180000
Fixed operating costs										
cost of labour										
Total operators per shift		2	2	2	2	2	2	2	2	2
number of shifts		4	4	4	4	4	4	4	4	4
Salary per annum		53750	53750	53750	53750	53750	53750	53750	53750	53750
Total cost of operating labour		430000	430000	430000	430000	430000	430000	430000	430000	430000
Total supervisors per shift		1	1	1	1	1	1	1	1	1
No. of shifts		4	4	4	4	4	4	4	4	4
Salary per annum		88000	88000	88000	88000	88000	88000	88000	88000	88000
Total cost of supervisory labour		352000	352000	352000	352000	352000	352000	352000	352000	352000
Total cost of labour per annum	COL	782000	782000	782000	782000	782000	782000	782000	782000	782000
Maintenance and repairs	0.01FCI	105000	105000	105000	108000	108000	108000	106000	106000	106000
Insurance	0.03FCI	316000	316000	316000	324000	324000	324000	317000	317000	317000
Patents and Royalties	0.02FCI	211000	211000	211000	216000	216000	216000	211000	211000	211000
Total fixed operating costs		1410000	1410000	1410000	1430000	1430000	1430000	1420000	1420000	1420000
Total Cost of operation	CO	6970000	7010000	7150000	6820000	6830000	6870000	11360000	11920000	13600000

Table E-8: Operating costs for AMD site 2 with increasing substrate costs.

Substrate		Molasses			Ethanol		
Increase in substrate cost		10%	20%	50%	10%	20%	50%
Description							
Variable operating costs	factor	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)	annual cost (R/yr)
electricity(kWh)		2700000	2700000	2700000	2760000	2760000	2760000
Disposal Costs		445000	445000	445000	111000	111000	111000
Hydrochloric cost		1040000	1040000	1040000	1120000	1120000	1120000
substrate cost		1460000	1590000	1990000	1530000	1670000	2080000
Total variable operating costs		5640000	5770000	6170000	5520000	5660000	6070000
Fixed operating costs							
cost of labour							
Total operators per shift		2	2	2	2	2	2
number of shifts		4	4	4	4	4	4
Salary per annum		53750	53750	53750	53750	53750	53750
Total cost of operating labour		430000	430000	430000	430000	430000	430000
Total supervisors per shift		1	1	1	1	1	1
No. of shifts		4	4	4	4	4	4
Salary per annum		88000	88000	88000	88000	88000	88000
Total cost of supervisory labour		352000	352000	352000	352000	352000	352000
Total cost of labour per annum	COL	782000	782000	782000	782000	782000	782000
Maintenance and repairs	0.01FCI	105000	105000	105000	108000	108000	108000
Insurance	0.03FCI	316000	316000	316000	324000	324000	324000
Patents and Royalties	0.02FCI	211000	211000	211000	216000	216000	216000
Total fixed operating costs		1410000	1410000	1410000	1430000	1430000	1430000
Total Cost of operation	CO	7060000	7190000	7590000	6950000	7090000	7510000