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**EFFECT OF HYDRAULIC RETENTION TIME AND ATTACHMENT MEDIA
ON SULFIDE PRODUCTION BY SULFATE REDUCING BACTERIA**

by

Beatriz Carolina Polo Christy

B.A.Sc.

A Thesis Submitted to the
Faculty of Graduate Studies and Research
through Civil & Environmental Engineering
in Partial Fulfillment of the Requirements for
the Degree of Master of Applied Science at the
University of Windsor

Windsor, Ontario, Canada

2001

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ABSTRACT

The effects of hydraulic retention time and attachment media on the rate of microbial sulfate reduction with lactate as carbon and energy source were investigated for sulfate reducing bacteria, SRB. The lactate to sulfate ratio was maintained below 2 for proper SRB activity. The Th.O.D.:N:P was kept at 100:5:1. The presence of cations, such as Na^+ and Mg^{2+} , below non-toxic concentrations was important to avoid any growth inhibition of SRB.

Batch anaerobic reactors were used in order to determine the stoichiometry of the biochemical reactions. Results indicated that 70% of the lactate fed was bio-oxidized and 30% was bio-synthesized, which showed that the energy production reaction predominated over the synthesis reaction in non-packed reactors.

Continuous mode operation of three upflow anaerobic suspended growth reactors yielded effluent sulfide concentrations as high as 190 mg S/L at the optimum conditions of 20 h of HRT, 1.5 m/d of upflow velocity and 6 kg/d/m³ of OLR, regardless of the presence of attachment media in the reactor. The critical HRT was 10 h, which provided an upward velocity of 3 m/d. Lower retention times showed a significant decrease in the effluent sulfide concentration and washout of biomass. Doubling the OLR increased proportionally the rate of sulfide production. The effluent pH ranged between 6.5 and 7.0. The ORP values were below -150 mV which indicated sulfide production and activity of SRB.

The substrate utilization rate constant, k , equal to μ/Y , was 2.53 h^{-1} on an average at 20 h of HRT and was obtained through the kinetic model developed for the continuous mode operation of upflow anaerobic reactors as a plug flow type.

Visual observation revealed the presence of settled biomass at the bottom. Therefore, major sulfide production occurred within the first 300 mm of the reactors' height. Using the equations developed in previous studies for metal applications, this active height of 300 mm would allow 100% removal of the heavy metal applied at influent concentrations of 48 mg Cr/L, 95 mg Cu/L, 90 mg Ni/L, 88 mg Zn/L, 44 mg Cd/L and 29 mg Pb/L.

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NOMENCLATURE

A	cross sectional area [L^2]
ASRB	acetate consuming sulfate reducing bacteria
COD	chemical oxygen demand
C	sulfide concentration [M/L^3]
C_o	initial sulfide concentration [M/L^3]
C_s	steady state sulfide concentration [M/L^3]
C_i	influent heavy metal concentration [M/L^3]
C_e	effluent heavy metal concentration [M/L^3]
CSTR	continuous (flow) stirred-tank reactor
H	reactor's height [L]
HLR	hydraulic loading rate [$L^3/L^2/T$]
HRT	hydraulic retention time also referred to as retention time [T]
K	rate constant [T^{-1}]
K'	rate constant [$L^3/M/T$]
k	substrate utilization rate constant = μ / Y [T^{-1}]
L	active volume depth [L]
M	heavy metal
OLR	organic loading rate [$M/T/L^3$]
ORP	oxidation-reduction potential
Q	volumetric flow rate [L^3/T]
S_{max}	maximum sulfide concentration [L^3/T]

SRB	sulfate reducing bacteria
S	sulfide
SS	suspended solids [M/L^3]
t_d	hydraulic retention time in active volume [T]
μ	specific growth rate [T^{-1}]
UA	upflow anaerobic reactor
UASB	upflow anaerobic sludge blanket reactor
UAFF	upflow anaerobic fixed film reactor
V	reactor's volume [L^3]
V_o	upflow velocity [L/T]
VSS	volatile suspended solids [M/L^3]
W_s	molecular mass of sulfide [M/Mmol]
W_m	molecular mass of heavy metal [M/Mmol]
X	biomass concentration [M/L^3]
X_o	initial biomass concentration [M/L^3]
X_s	steady state biomass concentration [M/L^3]
Y	yield coefficient [M/M]

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CHAPTER 1. INTRODUCTION

Anaerobic wastewater treatment constitutes an important biological treatment process in the management of large wastewater streams, and several systems have been developed to solve specific needs. The Fluidized Bed Reactor (FB), Anaerobic Filter (AF), Upflow Anaerobic Sludge Blanket Reactor (UASB) and Upflow Anaerobic Fixed Film Reactor (UAFFR) are the most common anaerobic treatment methods. In these systems, the anaerobic bacteria are immobilized on inert solid particles, e.g. plastic, or are held by an aggregation of the bacteria in a floc with certain settling properties.

The biochemical reaction of interest in the present study is the anaerobic conversion of organic matter and sulfates by means of the dissimilatory sulfate reduction associated with the growth of sulfate reducing bacteria, SRB. These obligate anaerobes use simple organic compounds, such as lactic acid, as electron donors and sulfate as the external electron acceptor, which is reduced to sulfide:

This bacterial production of sulfide has been recognized as an important reaction for removing heavy metals as insoluble metal sulfides from contaminated wastewaters:

where, M=heavy metal

Many industrial wastewaters, e.g. the ones generated in tanneries, electroplating processes, edible oil industries, mining and processing of metals, paper mills, potato starch factories and petroleum refining, contain considerable amounts of sulfate and/or heavy metals (McCartney *et al.*, 1990; Genschow *et al.*, 1996; Dries *et al.*, 1998; De Vegt *et al.*, 1999). The biological removal of sulfate and heavy metals as metal sulfides represents a very important treatment process and has remarkable advantages over other physicochemical methods (El Bayoumy *et al.*, 1999b):

- High removal efficiencies are possible with sulfide precipitation due to the low solubility of metal sulfides, lower than metal hydroxides.
- The applicability of some other physicochemical methods depends on the incorporation of pretreatment schemes or post-treatment processes in order to improve the overall treatment system performance.
- Smaller volumes of sludge are produced with sulfide precipitation.
- Metal sulfides do not redissolve within a wider pH range than metal hydroxides as shown in Figure 1.1.
- The cost of metal precipitants is no longer an issue, since sulfide precipitant is produced by means of SRB metabolism under certain environmental conditions in a reactor.

The biological oxidation of organic matter combined with the sulfate reduction to produce sulfide, requires the optimum conditions in a biological reactor. Therefore, it is important to provide the suitable environmental conditions for the SRB optimum

Figure 1.1 Solubility of Metal Hydroxides and Sulfides as a function of pH

(U.S. EPA, 1985 as found in McArdle *et al.*, 1988)

growth. The pH of the system, number of viable and active cells, temperature, upflow velocity that translates to effective biomass retention, substrate composition and the organic loading rate, are important parameters to optimize in order to maximize sulfide production (Speece, 1996). Low pH (<5-6) inhibits sulfate reduction activity and increases the solubility of metal sulfides. The upflow velocity, which is related to the

hydraulic retention time in a given cross sectional area, plays an important role in retaining a high concentration of the biomass in the reactor so that higher organic loading rates can be applied in these anaerobic treatment systems and specific reactions can be controlled and/or maximized.

With this background, the aim of the present study was to compare the process behavior of three UA reactors, one packed with polypropylene pall rings and the other two non-packed, to establish the ability of SRB to attach to an inert surface and form a biofilm that can improve the process of sulfate reduction. At the same time, a comparison between the three UA reactors was carried out varying the hydraulic retention time, HRT, in order to establish the optimum conditions for maximum sulfide production by SRB and to determine the critical upflow velocity at which the biomass is washed out from the bioreactors.

1.1 Objectives

- Compare the effect of packing material for attached growth with suspended growth on the performance of SRB in an upflow anaerobic reactor.
- Establish the effect of the hydraulic retention time on the sulfide production rate in an upflow anaerobic reactor.
- Determine the critical upflow velocity at which the biomass is washed out from the reactor.
- Determine the rate kinetics of sulfide production by SRB.

1.2 Scope

The scope of the present study was as follows:

1.2.1 Batch Culture in 2 L Reactors. Phase I

1.2.1.1 Obtain a healthy culture of SRB to be used as seed for subsequent experiments.

1.2.1.2 Establish the substrate composition and frequency of feeding to a batch culture of SRB.

1.2.2 Batch System in 10 L Reactors. Phase II

1.2.2.1 Establish the effect of packing material on the performance of SRB in 10 L batch anaerobic reactors.

1.2.2.2 Study the sulfide production in 10 L reactors under batch operation.

1.2.2.3 Establish the optimum substrate composition for the SRB growth.

1.2.2.4 Determine the SRB behavior at different depths in 10 L reactors under batch conditions.

1.2.2.5 Determine the stoichiometry for lactate oxidation, sulfate reduction, cell synthesis and overall system reactions.

1.2.3 Continuous System in 10 L Reactors. Phase III

- 1.2.3.1 Determine the effect of packing material on the performance of SRB in an upflow anaerobic reactor.
- 1.2.3.2 Establish the concentration gradients of sulfide and volatile suspended solids, VSS, along the height of the reactors under continuous flow conditions.
- 1.2.3.3 Establish the critical hydraulic retention time for maximum sulfide production and suspended growth of SRB.
- 1.2.3.4 Establish the critical upflow velocity for complete biomass washout from an upflow anaerobic reactor.
- 1.2.3.5 Determine the kinetic parameters for the sulfide production process in an upflow anaerobic reactor.

CHAPTER 2. LITERATURE REVIEW

2.1 Sulfate Reducing Bacteria

A specific group of anaerobes, collectively called sulfate reducing bacteria, SRB, carries out, under certain environmental conditions, the oxidation of organic substrates and the reduction of the sulfate ions, SO_4^{2-} . Sulfate ions act as electron acceptors during an energy-generating process and are reduced to sulfide. The simplified reaction is shown below (Hao *et al.*, 1996):

The distribution of different sulfide species, H_2S , HS^- and S^{2-} , is a function of pH. Therefore, it is extremely important to control the system's pH to drive the equilibrium to the specie of interest. The distribution of sulfide species as a function of pH is shown in Figure 2.1.

Three oxidation states of sulfur are found in significant amounts in nature (Hao *et al.*, 1996):

- (i) Oxidation state -2: R-SH sulfhydryl and sulfide HS^- .
- (ii) Oxidation state 0: elemental sulfur S.
- (iii) Oxidation state 6+: sulfate SO_4^{2-} , a very important ion, which is often found in different types of wastewater.

Figure 2.1 Sulfide Speciation as a function of pH (Hao *et al.*, 1996)

Analytically, three categories of sulfide in water and wastewater are distinguished (APHA, 1995; Mizuno *et al.*, 1998b):

- (i) Total sulfide: includes dissolved HS^- , S^{2-} , H_2S , and insoluble sulfide-S such as ferrous sulfide, FeS .
- (ii) Dissolved sulfide consists of ionized sulfide, HS^- and S^{2-} , and free H_2S .
- (iii) Un-ionized hydrogen sulfide gas, can be calculated from the concentration of dissolved sulfide, pH of the sample and ionization constant, K_s , of H_2S .

The un-ionized H₂S in the liquid phase is in equilibrium with the H₂S in the gas phase, approximately 26 mg/L H₂S are in the liquid phase for each 1% H₂S in the gas phase at 35 °C, while the pH determines the sulfide equilibrium in the liquid phase by the following reaction (Speece, 1996):

Microbial activities determine what is called the biological sulfur cycle, shown in Figure 2.2 (Brock and Madigan, 1991 as found in Hao *et al.*, 1996). One important characteristic of SRB in this cycle is that they can only degrade low-molecular-mass compounds like volatile acids, e.g. acetate, organic acids, e.g. lactate or pyruvate, and alcohols, e.g. ethanol. But the end products of the reactions depend on the SRB genera involved. *Desulfovibrio* genus, commonly named incomplete SRB, does not completely utilize lactate, but just degrades it to acetate. On the other hand, *Desulfotomaculum* genus can utilize acetate or in some cases glucose (Reis *et al.*, 1992; Hao *et al.*, 1996).

SRB are obligate anaerobes and are not inhibited by temporary exposures to oxygen. This is related as well with the redox potential of the system in which SRB are grown. Postgate (1984) refers a redox potential of –100 mV as necessary for the growth of SRB. SRB are adaptable to almost any environment, they tolerate temperatures from below -5 to 75° C and pH values ranging from 5.0 to 9.5 (Postgate, 1984; Perry, 1985).

Figure 2.2 Schematic Diagram of Sulfur Cycle (Brock and Madigan, 1991 as found in Hao *et al.*, 1996)

2.2 Competition for Organic Substrate between SRB and MPB

In the anaerobic treatment of wastewater, both sulfate reduction and methanogenesis can take place. Therefore, a competition between sulfate-reducing bacteria (SRB) and methane-producing bacteria (MPB) may occur because both are capable of using hydrogen and acetate or the organic substrate present. The importance of the competition between SRB and MPB relies on the type of wastewater that can be treated based on the

metabolism followed by a certain culture at certain environmental conditions. This competition is strongly dependant on the COD to sulfur ratio as well as the pH of the system (McCartney and Oleszkiewicz, 1993).

During normal anaerobic wastewater treatment, hydrogen and acetate are intermediate products and precursors to methane formation. The formers can function as electron donors for sulfate reduction, therefore, MPB and SRB may be considered competitors for these substrates. McCartney and Oleszkiewicz (1993) discussed the competition in terms of thermodynamic and kinetic factors. In terms of free energy changes for acetate and hydrogen, at standard conditions, SRB would out-compete MPB.

Nevertheless, the potential for MPB to out-compete SRB is present, when the electron donor (acetate, hydrogen or an organic) to sulfate ratio becomes high or, when a buildup of sulfide occurs. In the case of SRB, these bacteria out-compete MPB when the electron donor to sulfate ratio is low and if sulfide concentrations and pH are not high.

Choi and Rim (1991) have pointed out that SRB and MPB are in competitive relation for substrate utilization. They found that, with enough carbon source, as COD:SO₄²⁻ decreased to 1.7, the sulfate reduction efficiencies increased, and below 1.7, the efficiencies decreased, independent of the hydraulic retention time. When the ratio exceeded 2.7, methane producers began to predominate. These findings are very similar to those of Bhattacharya *et al.* (1996), who concluded that with high sulfate and low acetate concentrations (< 10 mg/L), SRB were predominant. Dries *et al.* (1998) reported a

complete sulfidogenesis, in which SRB accounted for 70-85% of the total COD uptake throughout their experiment, based on the COD to SO_4^{2-} as S ratio, COD:S, of 2.0-2.2, slightly alkaline conditions (pH= 7.9) and high sulfate loading rate of 10.4 g SO_4^{2-} as S/L/d.

McCartney and Oleszkiewicz (1993) cited the work of Isa *et al.* (1986a), which supported the theory that the COD to SO_4^{2-} ratio determined whether or not SRB would out-compete MPB. It is important to note that this ratio can be interpreted as a factor in the stoichiometric sulfate requirements for SRB metabolism and in the kinetics of substrate utilization. McCartney and Oleszkiewicz (1993) pointed out in their study that a lower COD: SO_4^{2-} drives the metabolic pathway to a complete oxidation of lactate via sulfate reduction, without intermediate products as propionate, coinciding with the findings of Harada *et al.* (1994). All related studies support the fact that a pathway taken is highly dependent on the environmental conditions provided, and if those change, the pathway involved can change as well.

Harada *et al.* (1994) found that the methane production was influenced by the sulfate levels in the feed solutions, having lower methane production at higher levels of sulfate (g SO_4^{2-} as S g COD imposed=0.4) due to the increment in sulfate reduction rate with time and was independent of the loading rate. This coincides with the work of Mizuno *et al.* (1994) who concluded that at the COD to SO_4^{2-} as S ratio of 6 or more, methane production was the predominant reaction, while at the COD to SO_4^{2-} as S ratio of 1.5, SRB utilized more than 50% of the total electron flow. Omil *et al.* (1997) found that a

decrease from 2.0 to 1.0 of the COD to sulfate ratio in the influent resulted in an increase of the fraction of acetate utilized by SRB to 40-50%, while in the long-term operation and at COD:sulfate of 0.5, a complete acetate removal by ASRB was accomplished.

Mizuno *et al.* (1994) used concentrations of organic and sulfate sources, 2500 mg COD/L as butyric acid and 1667 mg/L of sulfate with COD:SO₄²⁻ as S of 1.5. The researchers concluded that SRB did not out-compete MPB for acetate at a COD:SO₄²⁻ as S ratio greater than 3. In the present study, one of the conditions tested used 2500 mg Th.O.D./L as lactic acid and 1250 mg/L of sulfate with Th.O.D.:SO₄²⁻ of 2.

Harada *et al.* (1994) related some of their observations on the competition of SRB and MPB for acetate and hydrogen to V_M/K_M found in the literature, where V_M is the maximum specific acetate or hydrogen consumption rate and K_M is the half velocity constant for the specific substrate consumption. The higher the value of the ratio, the more favorable is the competition for a certain group of bacteria. SRB tend to out-compete MPB for both acetate and hydrogen, having a higher V_M/K_M . This contrasted with their own findings, in which just a small fraction of the COD eliminated was utilized for sulfate reduction from acetate. This is in agreement with Polprasert and Hass (1995) who concluded that acetic acid was not a major substrate for sulfate reduction in an anaerobic mixed culture.

Similarly, Isa *et al.* (1986a) found that acetate conversion by SRB were not competitive in a high-rate anaerobic reactor, arguing that SRB were not able to colonize and adhere as well as MPB to the carrier matrix and therefore were easily washed out. However, during

long-term operation, SRB became more competitive in comparison with MPB due to their higher affinity for substrate and higher substrate removal rate. El Bayoumy *et al.* (1999a) confirmed that in sulfate-poor environment, Th.O.D.:SO₄²⁻ higher than 2.25, MPB started to out-compete SRB. Therefore, in general, a sulfate-rich environment is preferred over a sulfate-poor environment.

2.3 Effect of Temperature

Visser *et al.* (1993) studied the effect of temperature shocks on the performance of a mesophilic (30 °C) UASB reactor fed with synthetic medium of acetate, propionate, butyrate and sulfate, in which competition between sulfate-reducing and methanogenic bacteria could take place. The effect of temperature was observed to be a function of the type of sludge involved, the exposure time to a certain temperature shock and the change in temperature applied. The authors observed that at 55 and 65 °C there was an important decrease of the mesophilic sludge activity, while the acetate oxidation was more sensitive than the propionate and butyrate ones. In the same way, sulfate reduction was less sensitive to temperature shocks than methanogenesis and, therefore, the sulfate reduction process could recover fairly quickly after a temperature shock.

Hao *et al.* (1996) reported an optimum temperature for SRB pure culture growth and consequently for high sulfate reduction rates, in the range of 28-32 °C, although for certain genera it was as high as 70 °C.

2.4 Attached Growth vs Suspended Growth

Reis *et al.* (1992) reported that inhibition of sulfide concentration for SRB was a function of the type of reactor involved. There was a slight inhibition in immobilized cell reactors because cells were better protected from the adverse environmental factors than in suspended growth reactors. McCartney and Oleszkiewicz (1993) referred to the work of Isa *et al.* (1986a) and Yoda *et al.* (1987) with the purpose of analyzing the competition of SRB and MPB in terms of differential bacterial attachment. The former had used a batch attached growth reactor while the latter had used a continuous-feed attached growth system. They found that the bacterial attachment determined the increase in MPB population as compared to the SRB population. Isa *et al.* (1986a) reported that the MPB population was 2000 times larger in the biofilm as compared to the effluent. In contrast, the SRB population was just 30 times larger in the biofilm as compared to the effluent. They concluded that SRB did not attach as readily to the support media and the washout was more evident

El Bayoumy *et al.* (1999b) concluded that the type and/or characteristics of the packing material did not affect the growth and production of the SRB in an Upflow Anaerobic Fixed-Film (UAFF) reactor. The authors also observed visually that the SRB biomass did not attach to the packing material and preferably settled down on the packing material. Higher bacterial concentration was observed near the bottom of the reactor.

2.5 Effect of Liquid Upflow Velocity

Although most researchers have reported results in terms of total COD and soluble COD removal efficiencies in an UASB reactor, the maximum sulfide production or maximum organic-COD removal by SRB, as a function of the hydraulic retention time has been established by just a few (Alphenaar *et al.*, 1993; Singh *et al.*, 1996). Alphenaar *et al.* (1993) studied the effect of hydraulic retention time and liquid upflow velocity on the granulation process in an UASB reactor, with no support media, and the competition for acetate between sulfate-reducing and methane-producing bacteria. They found that the fraction of organic material used by sulfate-reducers increased with time and at a hydraulic retention time of 40 h. The authors pointed out that, based on thermodynamic data with Gibbs free energies values, the oxidation of hydrogen and acetate with sulfate reduction, SRB driven, is more favorable than the oxidation of hydrogen and acetate with methane formation, MPB driven. Therefore, SRB may out-compete MPB as shown in reactions 2.3 through 2.6.

Biochemical Reaction	ΔG° (kcal/reaction)	
$4\text{H}_2 + \text{SO}_4^{2-} + \text{H}^+ \rightarrow \text{HS}^- + 4\text{H}_2\text{O}$	-38.0	2.3
$4\text{H}_2 + \text{HCO}_3^- + \text{H}^+ \rightarrow \text{CH}_4 + 3\text{H}_2\text{O}$	-32.7	2.4
$\text{CH}_3\text{COO}^- + \text{H}_2\text{O} \rightarrow \text{CH}_4 + \text{HCO}_3^-$	-28.2	2.5
$\text{CH}_3\text{COO}^- + \text{SO}_4^{2-} \rightarrow \text{HS}^- + 2\text{HCO}_3^-$	-39.5	2.6

But beside thermodynamic and kinetic factors, the competition between SRB and MPB depends on several parameters, such as the organic loading rate, theoretical oxygen demand:sulfate, pH and the retention of biomass in the reactor.

Alphenaar, *et al.* (1993) used three PVC UASB reactors with a liquid volume of 1.1 L. The height of the reactors was 400 mm and the diameter was 6 mm. The third UASB was followed by a continuously stirred, 5 L, PVC tank reactor in order to increase the retention time. In the second and third UASB reactors, effluent recycling was used to increase the upflow velocity. The recycling factor was 10. The organic substrate consisted of 50% acetic acid, 40% propionic acid and 10% sucrose, having an influent pH of 6.8 and COD to sulfate ratio of 0.5. The hydraulic retention times were 7.5, 6.9 and 40.2 h, while the upflow velocities were 0.05, 0.65 and 0.65 m/h, for UASB I, UASB II and UASB+CSTR, respectively. The start up period for this study was 50 days. The results showed that the amount of total-organic COD oxidized by SRB increased with time for the reactor sludge. The authors pointed out that a combination of an UASB/CSTR and a relatively longer HRT were more favorable for SRB with respect to acetate competition between SRB and MPB. The washout of both suspended-growing SRB and MPB occurred in UASB reactors operated at a HRT of 7-7.5 h, suggesting that the operation of anaerobic reactors at a longer HRT is favorable for SRB, growing either in dispersed or immobilized form. They also found that the hydraulic regime in the reactor played an important role in the concentrations of SRB, and a completely mixed system with recirculation enhanced the development of SRB in comparison with a plug-flow system.

Choi and Rim (1991) worked with laboratory anaerobic contact units and a municipal digester, which were operated at 35 °C with hydraulic retention times from 0.5-6 days and fed with synthetic substrate made of acetic acid supplemented with nutrients. The authors found that with longer hydraulic retention times, greater sulfate and COD reductions were obtained while the inhibitory concentrations of sulfide were higher.

Yoda *et al.* (1987) found that the percent of electron flow to SRB increased by increasing the hydraulic retention time, HRT, of the reactor. This contrasts with the findings of Mizuno *et al.* (1994), who reported that at the same COD:SO₄²⁻ as S, the electron flow used for SRB increased during steady-state conditions, from 51% to 67%, when the hydraulic retention time was changed from 20 to 5 days.

Singh *et al.* (1996) used 3 h of HRT, with an up-flow velocity of 1.3 m/h, in an UASB at an organic loading rate of 4 kg COD/m³*d, and obtained an effluent suspended solids, SS, concentration of 50 mg/L. The authors reported that a severe granule washout was noticeable at 3 h of HRT, but was lower than 30 mg/L for other retention times of 4 and 6 h with upflow velocities of 1 and 0.67 m/h, respectively. They also found that the soluble COD removal of 88% as well as the effluent sulfide concentration of 75 mg/L were fairly constant irrespective of the organic loading rate. In this study, more than 75% of the influent COD was reduced in the granular sludge bed zone of the reactor and the MLSS, COD and SO₄²⁻ concentrations tended to decrease along the reactor height. The highest activity of SRB was found in the first 100-300 mm of the reactor's height.

Agrawal *et al.* (1997b), found that the COD removal efficiency in an UASB reactor increased as the HRT was reduced from 28 to 9 h. The authors observed that a further decrease in HRT caused a complete failure of the process, due to the flotation of a considerable amount of sludge to the top of the reactor. Dries *et al.* (1998) reported that, at an upflow velocity of 20 m/h and 1.9 h of HRT, there was a significant loss of biomass into the effluent, causing a drop in the sulfate reduction rate and in the COD removal. This high value of liquid upflow velocity is contrasted with the one at which the study of De Smul and Verstraete (1999) was operated. The authors varied the liquid upflow velocity between 3 and 12 m/h, finding 3 m/h better for the retention of the biomass in the reactor and maintaining the SS concentration in the effluent below 20 mg/L.

Mizuno *et al.* (1998b) studied the effect of sulfate removal in anaerobic chemostat systems under steady-state condition. They observed that the sulfate removal efficiency increased with an increase in HRT that was varied between 2 and 12 h, with 2 h as the critical hydraulic retention time. The authors recommended a hydraulic retention time longer than 8 h. They also reported that the concentration of total sulfide, dissolved sulfide (HS^- , S^{2-} , H_2S) and free-hydrogen sulfide (H_2S) increased with an increase in HRT, reaching 170, 136 and 99 mg S /L, respectively.

2.6 Effect of Hydrogen Sulfide

One important factor that affects the growth and activity of SRB is sulfide, a metabolic product from sulfate reduction. The effect of the imposed environmental conditions, as

pH or the sulfide concentration, on the kinetic growth properties of SRB and MPB is very important; the former is a key factor in a sulfide inhibition mechanism resulting in the oscillating coexistence between SRB and MPB. Okabe *et al.* (1995) pointed out that pH, sulfide concentration, trace nutrients and substrate could not be maintained at the same levels over many generations because of the batch nature of the systems studied.

Choi and Rim (1991) reported the inhibitory concentrations of sulfide to be 120-140 mg/L for methane producers, and 160-200 mg/L for sulfate reducers in anaerobic contact units. They showed that COD was continuously removed at different organic loading rates, thereby producing different sulfide concentrations. This would suggest that the inhibitory effect of sulfide varied with the substrate. These researchers did not distinguish between ionized and un-ionized sulfide in their work.

Reis *et al.* (1992) studied the effect of pH and hydrogen sulfide on growth of SRB in batch reactors. Values of the specific growth rate, $\mu_{\max}$, and growth yield coefficients, Y , were calculated in the absence of hydrogen sulfide by continuous stripping of the system. The maximum values for both parameters were found at pH of 6.6. They also found that the hydrogen ion, H^+ , was the growth inhibitor for the SRB culture used in their study, since hydrogen sulfide, a potential inhibitor, was eliminated from the system. Reis *et al.* (1992) performed further experiments at constant pH values of 6.2 and 6.6, to verify if the inhibition on SRB was due to the direct toxicity of the sulfide produced or due to iron precipitation and consequently lack of iron for the microorganisms growth. They observed that the sulfide was the inhibitor of SRB and that it was a reversible process.

The sulfate uptake rate was increased during the lag and early logarithmic phases, after which a progressive decrease was observed due to the higher sulfide concentration. The authors reported the toxic concentration of hydrogen sulfide to be 547 mg/L. As the pH increased, hydrogen sulfide was the inhibitor of concern, so that at near neutral pH, SRB was affected by the hydrogen sulfide and not by the acetic acid produced in the incomplete oxidation of lactate. This agreed with the findings of McCartney *et al.* (1990), which supported that as pH increased, hydrogen sulfide became the inhibitor of concern because the acetate uptake rate for the reactors remained the same.

McCartney *et al.* (1990) have referred to the work of Rinzema and Lettinga (1988) who observed a significant decrease in propionate removal efficiency with hydrogen sulfide concentrations above 100 mg/L. Hilton and Oleszkiewicz (1988) pointed out that SRB, under batch conditions, were inhibited in proportion to the total sulfide concentration and not the hydrogen sulfide concentration. Nevertheless, McCartney *et al.* (1990) concluded that MPB were more sensitive to sulfide than the SRB, regardless of total sulfide concentration or pH.

McCartney and Oleszkiewicz (1993) reported un-ionized sulfide concentrations of 185 mg/L for 50% inhibition of SRB activity at pH 8 in serum bottles using a mixed culture in a lactate-sulfate medium, while at pH 7 concentrations below 300 mg/L did not inhibit SRB activity. Omil *et al.* (1997) found that an acclimated acetate-consuming SRB could operate under 100 and 800 mg/L free and total sulfide concentrations, respectively. Yamaguchi *et al.* (1999) reported 100 mg/L of un-dissociated sulfide as the limit for a

satisfactory COD removal performance. McCartney and Oleszkiewicz (1993) further concluded that a prolonged exposure to high sulfate, high lactate and elevated sulfide concentrations would affect the selection of a specific type of SRB species or the stimulation of a different metabolic pathway for SRB. The authors also concluded that SRB growing on a more complex substrate, like lactate, may be less sulfide sensitive than SRB grown on propionate, which agrees with the observation of Choi and Rim (1991).

Okabe *et al.* (1992) reported that sulfide inhibition of SRB was present when sulfide species, H_2S , HS^- and S^{2-} , combined with the iron in the cell, caused electron transport systems to stop activity. Therefore the pH of the system was very important because it determined the distribution of sulfide species. At pH 7, the H_2S to HS^- ratio was about 1:1 (pKa of H_2S is 7 at 25 °C). The percentage of un-ionized H_2S drops from 90% at pH 6 to 50% at pH 7 (Figure 2.1). The authors reported a noticeable inhibition by un-ionized H_2S , more than total sulfide. Okabe *et al.* (1995) separated the effect of sulfide inhibition into an effect on cell yield (growth) and on lactate utilization rate (activity) in a chemostat. They found that the growth had decreased at concentrations of 437 mg S /L, while the activity was not affected in the range of 332-437 mg S/L. The cell yield was found to be maximum at 108 mg S /L. In an earlier study by Okabe *et al.* (1992), it was reported that a total sulfide concentration of 280 mg/L dramatically decreased cellular production, and that lactate utilization and cellular production in a chemostat were strongly inhibited at total sulfide concentration of 600 mg/L.

Maillacheruvu and Parkin (1996) found that the acetate-utilizing SRB were more sensitive to sulfide toxicity and they concluded that the sulfide toxicity was a function of pH and the specific group of SRB involved. One important conclusion made by Hao *et al.* (1996) was that the different sensitivities to H_2S and dissolved sulfide were function of the organic substrate (electron donor) fed into the system and the type of anaerobic reactor used, an anaerobic filter or suspended growth reactors.

2.7 Heavy Metals Removal

Gundry *et al.* (1990) studied the removal of metals from electroplating wastewater based on an anaerobic degradation of organics by SRB, which used sulfate, SO_4^{2-} , as an electron acceptor and reduced them to sulfide, S^{2-} . The sulfide was then available to precipitate metals as metal sulfides. These researchers operated the anaerobic filters at a retention time of 24 h and fed them with a mixture of domestic wastewater and electroplating wastewater. They obtained a COD removal of 79%, while sulfate reduction showed the same behavior as COD. They also found 96-99% removal of the applied nickel in these anaerobic filters. The critical ORL for the production of sulfide in amount higher than that required to precipitate the metal of interest as the metal sulfide, was 0.35 kg COD/m³/d. However, the authors pointed out that above this OLR, the removal of the metal of interest, Ni, was within an acceptable percentage, suggesting that some other removal processes may have taken place, such as bio-adsorption or precipitation by other ions in the system. It is important to note that the deficiency of sulfide in the effluent was

overcome with an increase in the OLR, showing that the failure of the system was due to low organic content and not due to the toxicity of the metals in the plating waste.

El Bayoumy *et al.* (1999b) listed the advantages of metal sulfide precipitation over other methods as high efficiencies, production of lower sludge volume as compared with chemical precipitation, low solubility products and less tendency to resolubilize. The authors referred to the work of Dvorak *et al.* (1992) who used biological reactors in order to remove metals by means of sulfide precipitation with the sulfide produced by SRB, obtaining removals above 90%. They observed that the height of the reactor in which complete removal occurred depended on the influent metal concentration and that the rate of metal removal with height was relatively constant, with the consequent conclusion that, at low metal concentrations, the rate of sulfide production with height remained constant as well (El Bayoumy *et al.*, 1999b).

Hao *et al.* (1996) pointed out that the metal toxicity depended on experimental conditions like cell concentration, presence of complexing agents, metal mixtures, pH and type of the system, e.g. batch, sequencing batch reactor, pure culture, etc. It has been stated that some metals may also be precipitated and/or physically entrapped within the biomass (Hao *et al.*, 1996; El Bayoumy *et al.*, 1999b).

2.8 Operational Conditions

Various researchers have identified the optimum operating conditions for sulfide production by SRB. Wijaya (1993) showed that the optimum organic loading rate, OLR, and optimum phosphorous concentration for the SRB growth were 6 kg/d/m³ and 65 mg/L of P, respectively. El Bayoumy *et al.* (1999b) carried out a study to establish the optimum conditions for maximizing sulfide production by SRB to remove heavy metals. The following conditions were suggested:

- Th O D to sulfates ratio between 1.5 and 2.25.
- OLR of 6 kg C O D./d/m³ or higher, assuming COD:Th.O.D.=1.
- Nitrogen loading of 0.3-0.7 kg NH₄-N /d/m³.
- Phosphorus loading of 0.06 kg P/d/m³.
- Optimum Th O D.:N:P of 100:5:1.
- Hydraulic retention time, HRT, equal to or higher than 12 h, with a corresponding upflow velocity of 2.4 m/h.

The authors reported an ultimate sulfide concentration of 140 mg/L under these conditions. They also observed a linear increase in TOC removal with HRT, and this removal was maintained at about 50-60% until complete failure of the reactor occurred at critical metal loadings (El Bayoumy *et al.*, 1999b). It was pointed out that the sulfide production showed an increase with an increase in the total nitrogen concentration up to about 250 mg NH₄-N/L and it started to decrease at nitrogen concentrations between 250-

600 mg $\text{NH}_4\text{-N}$ /L. Higher nitrogen concentrations caused adverse effects. However, that was not the case for phosphorus when concentrations above 50 mg of P/L gave maximum sulfide production (El Bayoumy *et al.*, 1999b).

Okabe and Characklis (1991) reported that the limiting C to P ratio (w/w) for growth of *D. desulfuricans* was in the range of 400:1- 800:1. They also reported a limiting C to N ratio (w/w) for *D. desulfuricans* to be 45:1- 120:1, which was higher than that for an aerobic mixed population because *D. desulfuricans* partially oxidized lactate to acetate and CO_2 . Thus, cell production from substrate was approximately 10 times less than in aerobic systems and consequently nitrogen requirements were 10 times less. Hao *et al.* (1996) reported that sulfate and organic matter were used in a ratio of 2 to 1, but it was a function of the organic matter to be degraded.

CHAPTER 3. EXPERIMENTAL PROCEDURE

3.1 Experimental Set-Up

The following set-ups were used during this study:

Phase I. Growth of sulfate reducing bacteria in three 2 L parallel reactors.

Phase II. Batch studies in three 10 L parallel reactors.

Phase III. Continuous flow studies in three 10 L parallel reactors.

3.1.1 Experimental Set-Up During Phase I

The set-up for the first experimental phase consisted of three batch reactors, one of which was filled with 15.9 mm polypropylene pall rings with a porosity of 90% to provide a void volume of 1.7 L. The other two reactors were used without any packing material and the void volume of these reactors was 1.9 L. The batch reactors were constructed from 2 L Erlenmeyer flasks, capped and sealed with rubber stoppers, with one outlet for gas venting. The reactors were covered with aluminum foil to prevent any photosynthetic growth. Figure 3.1 shows the experimental set-up during Phase I.

All the three reactors were filled with feed solutions, with the composition established by El Bayoumy (1997) and shown in Table 3.1. The feed was based on 1 d and 4 d of retention time and the following optimum loading conditions based on stoichiometric equations:

Figure 3.1 Experimental Set-Up During Phase I

- $OLR = 6 \text{ kg/d/m}^3$.
- $Th.O.D. (g/L) = OLR * HRT$
- $Lactic \text{ Acid } (g/L) = Th.O.D. / 1.07$
- $NaOH (g/L) = 0.44 * Lactic \text{ Acid}$
- $SO_4^{2-} (g/L) = Th.O.D. / 2$
- $N (g/L) = Th.O.D. / 20$
- $P (g/L) = Th.O.D. / 100$

The reactors were seeded with a sulfide-smelling wastewater sample collected from the West Windsor Wastewater Treatment Plant. The wastewater sample had a black color, rotten egg odor, pH of 7.0 and 100 mL were added to each reactor to provide the SRB strain, which was confirmed by the SRB counting test. The mixed bacterial culture in wastewater was preferred over the pure SRB culture, because in actual treatment process, the industrial wastewater will contain a mixed culture. Deionized water was used for the preparation of the solutions, except for the third reactor in which tap water was used for comparison.

3.1.2 Experimental Set-Up During Phase II

3.1.2.1 Reactors

Three parallel transparent plexiglass 10 L reactors were used in batch mode. Each reactor had the following components:

1. Cylindrical reactors with an overall height of 1300 mm and an internal diameter of 102 mm. These reactors were kept inside a closed light-tight chamber made of dark gray polyvinyl chloride (PVC) material.
2. Connecting plastic tubes
3. Three sampling ports equally spaced at 300 mm to collect samples for analysis at different heights.

Table 3.1 Batch Substrate Composition A for Phase I

Substrate Component	1 d RT	4 d RT
Reactor I, II and III		
Lactic Acid (g/L)	5.57	22.26
NaOH (g/L)	2.45	9.80
MgSO ₄ *7H ₂ O (g/L) =2.56*SO ₄	7.68	30.72
NH ₄ Cl (g/L)=3.78*N	1.13	4.54
KH ₂ PO ₄ (g/L)=(4.387/4)*P	0.065	0.26
K ₂ HPO ₄ (g/L)=4* KH ₂ PO ₄	0.26	1.05

Reactor I. Packed. Deionized water for dilution.

Reactor II. Non-packed. Deionized water for dilution.

Reactor III. Non-packed. Tap water for dilution.

The experiments were conducted at room temperature 19-25 °C. Initially, two of the reactors, Reactor I and III, were filled with 15.9 mm polypropylene pall rings with a porosity of 90% to provide a void volume of 9.09 L, while the other reactor, non-packed,

had a void volume of 10.1 L. However, later on, it was decided to remove the packing material from Reactor III.

3.1.2.2 Seed Material

Once the average concentration of sulfide in Phase I had reached 100 mg/L, the sediment from Reactor II was used as a seed during the set up for Phases II and Phase III. The 10 L reactors were inoculated with 200 mL of the seed culture with a VSS concentration of 4300 mg/L and a VSS to SS ratio of 0.76. This culture showed the approximate concentration of SRB of 10^6 cells/mL when tested with an Immunoassay Test Kit for the detection of SRB.

3.1.2.3 Media

Two types of substrate composition were used during this stage:

1. Batch substrate composition A for Phase II as shown in Table 3.1 for 4 days of retention time. Final pH= 7.0 ± 0.1 .
2. Batch substrate composition B for Phase II as shown in Table 3.2. Final pH= 7.0 ± 0.1 .

The change in substrate composition and its sequence is described in the experimental procedure section.

Table 3.2 Batch Substrate Composition B for Phase II

Substrate Component (in order of addition)	g/L	g/L
NaOH	1.98	
Lactic Acid	4.5	4.85 as Th.O.D.
Sodium sulfate Na_2SO_4	2.36	1.6 as SO_4
Ammonium Sulfate $(\text{NH}_4)_2\text{SO}_4$	1.14	0.2425 as N, 0.829 as SO_4
Potassium Di-hydrogen Phosphate (KH_2PO_4)	0.07	0.02 as P
Potassium hydrogen Phosphate (K_2HPO_4)	0.18	0.0285 as P
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.03	
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.04	
SOLUTION OF LOW CONCENTRATION COMPOUNDS		
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.004	
$\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$	0.009	
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.008	
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.009	
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	0.006	
H_3BO_3	0.01	
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.008	
Trace elements: Fe, Ni, Cu, Zn, Mn, B, Co	0.002	

3.1.3 Experimental Set-Up During Phase III

3.1.3.1 Reactors

Three parallel plexiglass UA reactors were used in continuous flow mode. Each reactor had the following components:

1. Feed container. The substrate was pumped from a glass or plastic container, stirred by a magnetic or suitable device, to the bottom inlet of the reactor by means of a variable speed peristaltic pump. These pumps were calibrated at the beginning of the experiment and checked every 5 days by measuring the time required to collect a known volume of effluent from the reactor.
2. UA reactors. The transparent plexiglass reactors were kept inside a closed light-tight chamber made of dark gray polyvinyl chloride (PVC) material to avoid any photosynthetic sulfur bacteria growth that could disturb the desired environment in the reactors. All reactors used had the following dimensions:

Overall Height: 1300 mm

Distance between inlet and outlet: 1235 mm

Internal diameter: 102 mm

3. Effluent Glass Sampler Unit. The effluent sampler units were made of glass and had the following dimensions:

Height: 250 mm

Internal Diameter: 50 mm

These sampler containers had two controlled openings: one in the upper side of the unit for continuous withdrawal of reactor's effluent to the drain system and another one for collecting sample at the bottom of the unit.

4. Connecting Plastic Tubes.
5. Three sampling ports. Each reactor had three outlet ports, equally spaced at 300 mm, to collect samples for analysis at different heights.

Figure 3.2 shows the configuration of the reactors while Figures 3.3 and 3.4 show the actual experimental set up for Phase III.

The experiments were conducted at room temperature 19-25 °C, and the feed solutions were prepared daily to prevent any degradation, or in some cases, they were stored at 4 °C and then warmed up before use. An anaerobic environment was always maintained. The dissolved oxygen concentration in the effluent was always less than 0.5 mg/L, which is below the critical dissolved oxygen concentration of 1 mg/L for sulfate reduction (Hao *et al.*, 1996).

One of the reactors was filled with 15.9 mm polypropylene pall rings with a porosity of 90% to provide a void volume of 9.09 L. The characteristics of the packing material are presented in Table 3.3. The reactors were kept air-tight by using rubber stoppers and screws to control and close all the openings, and also were kept inside a closed light-tight dark gray PVC chamber during operation, except during sampling, to prevent the photosynthetic sulfur bacteria growth and consequently to prevent any changes in the desired environment inside the reactors.

Figure 3.2 Reactor Configuration. Phase III

Figure 3.3 Experimental Set Up During Phase III. View 1

Figure 3.4 Experimental Set Up During Phase III. View 2

Table 3.3 Packing Material Characteristics

Type	Polypropylene Fall Rings
Size (mm)	15.9
Surface Area (mm ² /mm ³)	10.8
Free Space (%)	90

3.1.3.2 Seed Material

Since the same reactors operated during Phase II were used for Phase III, no seeding procedure was followed. From the sulfide concentration obtained in Phase II it could be seen that an acclimatized SRB culture was present in the reactors to start the continuous mode.

3.1.3.3 Media

Initially, the 10 L reactors were fed under batch conditions with the medium described in Table 3.2. Once the sulfide concentration had reached 100 mg/L, the continuous flow mode was resumed and the substrate composition, shown in Table 3.4, was defined depending on the organic loading rate, OLR, and hydraulic retention time, HRT.

Table 3.4 Substrate Composition for Continuous Flow Mode

HRT (h)	5	7.5	10	10	12	20	30	50
Substrate Component (g/L)								
Lactic Acid	1.16	1.74	4.64	2.32	5.57	4.64	6.96	11.60
NaOH	0.51	0.77	2.04	1.02	2.45	2.04	3.06	5.1
SO ₄	0.63	0.94	2.50	1.25	3.00	2.50	3.75	6.25
Na ₂ SO ₄	0.58	0.87	2.33	1.17	2.80	2.33	2.41	5.83
MgSO ₄ • 7H ₂ O	0.04	0.06	0.17	0.08	0.20	0.17	0.25	0.42
CaCl ₂ • 2H ₂ O	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
N	0.06	0.09	0.25	0.13	0.30	0.25	0.38	0.63
(NH ₄) ₂ SO ₄	0.29	0.44	1.18	0.59	1.41	1.18	1.77	2.95
P	0.01	0.02	0.05	0.03	0.06	0.05	0.08	0.13
KH ₂ PO ₄	0.018	0.027	0.073	0.037	0.088	0.073	0.110	0.183
K ₂ HPO ₄	0.047	0.07	0.187	0.094	0.225	0.187	0.281	0.469
Trace elements Fe, Ni, Cu, Zn, Mn, B, Co	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Parameter								
Flow L/d	48.5	32.3	24.2	24.2	20.2	12.1	8.1	4.9
OLR (kg/d/m ³)	6	6	12	6	12	6	6	6
Th.O.D. (g/L)	1.25	1.88	5	2.50	6	5.00	7.50	12.50
Th.O.D. : SO ₄	2	2	2	2	2	2	2	2
Th.O.D. : N: P	100:5:1	100:5:1	100:5:1	100:5:1	100:5:1	100:5:1	100:5:1	100:5:1
KH ₂ PO ₄ : K ₂ HPO ₄	1:2	1:2	1:2	1:2	1:2	1:2	1:2	1:2

Lactic acid was used as the carbon source, and sodium sulfate and ammonium sulfate were used as the sulfate sources. The influent pH was 7.0 ± 0.1 . The Th.O.D.:sulfate and Th.O.D.:N:P were always kept at 2:1 and 100:5:1, respectively. The synthetic substrates were prepared daily and each feed tank was mixed continuously with a magnetic stirrer or any other suitable device.

3.2 Experimental Procedure

3.2.1 Experimental Procedure. Phase I

The main objective of this phase was to grow the sulfate reducing bacteria under batch conditions in order to use the strain to seed the 10 L reactors for Phases II and III. A healthy culture of the bacteria was indicated by the sulfide concentrations of 120-140 mg/L observed in previous studies (Wijaya, 1993; El Bayoumy, 1997).

In order to grow the SRB bacteria, each third day 15% of the volume of the 2 L reactors was replaced with fresh substrate solution and each week an analysis of sulfate, sulfide, pH and ORP was performed. The liquid content was completely mixed each time after refilling. The dilution water used in feeds for Reactors I and II was deionized water, while for Reactor III it was tap water.

This procedure was followed for 38 days. For the next 18 days the frequency of feeding was each third and fourth day with a weekly analysis. Then, for a week, the reactors were fed every second day. After these 63 days, the feeding was done each 8 days but the

substrate was concentrated, corresponding to 4 days of retention time. Also, deionized water was used for all substrate solutions. The reactors were covered with aluminum foil in order to eliminate light coming through. This was continued for additional 45 days but no analyses were done during this time.

This sequence permitted to achieve an average concentration of 100 mg/L for the Reactor II, which was conserved as the seed reactor for the subsequent studies. This concentration was checked every week and the pH was always kept at about 7.0. The feedings were done every 8 days, with a substrate solution based on 4 day of retention time. This substrate composition remained the same, until a change in substrate composition was made during the second phase of experiments. The new substrate composition is shown in Table 3.2.

3.2.2 Experimental Procedure. Phase II

A set of three 10 L parallel reactors was filled with the substrate with composition shown in Table 3.1 for a retention time of 4 days and was operated under batch conditions for 2 weeks. This was done in an attempt to grow the old strain of bacteria remaining from the previous experiments (El Bayoumy, 1997). A stable sulfide concentration in the three 10 L reactors was reached through different sequences of withdrawal-analysis-recharge.

1st sequence. Each reactor was inoculated with 200 mL of the sludge from Reactor II Phase I, which was equivalent to a VSS mass of 860 mg. These 200 mL of inoculum

were mixed with 1.3 L of feed solution, described in Table 3.1, for a retention time of 4 days. Each eight days, 1.5 L of reactor's contents was withdrawn from the 3rd port of the reactor for analysis of sulfide and recharged with an equivalent volume of fresh substrate solution. This was continued for 32 consecutive days.

2nd sequence. Each four days, 1.5 L of reactor's contents was withdrawn from the 3rd port of the reactor for analysis of sulfide and then recharged with an equivalent volume of fresh substrate solution. This continued for another 24 days. The packing material was removed from Reactor III on Day 44.

3rd sequence. In order to keep the bacteria active, a weekly withdrawal/recharge was done during 35 days and the sulfide concentration was monitored as well. At the end of this period and during four consecutive days with no recharges, analyses of sulfide, sulfate, VSS and ORP/pH were performed.

4th sequence. After the above sequence, it was decided to leave the reactors without any withdrawal or recharge for 12 days, and start the fourth sequence with a new formulation of substrate as presented in Table 3.2.

The sequence started with the withdrawal of 1.5 L of reactor's contents for analyses of sulfide, sulfate, VSS and pH/ORP, and recharge with the same volume with new substrate formulation. After 6 days of adaptation to the new formulation, the procedure of withdrawal-analysis-recharge was performed again for each column every third or fifth day. This was continued until 5 analyses for sulfide and VSS per reactor were done.

Analyses for ammonia-nitrogen and soluble chemical oxygen demand were also performed in between to have a perspective of the conditions of the reactors with respect to nitrogen content and consumption of organic matter.

Once the sulfide concentration reached 100 mg/L in all reactors, the continuous flow mode was started with the medium, shown in Table 3.4, for the desired organic loading rate, OLR, and hydraulic retention time, HRT.

3.2.3 Experimental Procedure. Phase III

The objectives of this phase were:

1. Determine the effect of packing material in comparison with suspended growth of SRB in an Upflow Anaerobic Reactor.
2. Establish the effect of the hydraulic retention time on the maximum sulfide concentration in the reactor.

In order to evaluate these parameters, two 10 L parallel reactors, one packed and the other non-packed, were run under identical conditions, while a third reactor non-packed was operated at a lower hydraulic retention time. The reactors were operated for a period of 120 days

In all the three reactors, a substrate with lactate as the sole carbon source was fed. Table 3.4 shows the composition of this substrate in which the concentration of lactic acid was based on its theoretical oxygen demand, Th.O.D., and the hydraulic retention time tested.

The Th.O.D.:sulfate and Th.O.D.:N:P were always kept as 2:1 and 100:5:1, respectively. The value of Th.O.D. was maintained at the stoichiometric value to allow complete organic removal through sulfate reduction. The organic loading rate was varied between 6 and 12 kg/d/m³ under different operating conditions as shown in Table 3.5.

Sulfide production was used as indicator of the SRB activity, VSS as estimation of growth of biomass and pH and ORP were measured to control the reactors' performance. These analyses were performed both on the effluent and a sample from the first port, 300 mm above the bottom of the reactor, until a steady state condition had been reached in each reactor, which generally occurred within 8 to 10 times the HRT. When the system had become stable, similar analyses were carried out on samples from the second and third ports. For each new set of conditions, it was necessary to change tubing in the system. Pump calibration was checked (Appendix A) and fresh feed solutions were used.

Table 3.5 UA Reactors Operating Conditions for Phase III

Reactor Void volume (L)	Packing Material	Set 1 HRT (h)	Set 2 HRT (h)	Set 3 HRT (h)	Set 4 HRT (h)		Set 5 HRT (h)	
I 9.09 L	Poly-propylene Pall Rings	50	30	20	NA	NA	NA	NA
II 10.1 L	None	50	30	20	NA	10	12	7.5
III 10.1 L	None	30	20	10	5	NA	NA	NA
OLR kg/d/m ³		6	6	6	6	12	12	12

3.3 Analyses

The analyses performed were:

- a) Sulfide. Sulfide production was investigated for suitable representation of SRB activity. This parameter was measured over the entire length of the study. The concentration of H_2S in the biogas was not determined.
- b) Volatile Suspended Solids, VSS. It was assumed that the VSS represented the biomass present in the reactor.
- c) Sulfate. Sulfate was measured as an indicator of the total sulfate consumed in the sulfide production process.
- d) pH and ORP. These tests were used as an indicator of the reactor's environmental conditions and as a controller of the reactor performance.
- e) Dissolved COD. These tests were performed when the level of organic substrate had to be estimated. However, sulfate concentrations were used as an indicator of the dissolved COD used in the sulfide production process.
- f) SRB count. SRB count was performed to detect the presence of SRB and the relative growth in the reactor.

All analytical procedures were performed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1995).

3.3.1 Sulfide

In order to determine the total sulfide concentration in the liquid samples, the Iodometric Method (4500-S²⁻ F) was used. This method is based on the titration of sulfide, which is oxidized by iodine in acid solution (APHA, 1995). The basic reactions are as follows (Jeffery *et al.*, 1989):

This method suffers interference from reducing substances that react with iodine, including thiosulfate, sulfite and various organic compounds. In order to suppress these interferences and to concentrate sulfide, a sample pretreatment was done by the precipitation of sulfide as ZnS with zinc acetate solution in alkaline medium (pH above 9.0) according to the standard procedure 4500-S²⁻ C. The pretreated sample was filtrated and this precipitate was collected and dissolved in water, excess of iodine solution was added in acidic conditions and titrated with a standardized thiosulfate (Na₂S₂O₃) solution.

During this procedure, it is important to take samples with minimum aeration and analyze immediately. The use of fresh standardized solutions of iodine and thiosulfate is extremely important for the precision of the results. The 95% confidence level of this analysis was found to be $\pm 5\%$ of the mean value as shown in Appendix C.

3.3.2 Volatile Suspended Solids

VSS were analyzed according to the Standard Method 2540 D (APHA, 1995). A liquid sample was filtered through a glass fiber filter of 1.5 μm pore size, and the residue was dried to a constant mass at 103-105 $^{\circ}\text{C}$. The retained mass on the filter paper showed the suspended solids. This dried residue was then ignited at 550 $^{\circ}\text{C}$. The mass lost on ignition showed the volatile suspended solids. VSS were assumed to represent the biomass present in the reactor. The ratio of VSS to SS, on an average, was between 0.7 and 0.8. The 95% confidence level of this analysis was found to be $\pm 8\%$ of the mean value as shown in Appendix C.

3.3.3 Sulfate

Sulfate was analyzed according to the Standard Turbidimetric Method 4500-SO₄²⁻ E (APHA, 1995). The basic principle of the method was the precipitation of sulfate ion, SO₄²⁻, in an acetic acid medium with barium chloride, BaCl₂, forming barium sulfate crystals. The light absorbance of the resulting solution was measured by a Ratio/XR turbidimeter Hach Model 43900. In order to determine the SO₄²⁻ concentration, the NTU

turbidity reading was compared with a standard curve prepared previously for different concentrations of standard sulfate solutions. The standard curve (see Appendix A) was prepared within a 0 to 40 mg SO_4^{2-} /L in order to keep method's accuracy and the stability of BaSO_4 suspensions (APHA, 1995). Whenever the sulfate concentration in the liquid samples exceeded the range of the calibration curve, an appropriate dilution of samples was made.

3.3.4 pH and ORP

The oxidation-reduction potential was measured with an ORP meter, Orion Model 290A, with a combined electrode and a temperature probe. The reading was taken after 10 min or until the instrument had reached equilibrium. The ORP meter was calibrated using a solution of a known ORP value. The standard ORP solution consisted of 39.21g $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, 48.22 g $\text{Fe}(\text{NH}_4)(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, 56.2 mL of H_2SO_4 , dissolved in 1L of distilled water. The standard ORP of this solution is 467 mV (APHA, 1995) and the actual ORP value obtained was 466 mV.

The pH was measured with a VWR Scientific Products pH meter Model 8100, which was calibrated each week with buffer solutions of pH 7.0 and pH 10.0.

3.3.5 Soluble COD

In order to determine the soluble Chemical Oxygen Demand in the liquid samples, the Closed Reflux Colorimetric Method (5220 D) was used.

The soluble portion was obtained after filtering the liquid sample through a glass fiber filter. The chemical oxygen demand is considered to be a measure of the oxygen required for the chemical oxidation of organic matter. This is done by means of a strong chemical oxidant, in this case a mixture of chromic and sulfuric acid was used.

The oxygen consumed after digestion in reaction culture tubes, was measured at 600 nm with a spectrophotometer, Model Spectronic 20D+, from Spectronic Instruments. The absorbance reading was then compared with a standard curve. This standard curve was prepared from five potassium hydrogen phthalate standards with COD equivalents from 100 to 500 mg O₂/L.

As the liquid samples also contained sulfide, a correction was made to the observed COD value by using the stoichiometry of the sulfide oxidation and the sulfide concentration measured in the sample.

3.3.6 Bacterial Count

An Immunoassay Test Kit Rapid Check II supplied by Strategic Diagnostics Inc. Newark, Delaware USA was used for the SRB count. It used purified antibodies attached to small particles that captured the enzyme adenosine-5'-phosphosulfate (APS) reductase which is characteristic of all SRB strains. The results of the test are expressed as cells/mL. The analysis shows total cell count and does not differentiate between live and dead cells. Also, it does not account for cells that have ruptured.

The test results are ambient temperature dependent so that the proper procedure was followed depending on the ambient temperature during the analysis.

CHAPTER 4. DEVELOPMENT OF STOICHIOMETRIC AND KINETIC EQUATIONS

In order to analyze and interpret the data, the following stoichiometric and kinetic equations were developed for this study.

4.1 Stoichiometric Equations

McCarty *et al.* (1969) had derived the following equations for organic compounds oxidation, sulfate reduction and bacterial cells synthesis considering, $C_5H_7O_2N$ as its chemical formula

Oxidation Equation for Lactate (Electron Donor)

assuming that all alkalinity is expressed as HCO_3^- , since the pH was kept at or below 7.0 (Peavy *et al.*, 1985).

Reduction Equation for Sulfate (Electron Acceptor)

Equation for Cell Synthesis

For respiration, addition of Equations 4.1 and 4.2 gives:

For synthesis, addition of Equations 4.1 and 4.3 gives:

4.2 Kinetic Model Equations

For the continuous mode operation of three upflow anaerobic reactors, kinetic models were developed to obtain the rate of sulfide production under the operational conditions established in this study. Two types of reactors were considered: continuous (flow) stirred-tank, CSTR, and plug flow. Due to limited mixing observed in the experiments, only plug flow reactor models were developed as shown below. The calculations and assumptions for each model are explained in the following sections.

4.2.1 Kinetic Model I: Biomass Production Rate

The following equation was used for biomass production rate:

$$\frac{dX}{dt} = \mu X \quad \dots\dots\dots 4.6$$

Where,

dX/dt = biomass production rate [mg/h/L]

μ = specific growth rate [h^{-1}]

X = biomass concentration [mg/L]

As shown in Figure 4.1, a mass balance for biomass in a differential volume, ΔV , in the active reactor element was carried out as follows:

$$\begin{array}{ccccccc} \text{Rate of} & = & \text{Rate of} & - & \text{Rate of} & + & \text{Rate of} \\ \text{Accumulation} & & \text{Biomass Inflow} & & \text{Biomass Outflow} & & \text{Biomass Production} \end{array}$$

$$\text{or Rate of Accumulation} = QX - Q\left(X + \frac{\Delta X}{\Delta h} \Delta h\right) + \mu X \Delta V$$

Under steady state conditions, Rate of Accumulation = 0 and $X=X_s$

After dividing by $\Delta V = A \Delta h$,

$$0 = -\frac{Q}{A} \frac{\Delta X_s}{\Delta h} + \mu X_s$$

when $\Delta h \rightarrow 0$

$$\frac{Q}{A} \frac{dX_s}{dh} = \mu X_s$$

Integrating between active volume with depth L in which biomass concentration increased from X_o to X_s ,

$$\int_{X_o}^{X_s} \frac{dX_s}{X_s} = \frac{A}{Q} \mu \int_0^L dh$$

$$\ln X_s - \ln X_o = \mu \frac{A \cdot L}{Q} = \mu \frac{V_{active}}{Q} = \mu t_d$$

$$\text{or} \quad \ln X_s = \mu \left(\frac{V_{active}}{Q} \right) + \ln X_o \quad \dots\dots\dots 4.7$$

Where,

X_o = inlet biomass concentration [mg/L]

X_s = steady state biomass concentration [mg/L]

V_{active} = active reactor's volume [m^3]

Q = volumetric flow rate [m^3/h]

$$t_d = \text{hydraulic retention time in active volume [h]} = \frac{V_{active}}{Q}$$

For collection and interpretation of results, VSS values were assumed to represent biomass concentration in the reactors.

Figure 4.1 Mass Balance Lay-Out in a Differential Volume Element

4.2.2 Kinetic Model II: Sulfide Production Rate

The following equation was used for sulfide production rate:

$$\frac{dC}{dt} = K C \quad \text{.....4.8}$$

Where,

dC/dt = sulfide production rate [mg/h/L]

K = rate constant [h^{-1}]

C = sulfide concentration [mg/L]

A mass balance for sulfide in a differential volume, ΔV , in the active reactor element was carried out as follows:

$$\begin{array}{ccccccc} \text{Rate of} & = & \text{Rate of} & - & \text{Rate of} & + & \text{Rate of} \\ \text{Accumulation} & & \text{Sulfide Inflow} & & \text{Sulfide Outflow} & & \text{Sulfide Production} \end{array}$$

$$\text{or Rate of Accumulation} = QC - Q(C + \Delta C) + KC \Delta V$$

Under steady state conditions, Rate of Accumulation = 0 and $C = C_s$

After dividing by $\Delta V = A \Delta h$,

$$0 = -\frac{Q}{A} \frac{\Delta C_s}{\Delta h} + KC_s$$

When $\Delta h \rightarrow 0$

$$0 = -\frac{Q}{A} \frac{dC_s}{dh} + KC_s$$

$$\frac{Q}{A} \frac{dC_s}{dh} = KC_s$$

Integrating between active volume with depth L in which sulfide concentration increased from C_o to C_s ,

$$\int_{C_o}^{C_s} \frac{dC_s}{C_s} = K \frac{A}{Q} \int_0^L dh$$

$$\ln[C_s] - \ln[C_o] = K \frac{A * L}{Q} = Kt_d$$

or

$$\ln C_s = K\left(\frac{V_{\text{active}}}{Q}\right) + \ln C_o \quad \text{.....4.9}$$

Where,

C_o = inlet sulfide concentration [mg/L]

C_s = steady state sulfide concentration [mg/L]

4.2.3 Kinetic Model III: Sulfide and Biomass Concentrations

The following equation was used for sulfide production based on sulfide and biomass concentrations:

$$\frac{dC}{dt} = K' C X \quad \dots\dots\dots 4.10$$

Where,

dC/dt = sulfide production rate [mg/h/L]

K' = rate constant [L/(mg*h)]

C = sulfide concentration [mg/L]

X = biomass concentration [mg/L]

A mass balance for sulfide in a differential volume, ΔV , in the active reactor element was carried out as follows

Rate of Accumulation	Rate of Sulfide Inflow	-	Rate of Sulfide Outflow	+	Rate of Sulfide Production
Rate of Accumulation	QC	-	$Q(C + \Delta C)$	+	$K' C X \Delta V$

Under steady state conditions Rate of Accumulation = 0, $X = X_s$ and $C = C_s$

After dividing by $\Delta V = A \Delta h$,

$$0 = -\frac{Q}{A} \frac{\Delta C_s}{\Delta h} + K' C_s X_s$$

When $\Delta h \rightarrow 0$

$$\frac{Q}{A} \frac{dC_s}{dh} = K' C_s X_s$$

Integrating between active volume with depth L in which sulfide concentration increased from C_o to C_s ,

$$\int_{C_o}^{C_s} \frac{dC_s}{C_s} = K' \bar{X}_s \frac{A}{Q} \int_0^L dh$$

$$\ln[C_s] - \ln[C_o] = K' \bar{X}_s \frac{A * L}{Q} = K' \bar{X}_s t_d$$

or $\ln C_s = K' \bar{X}_s \left(\frac{V_{\text{active}}}{Q} \right) + \ln C_o$ 4.11

Where,

C_o = initial sulfide concentration [mg/L]

$\bar{X}_s$ = average biomass concentration [mg/L]

4.3 Biomass Characterization

The SRB biomass produced in the reactor has been characterized in terms of its concentration and washout-out velocity.

4.3.1 Volatile Suspended Solids

It is assumed that the volatile suspended solids, VSS, indicated the biomass concentration present at a specific point in the reactor.

4.3.2 Washout Velocity

The upflow velocity in the reactor is given by:

$$V_o = \frac{Q}{A} \quad \text{.....4.12}$$

Where,

V_o = upflow velocity [m/h]

Q = volumetric flow rate [m^3/h]

A = cross sectional area [m^2]

It is assumed that whenever the upflow velocity, V_o , exceeded the settling velocity of the biomass, it was washed out of the reactor. On the other hand, whenever V_o was less than

the settling velocity of the biomass, it had gradually settled down. In case of the non-packed bed, the biomass had settled near the bottom of the reactor, whereas, in case of the packed bed, the settling occurred on the media surface and near the bottom of the reactor.

CHAPTER 5. RESULTS

5.1 Phase I

2 L reactors were operated in batch mode for SRB growth. After the first 48 days of cultivating bacteria and controlling their growth, a sulfide concentration of 120-130 mg/L was reached. Although there were fluctuations in this concentration in the next 20 days, in general the steady state was observed.

The sulfide concentration was considered to be the main indicator of SRB activity in the three batch reactors. After obtaining the optimum sulfide concentration, a set of other three parallel reactors was inoculated with this culture. The dilution water used for substrate preparation was deionized water for Reactors I and II and tap water for Reactor III. Tap water is known to contain residual chlorine, and if it is not removed by means of boiling or overnight evaporation, it can affect the bacterial growth. This was observed during the first 10 days of Phase I, when the concentration of sulfide in Reactor III was lower than in the other two reactors. Consequently, the tap water was boiled for 10 min, cooled overnight and then used for the preparation of new substrate. Each time a sample was withdrawn, it was replaced with a new substrate solution of equal volume.

Figure 5.1 shows the changes in sulfide concentration with time for the three 2 L reactors. The ORP and pH values over the same time are shown in Figures 5.2 and 5.3.

Figure 5.1 Sulfide Concentration vs Time (2 L Batch Reactors)

Figure 5.2 ORP vs Time (2 L Batch Reactors)

Figure 5.3 pH vs Time (2 L Batch Reactors)

The sulfide concentrations are the average values between two measurements. No VSS analyses were done during Phase I. However, as reported in Section 3.1.2.2, the settled biomass from Reactor II, which was used as the seed for Phase II, had a VSS concentration of 4300 mg/L and SS concentration of 5700 mg/L.

5.2 Phase II

The 10 L reactors in this phase were operated in a semi-continuous, draw-and-fill, manner and were fed with lactic acid as the sole carbon source. The main objective of this phase was to study the SRB growth under batch conditions and to determine the stoichiometry for the overall sulfates reduction. It was necessary to build up a healthy

culture and high concentration of SRB in the reactors before starting the next phase of the study. The Phase II study was performed in the following sequences:

1st Sequence and 2nd Sequence

As indicated in Chapter 3, in the first sequence the reactors were inoculated with a healthy SRB culture, and fed with new substrate solution once every eight days, while in the second sequence the same procedure was followed once every four days. The change in sulfide concentration is shown in Figure 5.4.

During batch operation, the packed reactor presented an overall better performance. In case of the third reactor, its performance was fairly similar to the other non-packed reactor once the packing material was removed. Although the progressions of sulfide concentration for the packed and the non-packed reactors were slightly different, it can be observed that the final concentration reached was roughly the same. Therefore, no significant difference in the behavior of the three reactors was considered.

The ORP and pH values, Figures 5.5 and 5.6, remained within the optimum ranges of -300 and -350 mV, and 6.5-7.0, respectively.

3rd Sequence

In order to keep the bacteria active in the reactors, a weekly withdrawal-fill operation was done during the next 35 days and the sulfide concentration was monitored during that

period. After that, analyses for sulfide, sulfate, VSS, ORP and pH were carried out for four consecutive days without refilling in between.

The sulfide progression for the 3rd and 4th sequences is presented in Figure 5.7. Similarly, VSS and pH results are plotted in Figures 5.8 and 5.9, respectively. It is evident that the sulfide concentration throughout that period remained fairly constant in each reactor. The highest sulfide concentration was found in Reactor I (packed bed), although its volatile suspended solids concentration was the lowest. This reflects that, in the packed reactor, the VSS remained low in the samples withdrawn because of the settling of solids on the packing material.

Figure 5.4 Sulfide Concentration vs Time (10 L Batch Reactors During 1st and 2nd Sequences)

Figure 5.5 ORP vs Time (10 L Batch Reactors During 1st and 2nd Sequences)

Figure 5.6 pH vs Time (10 L Batch Reactors During 1st and 2nd Sequences)

Figure 5.7 Sulfide Concentration vs Time (10 L Batch Reactors During 3rd and 4th Sequences)

Figure 5.8 VSS Concentration vs Time (10 L Batch Reactors During 3rd and 4th Sequences)

Figure 5.9 pH vs Time (10 L Batch Reactors During 3rd and 4th Sequences)

The sulfide, VSS, ORP and pH progressions over four consecutive days are presented in Figures 5.10, 5.11, 5.12 and 5.13, respectively. No significant changes were observed. These observations on each reactor, without filling in between, gave a perspective of the fluctuations in sulfide and VSS concentrations and the confidence in reproducing data.

4th Sequence

The fourth and last sequence of batch operation was carried out with a new substrate formulation to establish the relationship between sulfide production and VSS concentration. The three reactors were used and the consecutive analyses were done at 3 to 5 days interval in order to define the sulfide and VSS relationship and the trends along the reactors' height. Each reactor was provided with sampling ports equally spaced at

Figure 5.10 Sulfide Concentration vs Time (10 L Batch Reactors Consecutive)

Figure 5.11 VSS Concentration vs Time (10 L Batch Reactors Consecutive)

Figure 5.12 ORP vs Time (10 L Batch Reactors Consecutive)

Figure 5.13 pH vs Time (10 L Batch Reactors Consecutive)

300, 600, 900 and 1200 mm from the bottom of the reactor. The samples for these consecutive analyses were collected from the first (300 mm), second (600 mm) and third (900 mm) ports. The results and mass balance calculations are shown in Table 5.1.

In the mass balance calculations, the influence of the activity of the biomass settled at the bottom of the reactors was neglected since both sulfide and VSS concentrations in suspension at different ports were considered. This assumption was justified based on a separate study in which it was observed that the settling velocity of biomass in a batch SRB culture was very low and, therefore, most of the biomass produced in suspension remained in suspension during 3-5 day period used in the mass balance calculations.

The VSS and sulfide concentrations are the average values between two measurements. The sulfide concentrations were determined after the pretreatment of samples with zinc acetate solution in order to concentrate the sulfide and remove organic substances that could cause interferences. It was found that these sulfide concentration values were 85-90% of the values obtained without pretreatment. Since for each analysis, 0.5 L of sample was withdrawn per port (1.5 L total) and this resulted in wasting a certain amount of sulfide, the sulfide mass balance was based in the following equation:

$$\text{Mass of Sulfide Produced} = \sum V_i S_{\text{final}} - \left[\left(\sum_{n=1}^3 (V_i - 0.5) * S_{\text{initial}} \right) + 1.5 S_{\text{feed}} \right] \quad \dots\dots 5.1$$

Where,

V_i = initial reactor volume [L]

S_{initial} = initial sulfide concentration [mg/L]

S_{final} = final sulfide concentration [mg/L]

S_{feed} = feed sulfide concentration = 0 (assumed). [mg/L]

The same approach was taken for the VSS mass balance and the following equation was used:

$$\text{Mass of Sulfide Produced} = \sum V_i VSS_{\text{final}} - \left[\left(\sum_{n=1}^3 (V_i - 0.5) * VSS_{\text{initial}} \right) + 1.5 VSS_{\text{feed}} \right] \quad \text{.....5.2}$$

Where,

VSS_{initial} = initial VSS concentration [mg/L]

VSS_{final} = final VSS concentration [mg/L]

VSS_{feed} = feed VSS concentration = 0 (assumed). [mg/L]

The mass balance analysis and the average corresponding values for VSS and sulfide concentrations are shown in Table 5.1 and Figures 5.14, 5.15 and 5.16 for the three reactors. These average values and stoichiometric Equations 4.1 to 4.5 were then used for mass and electron balance calculations in order to determine the proportion in which the sulfate reduction and biosynthesis were taking place. Tables 5.2, 5.3 and 5.4 show mass and electron balances for VSS growth and sulfide production. From these calculations, it is found that about 70 % of the organic matter (lactate) was bio-oxidized and 30 % was bio-synthesized for Reactors II and III, both non-packed. Since samples collected from Reactor I (packed) for VSS did not include settled solids, these calculations were not considered to be representative.

Table 5.1 Sulfide and VSS Mass Production for 10 L Batch Reactors along Reactor Height (New Formulation)

Reactor I	pH	ORP	T (°C)	Sulfide mg S/L	Volume° L	Mass mg	VSS mg/L	Volume° L	Initial mass mg
Day	Port								
114	1 st	-355	20	154	2.45	376	13	2.45	32.3
	2 nd	-360	20	155	2.44	377	15	2.44	36.4
	3 rd	-353	20	152	2.70	411	12	2.70	32.7
				154*		1165**	13*		101**
117	1 st	-355	20	175	2.45	515	16	2.45	47.1
	2 nd	-354	20	176	2.44	518	17	2.44	50.6
	3 rd	-349	20	173	2.70	555	14	2.70	43.5
				175*		1588**	16*		141**
For Δt=3d	S produced (mg)	= 1588 - 1165 = 423				VSS produced (mg) = 141 - 101 = 40			
117				175	2.45	428	16	2.45	39.1
				176	2.44	430	17	2.44	42.0
				173	2.70	468	14	2.70	36.7
						1326**			118**

Table 5.1 Continued

Reactor I	Day	Port	pH	ORP	T (°C)	Sulfide mg S/L	Volume* L	Mass mg	VSS mg/L	Volume* L	Initial mass mg
								Final mass			Final mass
122		1 st	5.6	-343	22	185	2.45	545	18	2.45	52.0
		2 nd	5.6	-343	22	187	2.44	551	20	2.44	58.0
		3 rd	5.5	-339	22	184	2.70	589	15	2.70	49.3
						185*		1684**	18*		159**
For Δt=5d		S produced	(mg) = 1684 - 1326 = 359								
		Soluble COD	(mg O ₂ /L) = 6300								
								Initial mass			
122						185	2.45	452	18	2.45	43.2
						187	2.44	457	20	2.44	48.1
						184	2.70	497	15	2.70	41.6
								1406**			133**
								Final mass			Final mass
125		1 st	5.8	-345	20	202	2.45	595	25	2.45	73.6
		2 nd	5.9	-349	20	203	2.44	597	25	2.44	72.0
		3 rd	6.0	-351	20	200	2.70	639	26	2.70	83.2
						202*		1832**	25*		229**
For Δt=3d		S produced	(mg) = 1832 - 1406 = 426								
								VSS produced	(mg) = 229 - 133 = 96		

Table 5.1 Continued

Reactor 1	pH	ORP	T	Sulfide	Volume ^c	Mass	VSS	Volume ^c	Initial mass
Day	Port		(°C)	mg S/L	L	mg	mg/L	L	mg
125						Initial mass			Initial mass
				202	2.45	494	25	2.45	61.1
				203	2.44	495	25	2.44	59.8
				200	2.70	539	26	2.70	70.2
						1529**			191**
128	1 st	-356	20	209	2.45	616	29	2.45	86.6
	2 nd	-356	21	209	2.44	613	30	2.44	88.2
	3 rd	-355	21	208	2.70	667	28	2.70	90.9
				209*		1895**	29*		266**
For $\Delta t=3d$	S produced (mg)= 1895 - 1529 = 366					VSS produced (mg) = 266 - 191 = 75			

• Reduced volume due to sample withdrawal

* Average of 3 values

** Total of 3 values

Table 5.1 Continued

Reactor II		pH	ORP	T	Sulfide	Volume*	Mass	VSS	Volume*	Initial mass
Day	Port			(°C)	mg S/L	L	mg	mg/L	L	mg
114	1 st	6.3	-347	20	105	2.45	257	30	2.45	74.3
	2 nd	6.3	-349	20	105	2.44	257	40	2.44	97.6
	3 rd	6.3	-345	20	102	2.70	276	34	2.70	92.9
					104*		789**	35*		265**
117	1 st	6.2	-339	20	101	2.45	298	44	2.45	130.8
	2 nd	6.1	-343	20	110	2.44	323	46	2.44	135.2
	3 rd	6.2	-337	20	118	2.70	378	48	2.70	153.6
					110*		999**	46*		420**
For $\Delta t=3d$	S produced (mg) = 999 - 789 = 210							VSS produced (mg) = 420 - 265 = 155		
117					101	2.45	248	44	2.45	108.6
					110	2.44	268	46	2.44	112.2
					118	2.70	319	48	2.70	129.6
							835**			350**

Table 5.1 Continued

Reactor II	Port	pH	ORP	T (°C)	Sulfide mg S/L	Volume ^o L	Mass mg	VSS mg/L	Volume ^o L	Initial mass mg
							Final mass			Final mass
122	1 st	6.0	-336	20	121	2.45	356	64	2.45	189.7
	2 nd	6.0	-339	20	123	2.44	362	56	2.44	164.6
	3 rd	6.0	-333	20	124	2.70	395	63	2.70	202.2
					123*		1113**	61*		557**
For Δt=5d	S produced (mg)= 1113 - 835 = 278							VSS produced (mg) = 557 - 350 = 207		
	Soluble COD (mg O ₂ /L) = 6400							NH ₃ as N (mg/L)= 642		
							Initial mass			
122					121	2.45	296	64	2.45	157.5
					123	2.44	300	56	2.44	136.6
					124	2.70	333	63	2.70	170.6
							929**			465**
							Final mass			Final mass
125	1 st	6.1	-350	20	153	2.45	452	76	2.45	224.7
	2 nd	6.1	-353	20	151	2.44	443	75	2.44	221.7
	3 rd	6.1	-357	20	151	2.70	482	74	2.70	236.8
					152*		1377**	75*		683**
For Δt=3d	S produced (mg) = 1377 - 929 = 448							VSS produced (mg) = 683 - 465 = 218		

Table 5.1 Continued

Reactor II	pH	ORP	T	Sulfide	Volume*	Mass	VSS	Volume*	Initial mass
Day	Port		(°C)	mg S/L	L	mg	mg/L	L	mg
125						Initial mass			Initial mass
				153	2.45	375	76	2.45	186.6
				151	2.44	368	75	2.44	184.0
				151	2.70	407	74	2.70	199.8
						1150**			570**
128	1 st	6.0	20	160	2.45	471	86	2.45	Final mass
	2 nd	6.0	20	158	2.44	464	84	2.44	253.2
	3 rd	6.1	20	158	2.70	505	82	2.70	249.0
				159*		1440**	84*		262.5
For Δt=3d	S produced (mg)= 1440 - 1150 = 290						VSS produced (mg) = 765 - 570 = 195		765**

• Reduced volume due to sample withdrawal

* Average of 3 values

** Total of 3 values

Table 5.1 Continued

Reactor III	pH	ORP	T (°C)	Sulfide mg S/L	Volume* L	Mass mg	VSS mg/L	Volume* L	Initial mass mg
Day	Port								
114	1 st	5.9	-348	21	106	260	61	2.45	149.1
	2 nd	6.0	-351	21	103	251	91	2.44	222.0
	3 rd	6.0	-345	21	105	283	64	2.70	171.5
				105*		793**	72*		543**
						Final mass			Final mass
117	1 st	5.9	-345	20	121	356	91	2.45	268.6
	2 nd	5.9	-342	20	104	305	80	2.44	235.2
	3 rd	5.9	-339	20	79	253	81	2.70	258.6
				112*		915**	84*		762**
For $\Delta t=3d$	S produced (mg)= 915 - 793 = 122						VSS produced (mg)= 762-543 = 219		
117				121	2.45	296	91	2.45	223.0
				104	2.44	253	80	2.44	195.2
				79	2.70	214	81	2.70	218.2
						763**			636**

Table 5.1 Continued

Reactor III		pH	ORP	T	Sulfide	Volume*	Mass	VSS	Volume*	Initial mass
Day	Port			(°C)	mg S/L	L	mg	mg/L	L	mg
							Final mass			Final mass
122	1 st	5.9	-353	20	131	2.45	385	89	2.45	262.1
	2 nd	5.9	-352	20	125	2.44	366	90	2.44	264.6
	3 rd	5.9	-347	20	133	2.70	426	92	2.70	294.4
					130*		1178**	90*		821**
For Δt=5d	S produced (mg) = 1178 – 763 = 415							VSS produced (mg)= 821 – 636 = 185		
	Soluble COD (mg O ₂ /L) = 7700							NH ₃ as N (mg/L) = 666		
							Initial mass			
122					131	2.45	320	89	2.45	217.6
					125	2.44	304	90	2.44	219.6
					133	2.70	360	92	2.70	248.4
							983**			685.6**
							Final mass			Final mass
125	1 st	6.1	-351	20	153	2.45	449	107	2.45	315.1
	2 nd	6.1	-351	20	149	2.44	438	105	2.44	308.4
	3 rd	5.9	-347	20	148	2.70	474	99	2.70	315.5
					150*		1360**	104*		939**
For Δt=3d	S produced (mg) = 1360 – 983 = 377							VSS produced (mg)= 939- 686= 253		

Table 5.1 Continued

Reactor III	pH	ORP	T (°C)	Sulfide mg S/L	Volume ^o L	Mass mg	VSS mg/L	Volume ^o L	Initial mass mg
Day	Port								
125				153	2.45	373	107	2.45	261.6
				149	2.44	363	105	2.44	256.0
				148	2.70	400	99	2.70	266.2
						1136**			784**
128	1 st	-357	20	157	2.45	462	116	2.45	340.7
	2 nd	-353	20	155	2.44	457	113	2.44	333.4
	3 rd	-355	20	154	2.70	493	108	2.70	346.2
				155*		1412**	112*		1020**
For Δt=3d	S produced (mg)	= 1412 - 1136 = 276			VSS produced (mg) = 1020 - 784 = 236				

• Reduced volume due to sample withdrawal

* Average of 3 values

** Total of 3 values

Figure 5.14 Sulfide vs VSS Concentrations for Reactor I (packed). 4th Sequence

$$\text{Sulfide} = 3.07 \text{ VSS} + 123 \quad R^2 = 0.888$$

Figure 5.15 Sulfide vs VSS Concentrations for Reactor II (non-packed). 4th Sequence

$$\text{Sulfide} = 1.20 \text{ VSS} + 57.3 \quad R^2 = 0.956$$

Figure 5.16 Sulfide vs VSS Concentrations for Reactor III (non-packed). 4th sequence

$$\text{Sulfide} = 1.37 \text{ VSS} + 3.27 \quad R^2 = 0.962$$

Figure 5.17 Sulfide vs VSS Concentrations for Non-Packed Reactors II and III

TABLE 5.2 Mass and Electron Balances under Batch Mode for Reactor I (packed)

REACTOR I		Mass Balance										Electron Balance							
Average concentrations		Considering all VSS as bacterial cells, C ₅ H ₇ O ₂ N, mm 113										mm=32				mm=89			
		Volatile Suspended Solids						Sulfide				Lactate				e moles			
Volume	Time	Delta	Concen- tration	mass	Mass discarded	Delta	Delta	Concen- tration	mass	Mass discarded	Delta	Delta	Delta	Delta	Synthesis moles	Reduction moles	Oxidation moles		
L	d	t	mg/L	mg	mg	mass	mol	mg/L	mg	mg	mass	mol	mass	mol	%	%	%		
9.09	0		13	120				154	1398										
		3			20	45	0.400			231	423	13	844	9	8	106	114		
9.09	3		16	145				175	1590							7	93		
		3			24	39	0.347			262	354	11	708	8	7	88	95		
9.09	6		18	161				185	1682							7	93		
		3			27	93	0.824			278	432	14	923	10	16	108	124		
9.09	9		25	227				202	1836							13	87		
		3			38	74	1			303	367	11	777	9	13	92	105		
9.09	12		29	264				209	1900							12	88		
Average %																10	90		

TABLE 5.3 Mass and Electron Balances under Batch Mode for Reactor II (non-packed)

REACTOR II		Mass Balance										Electron Balance					
Average concentrations		Considering all VSS as bacterial cells (C.H.O.N: mm 11)										mm=89					
		Volatile Suspended Solids						Sulfide				Lactate		e moles			
Volume	Time	Delta	Concen- tration	mass	Mass discarded	Delta	Delta	Concen- tration	mass	Mass discarded	Delta	Delta	Delta	Synthesis moles	Reduction moles	Oxidation moles	
L	d	t	mg/L	mg	mg	mass	mol	mg/L	mg	mg	mass	mol	mol	mol	%	%	
10.1	0		35	353				104	1052								
		3			52	164	1		156	213	7	610	7	29	53	82	
10.1	3		46	465				110	1109					35	65		
		3			69	221	2		165	293	9	833	9	39	73	112	
10.1	6		61	616				123	1237					35	65		
		3			92	233	2		184	482	15	1199	13	41	120	162	
10.1	9		75	758				152	1535					26	74		
		3			113	203	2		228	294	9	811	9	36	73	109	
10.1	12		84	848				159	1601					33	67		
Average %														32	68		

TABLE 5.4 Mass and Electron Balances under Batch Mode for Reactor III (non-packed)

REACTOR III		Mass Balance										Electron Balance			
Average concentrations		Considering all VSS as bacterial cells, C ₅ H ₇ O ₂ N, mm 113										mm=89			
		Volatile Suspended Solids										Sulfide			
				Delta	Concen- tration mg/L	mass mg	Mass discarded mg	Delta	mass	Concen- tration mg/L	mass mg	Mass discarded mg	Delta	mass	mol
Volume	Time	Delta	Concen- tration mg/L	mass mg	Mass discarded mg	Delta	mol	Delta	mass	Concen- tration mg/L	mass mg	Delta	mass	mol	e moles
L	d	t													
10.1	0		72	726		105	1058								
		3		108	231	2		157	230	7	730	8	41	58	98
10.1	3		84	848		112	1131							41	59
		3		126	187	2		168	350	11	894	10	33	87	120
10.1	6		90	909		130	1313							27	73
		3		135	276	2		195	397	12	1099	12	49	99	148
10.1	9		104	1050		150	1515							33	67
		3		156	237	2		225	276	9	822	9	42	69	111
10.1	12		112	1131		155	1566							38	62
Average %															65

5.3 Phase III

The effect of packing material on the maximum sulfide production, used as an indicator of bacterial growth and performance of the reactor, was studied. Simultaneously, the effect of hydraulic retention time on the overall performance of the reactor was evaluated.

The first reactor contained packing material while the second and third had none. Seven different hydraulic retention times were tested: 50, 30, 20, 12, 10, 7.5 and 5 h. Two organic loading rates, 6 kg/d/m³ and 12 kg/d/m³, were also tested.

5.3.1 Set 1

Reactor I (packed). HRT= 50 h.

Reactor II (non-packed). HRT= 50 h.

Reactor III (non-packed). HRT= 30 h.

The reactors were operated for 28 days under these conditions and the organic loading rate was kept at 6 kg/d/m³ for the three reactors. Figures 5.18 and 5.19 show the data collected for sulfide concentrations in the effluent and first port, respectively. The steady state was reached at about 8 times the hydraulic retention time tested. Simultaneous data for the range and average of steady state VSS concentrations in effluent and first port are shown in Table 5.5. The average was calculated from the last three steady state values.

Figure 5.18 Effluent Sulfide Concentration vs Time During Continuous Operation (Set 1)

Figure 5.19 First Port Sulfide Concentration vs Time During Continuous Operation (Set 1)

Table 5.5 Range and Average of Steady State VSS Concentrations (Set 1)

Reactor	HRT (h)	Range of Steady State Concentrations		Average of Steady State Concentrations	
		Effluent VSS (mg/L)	First Port VSS (mg/L)	Effluent VSS (mg/L)	First Port VSS (mg/L)
Reactor I (packed)	50	49-80	58-90	63	81
Reactor II (non-packed)	50	41-91	50-89	62	65
Reactor III (non-packed)	30	15-41	18-58	25	42

The steady state effluent sulfide concentration with 50 h of HRT fluctuated between 130 and 150 mg/L. Similarly, the steady state sulfide concentrations near the bottom ranged between 120 and 150 mg/L. Generally, the sulfide concentrations in the packed reactor were slightly higher than in the non-packed reactor when operated at the same HRT. Also, no significant difference in effluent and first port concentrations of sulfide or VSS was observed, so concentrations can be assumed to be uniform along the reactor's height. The pH average was around 6.0 and the ORP ranged between -310 and -320 mV.

For the packed reactor, the steady state VSS concentration in the effluent with 50 h of hydraulic retention time was about 63 mg/L. This was slightly lower than 81 mg/L observed at the first port, 300 mm above the bottom of the reactor. The concentrations of VSS both in the effluent and the first port of the three reactors are not as high as it would be expected for the concentration of sulfide present. Therefore, it is inferred that major bacterial activity was taking place at the bottom of the reactor, within the first 300 mm,

where the biomass had settled down. The washout of VSS was similar both for the packed reactor and for the non-packed reactor at 50 h of HRT.

The comparison of the results at two different HRTs, 50 h and 30 h, while operating the reactors under the same organic loading rate and without packing material, shows that the effluent sulfide concentration with 30 h HRT had gone up to an average of 180 mg/L versus 135 mg/L with 50 h HRT, once the steady state had been reached. Also, with 30 h of HRT, the steady state VSS in the effluent was 25 mg/L versus 62 mg/L with 50 h of HRT. Again, this concentration was similar to the concentration at the first port of the reactor. It became evident that most of the biomass was active near the bottom, below 300 mm, and there was little washout of solids at 30 h of HRT.

5.3.2 Set 2

Reactor I (packed). HRT= 30 h.

Reactor II (non-packed). HRT= 30 h.

Reactor III (non-packed). HRT= 20 h.

The reactors were operated for 20 days under these conditions and the organic loading rate was kept at 6 kg/d/m³ for the three reactors. Figures 5.20 and 5.21 show the data collected for sulfide concentrations in the effluent and first port, respectively. The steady state was reached at about 8 times the hydraulic retention time tested. Simultaneous data for the range and average of steady state VSS concentrations in effluent and first port are shown in Table 5.6. The average was calculated from the last three steady state values.

Figure 5.20 Effluent Sulfide Concentration vs Time During Continuous Operation (Set 2)

Figure 5.21 First Port Sulfide Concentration vs Time During Continuous Operation (Set 2)

Table 5.6 Range and Average of Steady State VSS Concentrations (Set 2)

Reactor	HRT (h)	Range of Steady State Concentrations		Average of Steady State Concentrations	
		Effluent VSS (mg/L)	First Port VSS (mg/L)	Effluent VSS (mg/L)	First Port VSS (mg/L)
Reactor I (packed)	30	29-41	29-48	34	38
Reactor II (non-packed)	30	7-17	15-42	13	39
Reactor III (non-packed)	20	13-22	20-36	18	30

The steady state sulfide concentrations for the reactors, packed and non-packed, operated at 30 h of HRT ranged between 180-190 mg/L and the concentrations at the effluent and first port were fairly close. The steady state VSS concentration was 34 mg/L for the packed reactor at 30 h HRT and 13 mg/L for the non-packed reactor at the same hydraulic retention time of 30 h. The pH varied between 6.0 and 7.0 and the ORP ranged between -340 and -390 mV. Thus, the behavior of the two reactors is considered to be similar between them and also to Reactor III of Set 1 operated under the same conditions.

The non-packed reactor operating at 20 h of HRT showed a steady state sulfide concentration of 160 mg/L for the effluent sample while it was 120 mg/L for the first port. The corresponding steady state VSS concentrations for the effluent and first port were 18 mg/L and 30 mg/L, respectively. Therefore, the differences between the effluent and first port, both for sulfide and VSS concentrations, had become more significant with an increase in the upflow velocity. Although the sulfide concentrations had decreased at this higher flow rate and lower hydraulic retention time, the performance of the reactor

was acceptable and the washout of biomass was low. Further experiments were conducted at lower HRT in order to find out the critical flow rate at which the biomass would be washed out from the reactor.

5.3.3 Set 3

Reactor I (packed). HRT= 20 h.

Reactor II (non-packed). HRT= 20 h.

Reactor III (non-packed). HRT= 10 h.

The reactors were operated for 13 days under these conditions and the organic loading rate was kept at 6 kg/d/m^3 for the three reactors. Figures 5.22 and 5.23 show the data collected for sulfide concentrations in the effluent and first port, respectively. The steady state was reached at about 5 times the hydraulic retention time tested. Simultaneous data for the range and average of steady state VSS concentrations in effluent and first port are shown in Table 5.7. The average was calculated from the last three steady state values.

The non-packed reactor operating at 20 h of HRT showed a steady state sulfide concentration of 182 mg/L for the effluent sample while it was 160 mg/L for the first port. The pH and ORP were about 7.0 and -380 mV, respectively. The washout of VSS remained fairly low, 10-20 mg/L, both for the packed reactor and the non-packed reactor. The results confirmed the similar behavior of the packed and the non-packed reactors.

Figure 5.22 Effluent Sulfide Concentration vs Time During Continuous Operation (Set 3)

Figure 5.23 First Port Sulfide Concentration vs Time During Continuous Operation (Set 3)

Table 5.7 Range and Average of Steady State VSS Concentrations (Set 3)

Reactor	HRT (h)	Range of Steady State Concentrations		Average of Steady State Concentrations	
		Effluent VSS (mg/L)	First Port VSS (mg/L)	Effluent VSS (mg/L)	First Port VSS (mg/L)
Reactor I (packed)	20	16-24	15-26	16	25
Reactor II (non-packed)	20	10-16	16-36	11	30
Reactor III (non-packed)	10	16-23	11-41	18	17

The comparison of the results at two different HRTs, 20 and 10 h, while operating the reactors under the same conditions, shows that the steady state effluent sulfide concentration with 10 h HRT had gone down to 74 mg/L, which represents less than half of the steady state effluent sulfide concentration with 20 h HRT, 182 mg/L. However, the effluent VSS concentrations between these two hydraulic retention times were not significantly different

5.3.4 Set 4

Reactor II (non-packed). HRT= 10 h (OLR= 12 kg/d/m³)

Reactor III (non-packed). HRT= 5 h (OLR= 6 kg/d/m³)

In order to accomplish the objective of finding the critical flow rate at which the bacteria would be washed out from the anaerobic reactor, only two non-packed reactors were operated for 10 days under the above conditions, because the packing material did not represent a significant advantage in the performance of the reactors. Figures 5.24 and

5.25 show the data collected for sulfide concentrations in the effluent and first port, respectively. Simultaneous data for the range and average of steady state VSS concentrations in effluent and first port are shown in Table 5.8. The average was calculated from the last three steady state values.

The steady state effluent sulfide concentration of 165 mg/L observed with 10 h HRT and 12 kg/d/m³ OLR was nearly twice the steady state effluent sulfide concentration of 74 mg/L observed with 10 h HRT and 6 kg/d/m³ OLR.

The flow rate for 10 h HRT was found to be critical for the sulfide production up to the first port, where the steady state sulfide concentrations remained between 50-60 mg/L, for both organic loading rates. When the HRT was reduced to 5 h, a complete washout of the bacteria took place in the Reactor III within 48 hours and therefore its operation was stopped.

The difference in sulfide or VSS concentrations at different reactor's height was more evident for the reactor with a HRT of 10 h and OLR of 12 kg/d/m³. The steady state sulfide concentration in the effluent was 165 mg/L versus 53 mg/L in the first port. Similarly, the steady state VSS concentrations were not uniform between the two sampling points

Figure 5.24 Effluent Sulfide Concentration vs Time During Continuous Operation (Set 4)

Figure 5.25 First Port Sulfide Concentration vs Time During Continuous Operation (Set 4)

Table 5.8 Range and Average of Steady State VSS Concentrations (Set 4)

Reactor	HRT (h)	Range of Steady State Concentrations		Average of Steady State Concentrations	
		Effluent VSS (mg/L)	First Port VSS (mg/L)	Effluent VSS (mg/L)	First Port VSS (mg/L)
Reactor II (non-packed)	10	29-30	30-65	29	54
Reactor III (non-packed)	5	NA*	NA*	NA*	NA*

*Steady state condition was not reached due to continuous washout of biomass

5.3.5 Set 5

Reactor II (non-packed). HRT= 12 h; OLR 12 kg/d/m³.

Reactor II (non-packed). HRT= 7.5 h; OLR 12 kg/d/m³.

In order to confirm the previous findings, it was decided to test 12 h and 7.5 h of HRT at 12 kg/d/m³. This set of conditions was carried out in Reactor II (non-packed) since Reactor III (non-packed) had low biomass concentration as a consequence of its washout at 5 h of HRT previously tested. Figures 5.26 and 5.27 show the data collected for sulfide concentrations in the effluent and first port, respectively. Simultaneous data for the average of steady state VSS concentrations in effluent and first port are shown in Table 5.9. The average was calculated from the last three steady state values.

The non-packed reactor with 12 h HRT showed a better performance because the sulfide concentrations both in the effluent and first port were higher than the sulfide

concentrations reached at a higher flow rate and therefore a smaller HRT of 7.5 h. Since Set 5 was carried out immediately after Set 4 and the difference between the flow rates was not considerable, the reactor had reached the steady state quite fast. However, there were significant fluctuations in the first port sulfide concentrations. Decreasing the flow rate and, therefore, increasing the HRT from 10 to 12 h, while operating the reactors under the same organic loading rate of 12 kg/d/m^3 , increased the effluent sulfide concentration from 165 to 193 mg/L and the VSS washout was reduced from 30 to 26 mg/L. The difference in the effluent and first port sulfide concentrations for the reactor with 12 h HRT and 12 kg/d/m^3 ORL was significant, 193 mg/L for the effluent and 121 mg/L for the first port. A similar difference was also observed in the VSS concentrations, 26 mg/L for the effluent and 45 mg/L for the first port.

Figure 5.26 Effluent Sulfide Concentration vs Time During Continuous Operation (Set 5)

Figure 5.27 First Port Sulfide Concentration vs Time During Continuous Operation (Set 5)

Table 5.9 Average of Steady State VSS Concentrations (Set 5)

Reactor	HRT (h)	Average of Steady State Concentrations	
		Effluent VSS (mg/L)	First Port VSS (mg/L)
Reactor II (non-packed)	12	26	45
Reactor II (non-packed)	7.5	19	17

Increasing the flow and decreasing the HRT from 10 to 7.5 h, while operating the reactors under the same organic loading rate of 12 kg/d/m³, showed a change in the effluent sulfide concentration from 165 to 138 mg/L. One interesting observation was that the VSS concentration had become uniform along the reactor's height, 19 and 17 mg/L for the effluent and first port, respectively. The low VSS concentrations indicated the washout of the bacteria within a short period of time and thus the lack of solids in the reactor.

The pH ranged between 6.0 and 7.0 and the ORP varied between -320 and -370 mV. The SRB concentration was checked at the end of each run and it remained above 10⁶ cells/mL in the three reactors. The VSS to SS ratio varied between 0.7 and 0.8 throughout the experiments in Phase III in the three reactors.

Table 5.10 summarizes the results obtained during all five sets under different operating conditions. Figures 5.28, 5.29 and 5.30 show the results for steady state sulfide and VSS concentrations for each reactor at different hydraulic retention times and organic loading rates. The sulfide and VSS concentrations represent the average of the last three values obtained during the steady state operation of the reactors. Figure 5.31 shows the average curves for Reactor II and III, both non-packed, at different HRTs and OLRs.

Similar calculations of the rate of mass production for sulfide and VSS are summarized in Table 5.11 and plotted in Figures 5.32, 5.33 and 5.34. Figure 5.35 shows the average curves for Reactor II and III, both non-packed, at different HRTs and OLRs.

**Table 5.10 Summary of Steady State Sulfide and VSS Concentrations During
Continuous Operation (Phase III)**

Set	Sulfide Concentration mg/L		VSS Concentration mg/L		HRT h (Reactor)	OLR kg/d/m ³
	Effluent	First Port	Effluent	First Port		
1	142	155	63	81	50 (I)	6
	135	122	62	65	50 (II)	6
	180	166	25	42	30 (III)	6
2	188	183	34	38	30 (I)	6
	186	187	13	39	30 (II)	6
	159	120	18	30	20 (III)	6
3	195	177	16	25	20 (I)	6
	182	160	11	30	20 (II)	6
	74	64	18	17	10 (III)	6
4	165	53	29	54	10 (II)	12
	26	4	3	6	5 (III)	6
5	193	121	26	45	12 (II)	12
	138	56	19	17	7.5 (II)	12

**Table 5.11 Summary of the Rate of Mass Production for Sulfide and VSS During
Continuous Operation (Phase III)**

Set	Rate of Sulfide Production mg/d	Rate of VSS Production mg/d	HRT h (Reactor)	OLR kg/d/m³
1	771	344	50 (I)	6
	731	335	50 (II)	6
	1485	208	30 (III)	6
2	1613	289	30 (I)	6
	1577	112	30 (II)	6
	1925	222	20 (III)	6
3	2451	205	20 (I)	6
	2305	135	20 (II)	6
	1698	417	10 (III)	6
4	3815	675	10 (II)	12
	1261	146	5 (III)	6
5	3820	511	12 (II)	12
	4478	630	7.5 (II)	12

Figure 5.28 Steady State Sulfide and VSS Concentrations vs HRT. Reactor I (packed)

Figure 5.29 Steady State Sulfide and VSS Concentrations vs HRT. Reactor II (non-packed)

Figure 5.30 Steady State Sulfide and VSS Concentrations vs HRT.

Reactor III (non-packed)

Figure 5.31 Reactors II and III (non-packed) Average Curves

Figure 5.32 Rate of Sulfide and VSS Production vs HRT. Reactor I (packed)

Figure 5.33 Rate of Sulfide and VSS Production vs HRT. Reactor II (non-packed)

Figure 5.34 Rate of Sulfide and VSS Production vs HRT. Reactor III (non-packed)

Figure 5.35 Rate of Sulfide and VSS Production vs HRT. Reactors II and III (non-packed)

Average Curves

5.3.6 Kinetic Modeling

5.3.6.1 Kinetic Model I: Biomass Production Rate

As indicated previously, VSS were assumed to represent the biomass in the reactor. According to Equation 4.7 developed in Chapter 4 for mass balance, the logarithm of the steady state VSS concentration for the first port, X_s , was plotted against the actual retention time, V_{active}/Q in Figures 5.36, 5.37 and 5.38 for Reactor I (packed), Reactor II (non-packed) and Reactor III (non-packed), respectively. The specific growth rate for biomass, μ , was obtained from the slope of each plot. The active volume was assumed to be up to the first port because above this port the VSS concentration was fairly uniform.

Calculations for the biomass production rate are shown in Table 5.12 and the resulting models are given below:

$$\text{Reactor I: } \ln X_s = 0.212 \frac{V}{Q} + 2.32 \text{ where, } R^2=1.000 \quad \mu=0.212 \text{ h}^{-1} \text{ and } X_o=10.2 \text{ mg/L.}$$

$$\text{Reactor II: } \ln X_s = 0.125 \frac{V}{Q} + 2.81 \text{ where, } R^2=0.999 \quad \mu=0.125 \text{ h}^{-1} \text{ and } X_o=16.6 \text{ mg/L.}$$

$$\text{Reactor III: } \ln X_s = 0.194 \frac{V}{Q} + 2.39 \text{ where, } R^2=0.988 \quad \mu=0.194 \text{ h}^{-1} \text{ and } X_o=10.9 \text{ mg/L.}$$

The following equation for rate of substrate utilization was used to obtain the sulfide production rate from the biomass concentrations obtained above:

$$\frac{dL}{dt} = \frac{1}{Y} \frac{dX_s}{dt} = \frac{1}{Y} \mu X_s = \frac{\mu}{Y} X_s = k X_s \quad \dots\dots\dots 5.3$$

$$\frac{dC_s}{dt} = \left(\frac{1 \text{ mg/L sulfide}}{2.6 \text{ mg/L lactate}} \right) \frac{dL}{dt} = \frac{k}{2.6} X_s \quad \dots\dots\dots 5.4$$

Where,

dL/dt = lactate consumption rate under steady state conditions [mg/h/L]

dX_s/dt = biomass production rate under steady state conditions [mg/h/L]

Y = yield coefficient = $\frac{\text{mass of VSS produced/d}}{\text{mass of lactate consumed/d}} = \frac{\text{mass of VSS produced/d}}{2.6 \text{ mass of sulfide produced/d}}$

μ = specific growth rate [h^{-1}]

$k = \mu/Y$ = substrate utilization rate constant [h^{-1}]

X_s = steady state biomass concentration for the first port [mg/L]

dC_s/dt = sulfide production rate under steady state conditions [mg/h/L]

Calculations for k and the sulfide production rate are also shown in Table 5.12.

Table 5.12 Calculations for Model I: Biomass Production Rate

$\frac{dX}{dt} = \mu X$ $\ln X_s = \mu (V/Q) + \ln X_o$														
CONSIDERING VSS AT THE FIRST PORT (WITH CORRESPONDING VOLUME IN THE FIRST 300 mm)														
Internal diameter =				0.1020 m				Porosity of Packing Material in Reactor I = 90 %						
Radius =				0.051 m										
Cross sectional area =				0.0082 m ²										
Active volume =				2.45 L										
Reactor I														
HRT	Q	V/Q*	X _s		ln X _s	Q*X _s	Slope from Plot ln X _s vs V/Q		Q*C _s	2.6*mg S/d	mg VSS / mg S	u/Y	ln X _s = 0.212 V/Q + 2.32	dC _s /dt Eq. 5.4
h	L/d	h	mg/L			Mass VSS/d	μ	C _s	Mass S/d	Lactate used	Y	k	X _s Calculated	kg/d/m ³
50	5.43	9.75	81		4.39	440	0.212	155	840	2185	0.201	0.88	80.4	0.65
30	8.56	6.18	38		3.64	325		183	1569	4080	0.080	2.22	37.8	0.77
20	12.6	4.21	25		3.22	314	X _o	177	2228	5794	0.054	3.26	24.9	0.75
10	23.1	2.30	12		2.48	277	mg/L							
5	48.5	1.09	6		1.79	291	10.2							

*Retention time in active volume

Table 5.12 Continued

$\frac{dX}{dt} = \mu X$ $\ln X_s = \mu (V/Q) + \ln X_0$													
CONSIDERING VSS AT THE FIRST PORT (WITH CORRESPONDING VOLUME IN THE FIRST 300 mm)													
Internal diameter = 0.1020 m Porosity of Packing Material in Reactor I = 90 %													
Radius = 0.051 m													
Cross sectional area = 0.0082 m ²													
Active volume = 2.45 L													
Reactor III													
							Slope from Plot ln X _s vs V/Q	Q*C _s	2.6*mg S/d	mg VSS / mg S	w/Y	ln X _s = 0.194 V/Q + 2.39	dC _s /dt Eq. 5.4
HRT	Q	V/Q*	X _s	ln X _s	Q*X _s	μ	C _s	Mass S/d	Lactate used	Y	k	X _s Calculated	
h	L/d	h	mg/L		Mass VSS/d	1/h	mg/L	mg/d	mg/d	mg/mg	1/h	mg/L	kg/d/m ³
50	5.43	10.8	75	4.32	407	0.194							
30	8.25	7.13	42	3.74	348		166	1371	3566	0.098	1.81	43.5	0.73
20	12.1	4.85	30	3.39	359	X ₀	120	1456	3786	0.095	1.87	28.0	0.48
10	22.9	2.57	17	2.86	399	mg/L	64	1473	3829	0.104	1.70	18.0	0.28
5	48.5	1.21	6	1.79	291	10.9							
Critical upflow velocity= Q/A= 5.94 m/d													
Average μ= 0.177 1/h													
Average X ₀ = 12.6 mg/L													
Average k for 20 h = 2.53 1/h													
Q= 0.0485 m ³ /d													
A= 0.0082 m ²													

*Retention time in active volume

Figure 5.36 Model I: Biomass Production Rate. Reactor I (packed)

Figure 5.37 Model I: Biomass Production Rate. Reactor II (non-packed)

Figure 5.38 Model I: Biomass Production Rate. Reactor III (non-packed)

5.3.6.2 Kinetic Model II: Sulfide Production Rate

According to Equation 4.9 developed in Chapter 4 for mass balance, the logarithm of the steady state sulfide concentration for the first port, C_s , was plotted against the actual retention time, V_{active}/Q in Figures 5.39, 5.40 and 5.41 for Reactor I (packed), Reactor II (non-packed) and Reactor III (non-packed), respectively. The rate constant, K , was obtained from the slope of each plot. The active volume was assumed to be up to the first port because above this port the VSS concentration was fairly uniform.

The calculations for sulfide production rate are shown in Table 5.13 and the resulting models are given below:

Reactor I: $\ln C_s = -0.0274 \frac{V}{Q} + 5.33$ where, $R^2=0.733$ $K= -0.0274 \text{ h}^{-1}$ and $C_o= 206 \text{ mg/L}$.

Reactor II: $\ln C_s = -0.0504 \frac{V}{Q} + 5.42$ where, $R^2=0.538$ $K= -0.0504 \text{ h}^{-1}$ and $C_o= 226 \text{ mg/L}$.

Reactor III: $\ln C_s = 0.2080 \frac{V}{Q} + 3.68$ where, $R^2=0.968$ $K= 0.208 \text{ h}^{-1}$ and $C_o= 39.6 \text{ mg/L}$.

Figure 5.39 Model II: Sulfide Production Rate. Reactor I (packed)

Table 5.13 Calculations for Model II: Sulfide Production Rate

$\frac{dC}{dt} = K C$ $\ln C_s = K (V/Q) + \ln C_o$							
CONSIDERING VSS AT THE FIRST PORT (WITH CORRESPONDING VOLUME IN THE FIRST 300 mm) Internal diameter = 0.1020 m Porosity of Packing Material in Reactor I = 90 % Radius = 0.051 m Cross sectional area = 0.0082 m ² Active volume = 2.45 L							
Reactor I							
		up to 1st port	up to 1st port		Slope from Plot $\ln C_s$ vs V/Q	$\ln C_s = -0.0274 V/Q + 5.33$	$Q * C_{s \text{ calc}}$
HRT	Q	V/Q^*	C_s	$\ln C_s$	K	C_s calculated	Mass S/d
h	L/d	h	mg/L		1/h	mg/L	mg/d
50	5.43	9.75	155	5.04	-0.0274	158	858
30	8.56	6.18	183	5.21		174	1492
20	12.6	4.21	177	5.18	C_o	184	2311
10	23.1	2.30			mg/L		
5	48.5	1.09			206		
Reactor II							
		up to 1st port	up to 1st port		Slope from Plot $\ln C_s$ vs V/Q	$\ln C_s = -0.0504 V/Q + 5.42$	$Q * C_{s \text{ calc}}$
HRT	Q	V/Q^*	C_s	$\ln C_s$	K	C_s calculated	Mass S/d
h	L/d	h	mg/L		1/h	mg/L	mg/d
50	5.43	10.8	122	4.81	-0.0504	131	710
30	8.48	6.94	187	5.23		159	1349
20	12.7	4.64	160	5.08	C_o	179	2269
10	23.1	2.55			mg/L		
5	48.5	1.21			226		
Reactor III							
		up to 1st port	up to 1st port		Slope from Plot $\ln C_s$ vs V/Q	$\ln C_s = -0.0208 V/Q + 3.68$	$Q * C_{s \text{ calc}}$
HRT	Q	V/Q^*	C_s	$\ln C_s$	K	C_s calculated	Mass S/d
h	L/d	h	mg/L		1/h	mg/L	mg/d
50	5.43	10.8			0.2080		
30	8.25	7.13	166	5.11		175	1442
20	12.1	4.85	120	4.79	C_o	109	1319
10	22.9	2.57	64	4.17	mg/L	68	1548
5	48.5	1.21	4	1.39	39.6		
average K = NA 1/h							
average C_o = 157 mg/L							

*Retention time in active volume

Figure 5.40 Model II: Sulfide Production Rate. Reactor II (non-packed)

Figure 5.41 Model II: Sulfide Production Rate. Reactor III (non-packed)

5.3.6.3 Kinetic Model III: Sulfide and Biomass Concentrations

According to Equation 4.11 developed in Chapter 4 for mass balance, the logarithm of the steady state sulfide concentration for the first port, C_s , was plotted against $X_s V_{\text{active}}/Q$, where X_s represents the steady state VSS concentration for the first port, as shown in Figures 5.42, 5.43 and 5.44 for Reactor I (packed), Reactor II (non-packed) and Reactor III (non-packed), respectively. The rate constant, K' , was obtained from the slope of each plot. The active volume was assumed to be up to the first port because above this port the VSS concentration was fairly uniform.

The calculations for sulfide production rate are shown in Table 5.14 and the resulting models are given below:

$$\text{Reactor I: } \ln C_s = -0.0002 X_s \frac{V}{Q} + 5.23 \quad \text{where, } R^2=0.873 \quad K' = -0.0002 \text{ h}^{-1} \quad \text{and}$$

$$C_o = 187 \text{ mg/L}$$

$$\text{Reactor II: } \ln C_s = -0.0006 X_s \frac{V}{Q} + 5.26 \quad \text{where, } R^2=0.683 \quad K' = -0.0006 \text{ h}^{-1} \quad \text{and}$$

$$C_o = 192 \text{ mg/L}$$

$$\text{Reactor III: } \ln C_s = 0.0035 X_s \frac{V}{Q} + 4.11 \quad \text{where, } R^2=0.906 \quad K' = 0.0035 \text{ h}^{-1} \quad \text{and}$$

$$C_o = 60.9 \text{ mg/L}$$

Table 5.14 Calculations for Model III: Sulfide and Biomass Concentrations

$\frac{dC}{dt} = K' C X$ $\ln C_s = K' X_s (V/Q) + \ln C_o$								
CONSIDERING VSS AT THE FIRST PORT (WITH CORRESPONDING VOLUME IN THE FIRST 300 mm)								
Internal diameter =		0.1020 m		Porosity of Packing Material in Reactor I = 90 %				
Radius =		0.051 m						
Cross sectional area =		0.0082 m ²						
Active volume =		2.45 L						
Reactor I								
		up to 1st port			up to 1st port		Q* C _s	Slope from Plot ln C _s vs X _s *(V/Q)
HRT	Q	V/Q*	X _s	X _s *V/Q	C _s	ln C _s	Mass S /d	K'
h	L/d	h	mg/L		mg/L		mg/d	L/(mg*h)
50	5.43	9.75	81	878	155	5.04	440	-0.0002
30	8.56	6.18	38	261	183	5.21	325	
20	12.6	4.21	25	117	177	5.18	314	C _o
10	23.1	2.30	12	30.6			277	mg/L
5	48.5	1.09	6	7.27			291	187
Reactor II								
		up to 1st port			up to 1st port		Q* C _s	Slope from Plot ln C _s vs X _s *(V/Q)
HRT	Q	V/Q*	X _s	X _s *V/Q	C _s	ln C _s	Mass S/d	K'
h	L/d	h	mg/L		mg/L		mg/d	L/(mg*h)
50	5.43	10.8	65	700	122	4.81	351	-0.0006
30	8.48	6.94	39	271	187	5.23	331	
20	12.7	4.64	30	139	160	5.08	379	C _o
10	23.1	2.55	17	43.4				mg/L
5	48.5	1.21	6	7.27				192
Reactor III								
		up to 1st port			up to 1st port		Q* C _s	Slope from Plot ln C _s vs X _s *(V/Q)
HRT	Q	V/Q*	X _s	X _s *V/Q	C _s	ln C _s	Mass S /d	K'
h	L/d	h	mg/L		mg/L		mg/d	L/(mg*h)
50	5.43	10.8	75	813				0.0035
30	8.25	7.13	42	301	166	5.11	348	
20	12.1	4.85	30	143	120	4.79	359	C _o
10	22.9	2.57	17	44.9	64	4.17	399	mg/L
5	48.5	1.21	6	7.28	4	1.39	291	60.9
Critical upflow velocity= Q/A= 5.93 m/d					Q = 0.0485 m ³ /d A = 0.0082 m ²			

*Retention time in active volume

Figure 5.42 Model III: Sulfide and Biomass Concentrations. Reactor I (packed)

**Figure 5.43 Model III: Sulfide and Biomass Concentrations.
Reactor II (non-packed)**

Figure 5.44 Model III: Sulfide and Biomass Concentrations.

Reactor III (non-packed)

CHAPTER 6. DISCUSSION

During the present work, three basic operating parameters were studied in order to define the optimum conditions for the operation of an upflow anaerobic reactor, with SRB as the dominant bacteria. These three operating parameters were: feed composition, presence of packing material and hydraulic retention time, which was related to the upflow velocity. The optimization of these parameters was carried out in order to maximize the sulfide production, which was used as an indicator of the reactor performance and the SRB activity.

6.1 Analytical Techniques

An important analysis during the experiments was the sulfide analysis, which followed the Standard Method 4500-S²⁻ F, as described in Section 3.3.1. All the samples were pretreated to eliminate interferences and to concentrate sulfide. The analysis results represented 90% of the total sulfide observed in the sample without pretreatment.

All the analyses during the continuous operation of the reactors were carried out once every second day, until the steady state was reached, generally after 5 to 8 times the hydraulic retention time tested. The steady state is defined as keeping the fluctuations in concentrations within the statistical variation for each specific analysis as calculated in Appendix C. The steady state sulfide and VSS concentrations used for analysis of data were the average of the last three values.

6.2 Phase I

Since the bacteria were in the acclimatization period, a less concentrated substrate was used. An increase in sulfide concentration was observed after about 20 days showing an acclimatized SRB culture. After the first 40 consecutive days, the stationary phase was reached, which was confirmed by stable ORP and pH values. It can be seen that obtaining a healthy SRB culture is a long process, basically due to the type of substrate used and the type of seed used to start the reactors.

In an acetate fed reactor, Omil *et al.* (1997) detected sulfidogenic acetate degradation after 50 days, while just 10% of the acetate was degraded by the ASRB after 100 days. The researchers concluded that methods that rely on the development of an SRB population with time would require a long process.

For a healthy SRB activity in the sulfide production and the corresponding reduction of sulfate, factors like pH and ORP had to be kept within the optimum values. Even though sulfide concentrations were always detected, a healthy SRB activity was considered only after a sulfide concentration of 120-130 mg/L was reached in the 2 L reactors. Significant fluctuations in the results were noticed until the sulfide concentration gradually reached the steady state.

6.3 Phase II

One of the most important limitation in the batch study of the 10 L reactors was the channeling within the reactors. Since no mixing device was used, channeling of the feed solution within the sludge bed could have taken place during the experiment. The samples were not representative of the reactors' content because there was no mixing available to provide a uniform sample.

6.3.1 Substrate Composition

It was observed that the reactors performed better and higher sulfide concentrations were obtained when the initial substrate composition was changed. Although the Th.O.D.:SO₄ and the Th.O.D.:N:P were the same in all formulations, five main changes were made:

1. Sulfate Source. Magnesium sulfate (MgSO₄*7H₂O), used in the first batch substrate formulation, was changed to ammonium sulfate ((NH₄)₂SO₄) and sodium sulfate (Na₂SO₄), thereby eliminating the high concentrations of magnesium (above 3000 mg/L), which may have caused strong inhibition in the anaerobic process (Speece, 1996).
2. Nitrogen Source. Ammonium chloride was replaced by ammonium sulfate, which functioned both as sulfate source and nitrogen source. The optimum nitrogen concentration for SRB growth reported by El Bayoumy (1997) was between 250

and 500 mg/L. The first batch formulation had nitrogen concentrations above this level, while the new substrate nitrogen content was within the optimum limits.

3. Sodium Concentration. A moderate inhibition was probably caused by the sodium concentration of 3500-5500 mg/L in the first batch substrate formulation. The sodium concentration was reduced to 1900 mg /L in the new formulation and was maintained below the moderate inhibitory concentrations (Speece, 1996).
4. Buffers Ratio. A Th.O.D. to P ratio of 100:1 was kept for the new formulation, but the ratio between the potassium di-hydrogen phosphate (KH_2PO_4) and di-potassium hydrogen phosphate (K_2HPO_4) was changed from 1:4 to 1:2.
5. Trace Elements Iron, Nickel, Copper, Zinc, Manganese, Boron and Cobalt were added as trace elements in the new formulation since deionized water was used for the preparation of all the solutions.

6.3.2 Profiles of Sulfide and VSS

Profiles of sulfide and VSS were studied along the reactors' height during the 4th sequence of Phase II. No significant difference in concentrations along the reactors' height was found during the batch operation of the three 10 L reactors. However, Reactor I (packed) showed more uniform sulfide and VSS concentrations along its height as compared to the other reactors. It should be noted that the VSS concentrations obtained were not representative because the samples did not include solids settled within the packing material. Additionally, the biomass in Reactor I (packed) had settled more uniformly throughout the reactor and therefore had produced higher sulfide

concentrations with lower VSS in suspension. Figures 5.14, 5.15 and 5.16 show sulfide and VSS concentrations for Reactor I (packed), Reactor II (non-packed) and Reactor III (non-packed), respectively. Figure 5.17 show the combined results for Reactor II and III. The sulfide and VSS concentrations plotted for each reactor represent the average along the reactor's height for each day of analyses during the 4th sequence. Although Reactor II and Reactor III, both non-packed, were started at different VSS concentrations, they showed similar correlations between sulfide and VSS concentrations.

The observed VSS concentrations corresponded to the VSS in suspension. However, the observed VSS concentrations in suspension did not represent the actual biomass present in the reactors because a portion of the biomass had settled down at the bottom of the reactors. The influence on sulfide production of this settled biomass was neglected for mass balance calculations since it was observed that the settling velocity of the biomass was low enough to allow solids to remain in suspension during 3 to 5 day period of time used in the study.

6.3.3 Stoichiometry

Stoichiometrical analyses and kinetic rates were used to determine important microbial reaction characteristics. First, the mass and electron balances of VSS and sulfide were carried out using the results from the batch operation of the three 10 L anaerobic reactors.

Dissimilatory sulfate reduction can be represented by the following stoichiometric equations:

1. Energy: oxidation of an organic carbon source. In the present study, lactate was the electron donor.
2. Sulfate reduction: sulfate functioned as the electron acceptor.
3. Synthesis: formation of bacterial cells considered as VSS.

The appropriate equations were developed in Chapter 4 and from the results of the mass and electron balance calculations presented in Chapter 5, Tables 5.2, 5.3 and 5.4, it was possible to obtain the proportions in which the reactions mentioned above were taking place. It was determined that about 70 % of the organic matter (lactate) was bio-oxidized, while 30 % was bio-synthesized in non-packed reactors.

The overall stoichiometric equation was obtained by summing the oxidation-reduction equation with the synthesis equation in the ratios mentioned above:

Multiplying Equation 4.4 by 0.7 gives:

Multiplying Equation 4.5 by 0.3 gives:

Summing Equation 6.1 and Equation 6.2 gives:

.....6.3

Dividing Equation 6.3 by 0.088 gives the overall sulfate reduction equation:

.....6.4

Using the following molecular masses in Equation 6.4 gives the ratios in which sulfates, lactate and sulfide were reacting:

MM lactate ion=89

MM SO_4^{2-} ion=96

MM S^{2-} ion=32

MM $\text{C}_5\text{H}_7\text{O}_2\text{N}$ as cells= 113

Thus,

Dividing by 9.6,

Therefore,

$$\frac{\text{Sulfide}}{\text{Sulfate}} = \frac{1}{3} \quad \text{.....6.5}$$

$$\frac{\text{Lactate}}{\text{Sulfate}} = 0.87 \quad \text{.....6.6}$$

$$\frac{\text{Lactate}}{\text{Sulfide}} = 2.6 \quad \text{.....6.7}$$

The Th.O.D., defined as the amount of oxygen in mg/L required to stabilize an equivalent amount of organic matter in mg/L, was calculated by the following equations:

Where,

$$\text{Th.O.D.} = \frac{96}{89} * \text{Lactate} = 1.08 * \text{Lactate} \quad \text{.....6.9}$$

Therefore,

$$\frac{\text{Th.O.D.}}{\text{SO}_4} = 0.87 * 1.08 = 0.94 \quad \text{.....6.10}$$

These balanced equations indicate that the energy production reaction dominated over the synthesis reaction. It is shown that the experimental lactate to sulfate ratio was equal to 0.87 and the corresponding Th.O.D.:SO₄ was equal to 0.94, which is below 2 as suggested by Annachhatre and Suktrakoolvair (2001).

6.4 Phase III

All reactors were operated in a continuous flow mode in this phase of the study. The data used for discussion are those obtained after steady state, which generally was reached in 5-8 times the hydraulic retention time.

Under continuous operation, the optimization of OLR, Th.O.D.:SO₄, total nitrogen loading rate, total phosphorus loading rate and Th.O.D.:N:P, is essential in order to operate a SRB system efficiently. Therefore, the optimum values of these parameters were kept the same as recommended by El Bayoumy (1997).

Other important operating parameters include the nature of support media for bacterial growth, attached or suspended, the hydraulic retention time and the biomass characteristics. These parameters were varied in this phase to maximize the sulfide production and, therefore, optimize SRB activity in an upflow anaerobic suspended growth reactor. The concentrations of possible inhibitory cations, such as Mg and Na, and nitrogen concentrations were kept within acceptable limits: 3-42 mg/L, 480-4800 mg/L and 60-630 mg/L, respectively.

6.4.1 General

Table 5.10 summarizes the steady state data collected from first port and effluent under different operating conditions of HRT and OLR.

The sulfide concentrations at the first port and the effluent were similar, within statistical variations, indicating that the active volume of the reactor for biomass growth and sulfide production was within the first 300 mm of reactor's height. However, when the OLR was increased from 6 to 12 kg/d/m³, the sulfide concentration gradient between the first port and the effluent clearly indicates that the active volume had increased to above the first port.

As mentioned previously, VSS concentrations do not strictly represent the concentration of active biomass because a portion of the biomass had settled down at the bottom of the reactors. The VSS concentrations were generally higher at the first port than in the effluent showing a slight gradient along the column height.

When the HRT was reduced by increasing the upflow velocity, the VSS concentrations in the reactors were reduced indicating higher washout of biomass.

6.4.2 Support Media

Two reactors were run in parallel under similar conditions, with one packed reactor, I, and the other non-packed, II. The support media or packing material consisted of 16 mm polypropylene pall rings with a porosity of 90 %.

The packed reactor showed comparatively less variations in the sulfide and VSS concentrations during all the experiments at different hydraulic retention times and

different depths. An interesting finding, as shown in Table 5.10, was that the steady state sulfide and VSS concentrations for the packed and the non-packed reactors, operated under the same conditions, were similar, and the variations in concentrations observed in the two reactors are considered to be statistical variations.

It is noteworthy that the observed VSS concentrations corresponded to the biomass that was in suspension along the reactors' height and that was washed out from the reactor. The actual VSS concentration may have been higher because a portion of the biomass had settled at the bottom within the first 300 mm of the reactors' height.

The conclusions of several other studies agree with the findings of the present work. Alphenaar *et al* (1993), Omil *et al.* (1997) and Dries *et al.* (1998) observed that SRB had poor attachment properties and the presence of a support media did not improve the performance of the system

Therefore, after considering the additional cost of packing material, it is concluded that there is no significant advantage in using packed bed reactors for SRB growth.

6.4.3 Hydraulic Retention Time

The effect of hydraulic retention time on the performance of three upflow anaerobic reactors was studied by varying the HRT between 5 and 50 h.

From the results shown in Table 5.10 and Figures 5.28, 5.29 and 5.30, it can be observed that both packed and non-packed reactors presented a similar behavior. Figure 5.31 shows that 20-30 h was the optimum hydraulic retention time range for sulfide production. At lower HRT, the sulfide concentration and the mass of sulfide produced per day decreased due to the higher washout of biomass and the reduced time available for the sulfide production reaction. On the other hand, at higher HRT the sulfide concentration and the mass of sulfide produced per day decreased. It is believed that the biomass was fluidized at 20-30 h of HRT, whereas it remained as a compact bed at 50 h of HRT. A fluidized bed provided much higher contact between the substrate and the active bacteria as compared to the compact bed.

The maximum sulfide concentrations observed are given below:

1. Reactor I, packed, achieved the maximum concentration of 195 mg S/L at 20-30 h of retention time.
2. Reactor II, non-packed, achieved the maximum concentration of 186 mg S/L at 20-30 h of retention time.
3. Reactor III, non-packed, achieved the maximum concentration of 180 mg S/L at 20-30 h of retention time.

The average of these three maximum sulfide concentrations is 187 mg S/L.

The maximum sulfide concentrations were similar in all three reactors and within the statistical variations of the tests. The combined effect of the hydraulic retention time and

the packing material shows that the maximum sulfide concentration was reached within the same range of retention time for the packed reactor as for the non-packed reactor.

It is also observed that the VSS concentrations in the effluent and first port in all reactors had increased with an increase in HRT because there was more time for bacterial growth and, therefore, higher biomass concentration was present in the reactor. On the other hand, VSS had rapidly decreased due to washout of biomass when the HRT was below 10 h.

The maximum rates of sulfide production are listed below:

1. Reactor I (packed) achieved the maximum rate of 2450 mg S/d at 20 h of retention time.
2. Reactor II (non-packed) achieved the maximum rate of 2310 mg S/d at 20 h of retention time.
3. Reactor III (non-packed) achieved the maximum rate of 1930 mg S/d at 20 h of retention time.

The average of these three maximum rates of sulfide production is 2230 mg S/d.

The maximum rates of sulfide production were similar regardless of the type of reactor, packed or non-packed, as shown in Table 5.11 and Figures 5.32, 5.33 and 5.34. The variations in results between different reactors are considered to be statistical variations. It can be seen that the maximum rate of sulfide production was not dependent on the type

of SRB growth involved, attached or suspended, and it was achieved at an optimum retention time of 20 h as shown in Figure 5.35.

It is concluded that the hydraulic retention time is an important parameter in the design and operation of anaerobic reactors. The optimum HRT ranged between 20 and 30 h. Below 10 h of HRT, the SRB activity, and therefore the concentration of sulfide, became so low that the overall performance of the system was not satisfactory. The loss of SRB activity at HRT below 10 h was mainly due to the washout of biomass caused by the high upflow velocity.

The hydraulic retention time, HRT, is related to the hydraulic loading rate, HLR and the upflow velocity, V_o , as shown below:

$$\text{HRT} = \frac{V}{Q} = \frac{A * H}{Q} = \frac{H}{\left(\frac{Q}{A}\right)} = \frac{H}{\text{HLR}} = \frac{H}{V_o} \quad \text{.....6.11}$$

Where,

HRT = hydraulic retention time (d)

V = reactor's volume (m^3)

Q = flow rate (m^3/d)

A = cross sectional area (m^2)

H = reactor's height (m)

HLR = hydraulic loading rate ($\text{m}^3/\text{m}^2/\text{d}$)

V_o = upflow velocity (m/d)

In these reactors, the maximum rate of sulfide production had been reached at 1.5 m/d of V_o , 20 h of HRT. This value showed an optimum performance of the system along with a small VSS washout. On the other hand, there was a complete washout at 5 h of HRT and 5.9 m/d of V_o . El Bayoumy (1997) had recommended 11 h as the optimum HRT for an UAFF reactor.

In a supporting study, Dvorak *et al.* (1992) found that the hydraulic flow rate significantly affected the performance of the anaerobic system. At shorter residence times, the upflow anaerobic reactors were not as effective not only because there was insufficient time for bacterial activity to remove metals but it also dislodged solids and prevented the precipitates from settling. On the other hand, longer residence times may limit the reactor to small influent loads of metals.

6.4.4 Effect of Organic Loading Rate

Table 5.10 and Figure 5.31 show that when the organic loading rate was doubled from 6 kg/d/m³ to 12 kg/d/m³, at the same hydraulic retention time and upflow velocity, the mass of sulfide produced, as measured in the effluent, had also increased proportionately. Similarly, the mass of VSS produced in the effluent had increased with an increase in OLR. The rate of sulfide production under different organic loading rates at 10 h HRT are listed below:

First Port

1. Reactor III (non-packed) produced 1470 mg S/d at 6 kg/d/m³ of organic loading rate.
2. Reactor II (non-packed) produced 1230 mg S/d at 12 kg/d/m³ of organic loading rate.

Effluent

1. Reactor III (non-packed) produced 1700 mg S/d at 6 kg/d/m³ of organic loading rate.
2. Reactor II (non-packed) produced 3820 mg S/d at 12 kg/d/m³ of organic loading rate.

It was observed that doubling the OLR increased the active volume. At 300 mm, first sampling port, the mass of sulfide produced with 12 kg/d/m³ was similar to the one obtained with 6 kg/d/m³. However, at the effluent port, 12 kg/d/m³ of OLR produced more than twice the sulfide mass as compared to 6 kg/d/m³ of OLR. Singh *et al.* (1996) have reported that at higher OLR, the settling characteristics of the biomass were poorer and it was possible an increase in the solids distribution and, therefore, an increase in active height.

Since the rate of lactate consumption is proportional the concentration of lactate present, when the latter is increased the rate of organic matter consumption increases proportionally. From Equation 5.4, it can be observed that the rate of lactate consumption

is proportional to the rate of sulfide production and therefore, doubling the OLR would double the rate of sulfide production.

It was confirmed that the sulfide decreased as the HRT was decreased, regardless of the OLR. This was observed within a hydraulic retention time range of 7.5 and 12 h in upflow anaerobic suspended growth reactors.

6.4.5 pH and ORP

Mizuno *et al.* (1998b) has mentioned that the pH value is a key factor affecting sulfate reduction. The pH and ORP in the reactors were checked throughout the experiments. It was observed that the effluent pH ranged between 6.5 and 7.0 and the ORP values fluctuated between -300 and -390 mV, which are within the optimum limits reported for a healthy SRB environment. The substrate composition with its pH (7.0 ± 0.1) and its buffering capacity, helped in maintaining the effluent pH in the range mentioned.

Sulfate reducers prefer an environment around pH 7.0 and are usually inhibited at pH values lower than 6.0 or higher than 9.0 (Perry, 1995). Mizuno *et al.* (1998b) has stated that a low pH is not recommended because of possible inhibition to SRB growth facilitated by the formation of H_2S according to the following equation:

Similarly, the ORP values below -150 mV are an indicator of sulfide production and activity of SRB (Postgate, 1984).

6.4.6 Kinetics

In this study, the total sulfide concentration in the reactors was below the toxic and inhibitory concentrations reported in the literature, i.e. 250-600 mg S/L (Okabe *et al.*, 1992; Reis *et al.*, 1992; Okabe *et al.*, 1995). Additionally, the pH was kept between 6.5 and 7.0, which means that the hydrogen sulfide concentration was less than 50% of the total sulfide concentration. Thus, the concentration of hydrogen sulfide, a strong inhibitor for SRB growth, was minimized through the pH control within a narrow range.

The results from the upflow anaerobic reactors, in continuous mode operation, were used to develop the kinetic models.

The biochemical transformations in the reactors are mathematically described by mass balance equations in Chapter 4. The mass balances were based on a plug flow system by considering a differential volume element. An important design parameter is the rate constant of the process, which was obtained through the plug flow reactor model up to the first port, 300 mm above the influent. This was done based on the fact that the concentrations above this port were fairly uniform, confirming that significant sulfide production was taking place within the first 300 mm of the reactor's height. Therefore, the actual hydraulic retention time up to that port was used in calculations.

6.4.6.1 Biomass Production

The kinetic rate constant for the biomass growth was calculated from the best-fit slope of the $\ln X_s$ plotted versus calculated hydraulic retention time, Figures 5.36, 5.37 and 5.38, where X_s represents the VSS steady state concentration at the first port. According to the calculations in Table 5.12 for Model I, the average value of the specific growth rate, μ , was 0.177 h^{-1} .

The values for Y and k depended on the hydraulic retention time as shown in Table 5.12. The yield coefficient decreased as the hydraulic retention time decreased, whereas the substrate utilization rate constant increased with a decrease in hydraulic retention time.

6.4.6.2 Sulfide Production

Based on the values for Y and k obtained above, the corresponding rates for sulfide production at different hydraulic retention times were calculated from the Equation 5.4 developed previously. The values for X_s were calculated using the corresponding model for each reactor as shown in Section 5.3.6.1.

This shows a similarity with McCartney *et al.* (1990) study. They reported that lactate removal in batch reactors was essentially independent of sulfide concentration.

The sulfide production rates were also calculated using Kinetic Model II developed in Section 5.3.6.2. In this model, it was assumed that the rate of sulfide production was

directly proportional to the sulfide concentration. The correlations for Model II in Figures 5.39, 5.40 and 5.41 did not show as good fit as correlations for Model I.

The Kinetic Model III developed in Section 5.3.6.3, based on the rate of sulfide production being proportional to the product of biomass and sulfide concentrations, did not show satisfactory sulfide production rate calculations and therefore the calculations were neglected.

Using another approach, the rate of sulfide production per active volume can be calculated using the observed steady state sulfide concentrations at different hydraulic retention times with their corresponding flow rates. These calculations are shown in Table 6.1.

El Bayoumy (1997) had established the following empirical equation for the sulfide production rate in packed reactors:

$$\frac{dS}{dt} = \frac{S_{\max}}{10^3 * HRT} [1 - e^{(-0.75 * OLR)}] \quad \text{.....6.13}$$

Where,

dS/dt = sulfide production rate [kg/d/m³]

$S_{\max}$ = maximum sulfide concentration [mg/L]

HRT = hydraulic retention time [d]

OLR = organic loading rate [kg/d/m³]

El Bayoumy (1997) had found a maximum sulfide concentration of 140 mg/L in his experiments, considering the entire reactor volume as the active volume in developing Equation 6.13.

Calculations for sulfide production rate according to Equation 5.4 and 6.13 are shown in Table 6.1.

Table 6.1 Sulfide Production Rate Calculated by Different Methods

HRT	OLR	C _s Observed Steady State Sulfide Concentration at the first port	Q	$\frac{Q * C_{s \text{ observed}}}{\text{Active volume}}$	dCs/dt Equation 5.4	dS/dt Equation 6.13
(h)	kg/d/m ³	mg/L	L/d	kg/d/m ³	kg/d/m ³	kg/d/m ³
Reactor I						
50	6	155	5.43	0.34	0.65	0.38
30	6	183	8.56	0.64	0.77	0.70
20	6	177	12.6	0.91	0.75	1.00
Reactor II						
50	6	122	5.43	0.27	0.52	0.27
30	6	187	8.48	0.65	0.81	0.64
20	6	160	12.7	0.83	0.68	0.82
Reactor III						
30	6	166	8.25	0.56	0.73	0.55
20	6	120	12.1	0.59	0.48	0.59
10	6	64	22.9	0.60	0.28	0.59

Basically four factors can explain the differences found in sulfide production calculated with different methods:

- Type of substrate. Different feed compositions were used in El Bayoumy (1997) studies and the present research work.
- SRB seed culture.
- Reactor's active volume considered in the modeling.
- Variables considered in the different kinetic models.

6.4.7 Comparison between Batch and Continuous Flow Reactors

The present study shows that a better performance and higher sulfide concentrations were reached with the continuous system as compared to the batch operation.

McCartney and Oleszkiewicz (1993) referred the work of Karhadkar *et al.* (1987) in which it was concluded that it was difficult to achieve a specific aqueous sulfide concentration in batch systems due to the precipitation process and gaseous hydrogen sulfide equilibrium. Karhadkar *et al.* (1987) also noted that the batch reactors performed better with sulfide addition because sulfide was the growth-limiting factor while McCartney and Oleszkiewicz (1993) suggested that the oxidation-reduction potential of the media was lowered by the sulfide addition. Okabe *et al.* (1995) observed SRB growth rates for batch cultures lower than those for continuous cultures.

6.4.8 Active Height

During the continuous operation of the three 10 L anaerobic reactors, the sulfide concentrations were uniform at different ports along the reactors' height. It is believed that due to the presence of a portion of active sludge in the lower portion of the reactors, which was confirmed by visual observations, major sulfide production occurred within the first 300 mm of the reactor's height. When the hydraulic retention time was reduced to 10 h, a gradient of sulfide concentrations along the reactor's height was observed, indicating that this hydraulic retention time had started limiting the optimum performance of the system.

Gundry *et al.* (1990) also concluded that the active removal of nickel as NiS, and regardless of its initial concentration in the influent, occurred in the lower part of an anaerobic filter. Chen *et al.* (1994) observed in an upflow anaerobic porous media reactor fed with lactate, that most of the lactate was consumed at the front part of the column, and the measurements of SRB biomass on the solid phase, sand, and in the liquid phase, reflected that the maximum concentration of SRB in the solid phase was at the front part of the column, while the maximum in the liquid phase occurred slightly further downstream indicating a SRB detachment phenomenon.

Rivera (1983) studied the heavy metal removal in an upflow anaerobic packed-bed bioreactor, finding that the soluble metal removal took place mainly in the lower regions, within the first 500 mm of the column. The author observed that at steady-state

conditions, more than 80% of the soluble COD was removed within the first 300 mm of the column with 120 mm diameter, and concluded that most of the biosolids were located at the bottom of the column.

6.4.9 Heavy Metals Removal

El Bayoumy (1997) developed equations to calculate the critical reactor height required to remove certain heavy metals i.e. chromium, copper, nickel, zinc, cadmium and lead. Based on that critical height, which is a function of the initial concentration of the heavy metal, and considering the mass of sulfide required to remove certain mass of heavy metal, the reactor design could be established.

The mass of sulfide required for metal precipitation as metal sulfide can be calculated according to the following equation given by El Bayoumy (1997):

$$\Delta S_r = \Delta M_r * \left[\frac{W_s}{W_m} \right] = \left[(C_i - C_e) * \frac{W_s}{W_m} \right] * Q_i * \Delta t \quad \text{.....6.14}$$

Where,

ΔS_r = mass of sulfide required for heavy metals removal [mg]

ΔM_r = mass of heavy metal to remove [mg]

W_s = molecular mass of sulfide [mg/mgmol]

W_m = molecular mass of heavy metal [mg/mgmol]

C_i = influent heavy metal concentration [mg/L]

C_e = effluent heavy metal concentration [mg/L]

Q_i = flow rate [L/d]

Δt = time [d]

Considering the optimum retention time of 20 h with a corresponding flow of 12.7 L/d, influent metal concentrations of 75-100 mg Cr/L, 200-350 mg Cu/L, 150-200 mg Ni/L, 150-200 mg Zn/L, 50-75 mg Cd/L and 40-50 mg Pb/L, which are within the non-toxic levels as recommended by El Bayoumy (1997), would be >90% removed in an upflow anaerobic suspended growth reactor with maximum sulfide concentrations of 190 mg/L.

Using the equations developed previously by El Bayoumy (1997) for metal applications, an active height of 300 mm would allow 100% removal of the heavy metal applied at an influent concentrations of 48 mg Cr/L, 95 mg Cu/L, 90 mg Ni/L, 88 mg Zn/L, 44 mg Cd/L and 29 mg Pb L

CHAPTER 7. CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

1. A long process was required to develop a healthy SRB culture, basically due to the type of substrate used and the type of seed used to start the batch anaerobic reactors. A healthy SRB activity was considered only after a sulfide concentration of 120-130 mg/L was reached in the batch anaerobic reactors.
2. No significant difference in concentrations along the reactors' height was found during the batch operation of three 10 L reactors regardless of the type of SRB growth involved, attached or suspended. However, a portion of the biomass had settled at the bottom.
3. From the mass and electron balances during the batch study, it was observed that 70% of the lactate fed was bio-oxidized while the 30% was bio-synthesized, which indicates that the energy production reaction predominated over the synthesis reaction in non-packed reactors.
4. The experimental lactate to sulfate ratio was equal to 0.87 and the corresponding Th.O.D.:SO₄ was equal to 0.94, which is below 2 as suggested by Annachhatre and Suktrakoolvait (2001).

5. In the continuous operation of three 10 L anaerobic reactors, an acclimatized culture of sulfate reducing bacteria, SRB, could reduce the inflow sulfates to sulfide effluent concentrations up to 180-190 mg of S/L, both in packed and non-packed reactors. The effect of the hydraulic retention time on the sulfide production, used as an indicator of an upflow anaerobic suspended growth reactor performance, was evident.
6. The SRB had poor attachment properties and the presence of a support media did not show any increase in effluent sulfide concentrations and thus any improvement in the performance of the system. The additional cost of packing material is not justified.
7. The maximum rate of sulfide production, 2230 mg S/d on an average, was not dependent on the type of SRB growth involved, attached or suspended, and it was achieved at an optimum HRT of 20 h with a corresponding upflow velocity of 1.5 m/d. Below 10 h of HRT, the SRB activity, and therefore the concentration of sulfide, became so low that the overall performance of the system was not satisfactory. The loss of SRB activity at HRT below 10 h was mainly due to the washout of biomass caused by the high upflow velocity, above 3 m/d.
8. It was observed that doubling the OLR increased proportionally the rate of sulfide production. Similarly, the active volume had increased as a result of poorer

settling characteristics of the biomass and, therefore, an increase in the solids distribution.

9. The substrate utilization rate constant, k , which was obtained through a plug flow reactor model for VSS up to the first port, 300 mm above the reactor's bottom, was found to be 2.53 h^{-1} on an average, for the optimum retention time of 20 h. The average specific growth rate, μ , was 0.177 h^{-1} .

7.2 Recommendations

1. Experiments should be conducted to determine the actual metal application for the operation of an upflow anaerobic suspended growth reactor under the conditions studied.
2. Further work is required to determine biomass concentrations near the bottom of the reactor and its influence on the performance of the system.
3. Studies are required on the behavior of SRB fed with a specific industrial wastewater, to determine the suitability of this system for those particular carbon and sulfate sources.
4. The scale-up study of the present design should be considered and further work is required to establish the necessary procedures for start-up, adaptation and optimum performance at an industrial scale.
5. Studies are required to determine the effect of temperature in the performance of the system.

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APPENDIX A

CALIBRATION CURVES

The following calibration curves were prepared during this study.

A.1 Pump Calibration

For substrate pumping during the continuous mode operation of the upflow anaerobic reactors, two peristaltic pumps were used. These were calibrated every week in order to control any fluctuations in the flow rate. The calibration was done by measuring the flow rate at different pump controller settings and plotting the flow rate vs the setting. Figures A.1 and A.2 show typical calibration curves.

Controller 1
(one head for Reactor III)

Setting	Flow mL/min
0	3
1	14
2	30
3	52
4	69
5	86
6	108
7	130
8	150
9	164
10	168

Figure A.1 Pump Calibration Curve. Pump 1.

Controller 2
(two heads for Reactors
I and II)

Setting	Flow mL/min
0	15
1	14
2	31
3	40
4	66
5	87
6	108
7	126
8	144
9	162
10	170

Figure A.2 Pump Calibration Curve. Pump 2.

A.2 Sulfate Calibration Curve

Sulfate was analyzed according to the Standard Turbidimetric Method 4500-SO₄²⁻ E (APHA, 1995). In order to determine the SO₄²⁻ concentration, the NTU turbidity reading was compared with a standard curve prepared previously for different concentrations of standard sulfate solutions. The standard curve was prepared according to the Standard Methods (APHA, 1995) and is shown in Figure A.3. The equation obtained from the linear fitting of the points was used to calculate the sulfate concentration, x , based on the NTU, y , reading. The sulfate calibration curve was updated every two weeks or when a new batch of analyses was performed.

Figure A.3 Sulfate Calibration Curve

APPENDIX B

DATA

B.1 Phase I

Table B.1 Sulfide Production During Phase I (2 L Batch Reactors)

Time (days)	Sulfide Concentration (mg/L)		
	Reactor I	Reactor II	Reactor III
5	63	41	18
10	78	79	57
15	75	66	57
20	24	35	54
26	72	50	54
32	80	76	88
38	95	98	123
46	123	130	123
56	99	114	84
63	100	115	90

Reactor I. Packed. Deionized water for dilution.

Reactor II. Non-packed. Deionized water for dilution.

Reactor III. Non-packed. Tap water for dilution.

Table B.2 ORP Progression During Phase I (2 L Batch Reactors)

Time (days)	ORP (mV)		
	Reactor I	Reactor II	Reactor III
5	-366 (25 °C)	-345 (25 °C)	-351 (25 °C)
10	-304 (25 °C)	-314 (25 °C)	-295 (25 °C)
15	-335 (23 °C)	-323 (23 °C)	-308 (20 °C)
20	-370 (19 °C)	-371 (20 °C)	-359 (19 °C)
26	-367 (25 °C)	-375 (24 °C)	-371 (25 °C)
32	-372 (20 °C)	-375 (19 °C)	-372 (20 °C)
38	-381 (22 °C)	-381 (23 °C)	-381 (22 °C)
46	-382 (21 °C)	-383 (19 °C)	-383 (19 °C)
56	-379 (19 °C)	-381 (22 °C)	-379 (22 °C)
63	-380 (20 °C)	-380 (20 °C)	-380 (19 °C)

Table B.3 pH Progression During Phase I (2 L Batch Reactors)

Time (days)	pH		
	Reactor I	Reactor II	Reactor III
5	7.0	7.0	7.0
10	7.0	7.0	6.7
15	6.5	7.0	6.5
20	7.0	7.0	7.0
26	7.0	7.0	6.7
32	6.8	7.1	6.9
38	7.0	7.0	7.0
46	7.0	7.0	7.0
56	6.9	7.1	6.6
63	6.5	7.0	6.5

B.2 Phase II

Table B.4 Sulfide Production During Phase II (1st and 2nd Sequences)

Time (days)	Sulfide Concentration (mg/L)		
	Reactor I	Reactor II	Reactor III
0	0	0	0
7	32	12	2
14	35	21	3
21	32	5	8
28	67	47	38
32	88	70	81
36	97	67	75
40	78	66	71
44	81	47	60
48	88	50	51
52	84	72	62
56	85	80	66

Reactor I. Packed.

Reactor II. Non-packed.

Reactor III Packed. Packing material removal on Day 44.

Table B.5 ORP Progression During Phase II (1st and 2nd Sequences)

Time (days)	ORP (mV)		
	Reactor I	Reactor II	Reactor III
7	-328 (25 °C)	-328 (25 °C)	-290 (25°C)
14	-336 (20 °C)	-278 (20 °C)	-283 (20 °C)
21	-329 (20 °C)	-301 (19 °C)	-288 (19 °C)
28	-326 (19 °C)	-297 (18 °C)	-314 (19 °C)
32	-327 (19 °C)	-318 (19 °C)	-319 (19 °C)
36	-315 (23 °C)	-323 (23 °C)	-324 (23 °C)
40	-326 (19 °C)	-322 (19 °C)	-352 (19 °C)
44	-325 (19 °C)	-299 (19 °C)	-350 (19 °C)
48	-343 (18 °C)	-288 (19 °C)	-342 (18 °C)
52	-350 (19 °C)	-292 (19 °C)	-345 (19 °C)
56	-351 (19 °C)	-363 (18 °C)	-353 (18 °C)

Table B.6 pH Progression During Phase II (1st and 2nd Sequences)

Time (days)	pH		
	Reactor I	Reactor II	Reactor III
0	7.1	7.1	6.5
7	6.8	6.7	6.7
14	6.9	7.0	6.8
21	7.0	6.5	6.8
28	7.0	6.7	6.8
32	7.0	6.8	7.0
36	7.0	6.7	7.0
40	7.0	6.5	7.0
44	7.0	7.0	7.0
48	7.0	7.0	7.0
52	7.0	6.0	7.0
56	7.0	7.0	7.0

Table B.7 Sulfide Production During Phase II (3rd and 4th Sequences)

Time (days)	Sulfide Concentration (mg/L)		
	Reactor I	Reactor II	Reactor III
60	125	74	113
64	120	32	45
69	141	82	73
72	139	89	69
76	129	75	65
88	136	89	83
92	144	101	110
96	170	107	119
108	187	114	142
114	152	102	105
117	173	118	79
122	184	124	133
125	200	151	148
128	208	158	154
149	164	185	169
154	154	177	138

Reactor I. Packed.

Reactor II Non-packed.

Reactor III Non-packed.

Table B.8 VSS Production During Phase II (3rd and 4th Sequences)

Time (days)	VSS Concentration (mg/L)		
	Reactor I	Reactor II	Reactor III
60	54	31	81
64	21	17	85
69	21	17	84
92	11	22	55
96	12	25	50
108	12	33	56
114	12	34	64
117	14	48	81
122	15	63	92
125	26	74	99
128	28	82	108
149	14	48	94
154	11	38	62
160	11	28	64

Table B.9 pH Progression During Phase II (3rd and 4th Sequences)

Time (days)	pH		
	Reactor I	Reactor II	Reactor III
60	6.4	6.2	6.4
64	7.0	7.0	7.0
76	6.5	6.3	6.2
88	6.1	6.2	6.1
92	6.2	6.2	6.0
96	6.4	6.4	6.3
108	6.1	6.4	6.2
114	6.1	6.3	6.0
117	5.7	6.2	5.9
122	5.5	6.0	5.9
125	5.8	6.0	6.0
128	5.9	6.0	6.0
149	5.0	5.1	5.0
154	5.3	5.3	5.1
160	5.0	5.0	5.0

Table B.10 Sulfide Production During Phase II (Consecutive)

Time (days)	Sulfide Concentration (mg/L)		
	Reactor I	Reactor II	Reactor III
0	144	101	110
1	147	95	91
2	150	99	99
3	158	101	103
4	170	107	119

Table B.11 VSS Production During Phase II (Consecutive)

Time (days)	VSS Concentration (mg/L)		
	Reactor I	Reactor II	Reactor III
0	11	22	55
1	15	22	51
2	16	25	48
3	14	24	48
4	12	25	50

Table B.12 ORP Progression During Phase II (Consecutive)

Time (days)	ORP (mV)		
	Reactor I	Reactor II	Reactor III
0	-346 (23 °C)	-309 (23 °C)	-337 (24 °C)
1	-362 (23 °C)	-312 (23 °C)	-340 (23 °C)
2	-363 (22 °C)	-328 (22 °C)	-300 (23 °C)
3	-341 (22 °C)	-301 (22 °C)	-307 (22 °C)
4	-350 (19 °C)	-315 (20 °C)	-287 (20 °C)

Table B.13 pH Progression During Phase II (Consecutive)

Time (days)	pH		
	Reactor I	Reactor II	Reactor III
0	6.2	6.2	6.0
1	6.1	6.2	6.1
2	6.1	6.2	6.2
3	6.2	6.3	6.2
4	6.4	6.4	6.3

Table B14. VSS and Sulfide Relation for Reactor I (New Substrate Formulation)

VSS (mg/L)	Sulfide (mg/L)
13	154
16	175
18	185
25	202
29	209

Table B15. VSS and Sulfide Relation for Reactor II (New Substrate Formulation)

VSS (mg/L)	Sulfide (mg/L)
35	104
46	110
61	123
75	152
84	159

Table B.16 VSS and Sulfide Relation for Reactor III (New Substrate Formulation)

VSS (mg/L)	Sulfide (mg/L)
72	105
84	112
90	130
104	150
112	155

B.3 Phase III

Table B.17 Effluent and First Port Sulfide Concentrations. Set 1

SULFIDES (mg/L): EFFLUENT				SULFIDES (mg/L): FIRST PORT			
Time (d)	Reactor I (50 h)	Reactor II (50 h)	Reactor III (30 h)	Time (d)	Reactor I (50 h)	Reactor II (50 h)	Reactor III (30 h)
0	144	102	164	0	137	72	160
2	106	61	56	2	46	68	56
4	31	38	15	4	44	46	41
7	32	47	32	7	50	68	52
9	54	77	72	9	70	59	55
11	56	78	154	11	68	49	82
13	83	92	170	13	114	101	151
15	102	114	181	15	150	114	167
17	125	135	174	17	154	104	163
19	137	132	192	19	165	119	190
21	155	141	176	21	165	144	190
23	142	130	186	23	153	121	195
26	142	128	180	26	152	116	149
28	142	146	174	28	160	130	155
average last 3 values	142	135	180	average last 3 values	155	122	166

Table B.18 Effluent and First Port VSS Concentrations. Set 1

VSS (mg/L): EFFLUENT				VSS (mg/L): FIRST PORT			
Time (d)	Reactor I (50 h)	Reactor II (50 h)	Reactor III (30 h)	Time (d)	Reactor I (50 h)	Reactor II (50 h)	Reactor III (30 h)
0	17	45	84	0	7	36	52
2	15	48	10	2	10	36	25
4	46	17	20	4	57	29	29
7	29	43	52	7	23	54	34
9	38	27	53	9	31	29	24
11	22	40	22	11	21	19	16
13	49	60	27	13	50	68	40
15	52	53	30	15	57	48	27
17	49	41	25	17	62	63	26
19	50	53	19	19	58	89	24
21	62	49	18	21	89	54	18
23	54	49	15	23	90	63	23
26	56	45	19	26	67	50	46
28	80	91	41	28	86	81	58
average last 3 values	63	62	25	average last 3 values	81	65	42

Table B.19 Effluent and First Port pH Values. Set 1

pH: EFFLUENT				pH: FIRST PORT			
Time (d)	Reactor I (50 h)	Reactor II (50 h)	Reactor III (30 h)	Time (d)	Reactor I (50 h)	Reactor II (50 h)	Reactor III (30 h)
0	6.3	6.4	5.7	0	6.3	6.2	6.2
2	6.1	6.7	6.7	2	6.4	6.5	6.4
4	6.2	6.8	6.3	4	5.7	5.8	6.3
7	6.0	6.2	6.1	7	5.7	5.5	6.3
9	6.1	6.5	6.3	9	6.0	6.4	6.4
11	6.2	6.3	6.5	11	6.0	6.3	6.5
13	6.0	6.1	6.4	13	5.8	6.1	6.4
15	5.9	6.1	6.2	15	5.8	5.9	6.4
17	5.9	6.0	6.4	17	5.7	6.0	6.4
19	6.1	6.2	6.5	19	5.7	5.9	6.3
21	5.9	6.0	6.5	21	5.6	5.6	6.2
23	5.8	5.9	6.2	23	5.6	5.7	6.1
26	5.9	6.1	6.1	26	5.6	6.0	5.9
28	5.6	5.7	6.0	28	5.3	5.3	5.4
average last 3 values	5.8	5.9	6.1	average last 3 values	5.5	5.7	5.8

Table B.20 Effluent ORP Values. Set 1

ORP (mV): EFFLUENT				TEMPERATURE (°C)		
Time (d)	Reactor I (50 h)	Reactor II (50 h)	Reactor III (30 h)	Reactor I (50 h)	Reactor II (50 h)	Reactor III (30 h)
0	-322	-314	-345	19	19	19
2	-307	-303	-286	21	21	21
4	-230	-278	-230	22	22	22
7	-230	-281	-243	20	20	20
9	-250	-289	-266	20	20	20
11	-261	-291	-331	19	19	18
13	-271	-289	-347	20	20	20
15	-296	-307	-356	21	21	21
17	-303	-311	-365	19	19	19
19	-306	-312	-363	19	19	19
21	-308	-315	-366	19	19	19
23	-314	-318	-363	20	20	20
26	-303	-314	-342	18	18	19
28	-302	-336	-379	19	19	20
average last 3 values	-306	-323	-361	19	19	19

Table B.21 First Port ORP Values. Set 1

ORP (mV): FIRST PORT				TEMPERATURE (°C)		
Time (d)	Reactor I (50 h)	Reactor II (50 h)	Reactor III (30 h)	Reactor I (50 h)	Reactor II (50 h)	Reactor III (30 h)
0	-324	-316	-348	19	19	19
2	-275	-310	-294	21	21	21
4	-232	-285	-223	22	22	22
7	-253	-289	-231	20	20	20
9	-266	-288	-282	20	20	20
11	-280	-292	-303	19	19	18
13	-281	-309	-331	20	20	20
15	-309	-317	-344	21	21	21
17	-314	-317	-349	20	20	20
19	-318	-317	-351	19	19	19
21	-320	-323	-354	20	20	20
23	-319	-320	-351	20	20	20
26	-307	-317	-348	18	19	19
28	-309	-340	-370	19	19	20
average last 3 values	-312	-325	-356	19	19	20

**Table B.22 Analyses along Reactors' Height
During Steady State. Set 1**

Sulfide (mg/L)			
Sampling Port	Reactor I (50 h)	Reactor II (50 h)	Reactor III (30 h)
effluent	142	166	164
3rd port	160	207	187
2nd port	156	190	201
1st port	160	198	155

VSS (mg/L)			
Sampling Port	Reactor I (50 h)	Reactor II (50 h)	Reactor III (30 h)
effluent	80	91	41
3rd port	83	61	46
2nd port	67	64	51
1st port	86	81	58

pH			
Sampling Port	Reactor I (50 h)	Reactor II (50 h)	Reactor III (30 h)
effluent	5.6	5.7	6.0
3rd port	5.4	5.5	5.6
2nd port	5.3	5.5	5.3
1st port	5.3	5.3	5.4

ORP (mV)			
T (°C)			
Sampling Port	Reactor I (50 h)	Reactor II (50 h)	Reactor III (30 h)
effluent	-302	-336	-379
	19	19	20
3rd port	-311	-338	-375
	19	19	20
2nd port	-308	-337	-377
	19	20	20
1st port	-309	-340	-370
	19	19	20

Table B.23 Effluent and First Port Sulfide Concentrations. Set 2

SULFIDES (mg/L): EFFLUENT				SULFIDES (mg/L): FIRST PORT			
Time (d)	Reactor I (30 h)	Reactor II (30 h)	Reactor III (20 h)	Time (d)	Reactor I (30 h)	Reactor II (30 h)	Reactor III (20 h)
0	163	173	137	0	148	161	159
2	66	158	147	2	51	161	153
4	103	170	146	4	105	177	137
6	127	160	158	6	143	172	126
8	155	190	140	8	144	181	99
10	178	190	167	10	156	185	118
13	187	195	155	13	179	171	121
15	186	193	166	15	181	187	110
17	189	180	150	17	183	188	120
20	190	185	160	20	186	187	130
average last 3 values	188	186	159	average last 3 values	183	187	120

Table B.24 Effluent and First Port VSS Concentrations. Set 2

VSS (mg/L): EFFLUENT				VSS (mg/L): FIRST PORT			
Time (d)	Reactor I (30 h)	Reactor II (30 h)	Reactor III (20 h)	Time (d)	Reactor I (30 h)	Reactor II (30 h)	Reactor III (20 h)
0	73	83	38	0	106	94	38
2	36	51	85	2	37	29	24
4	46	52	14	4	47	16	28
6	43	28	16	6	33	34	34
8	16	4	14	8	22	11	19
10	30	7	14	10	29	15	27
13	41	11	17	13	33	25	33
15	34	11	13	15	34	35	20
17	29	12	22	17	48	40	33
20	38	17	20	20	31	42	36
average last 3 values	34	13	18	average last 3 values	38	39	30

Table B.25 Effluent and First Port pH Values. Set 2

pH: EFFLUENT				pH: FIRST PORT			
Time (d)	Reactor I (30 h)	Reactor II (30 h)	Reactor III (20 h)	Time (d)	Reactor I (30 h)	Reactor II (30 h)	Reactor III (20 h)
0	5.6	4.9	5.9	0	5.4	4.6	5.0
2	6.3	6.7	6.9	2	6.2	6.6	6.8
4	6.2	6.6	6.8	4	5.8	6.4	6.4
6	5.9	6.5	6.7	6	5.7	6.3	6.3
8	5.9	6.5	6.2	8	5.7	6.4	6.1
10	6.1	6.7	6.6	10	6.0	6.5	6.5
13	6.2	6.7	6.7	13	6.0	6.6	6.6
15	6.3	6.8	6.6	15	6.1	6.6	6.6
17	6.2	6.9	6.6	17	6.1	6.4	6.5
20	6.3	6.7	6.7	20	6.2	6.6	6.5
average last 3 values	6.3	6.8	6.6	average last 3 values	6.1	6.5	6.5

Table B.26 Effluent ORP Values. Set 2

ORP (mV): EFFLUENT				TEMPERATURE (° C)		
Time (d)	Reactor I (30 h)	Reactor II (30 h)	Reactor III (20 h)	Reactor I (30 h)	Reactor II (30 h)	Reactor III (20 h)
0	-332	-371	-378	19	19	19
2	-305	-363	-369	21	21	21
4	-327	-386	-374	21	21	21
6	-322	-381	-363	19	19	19
8	-316	-378	-352	20	20	20
10	-328	-381	-363	19	19	19
13	-338	-386	-368	18	18	18
15	-341	-387	-369	18	18	18
17	-343	-388	-372	22	22	22
20	-354	-391	-377	19	19	18
average last 3 values	-346	-388	-373	20	20	19

Table B.27 First Port ORP Values. Set 2

ORP (mV): FIRST PORT				TEMPERATURE (° C)		
Time (d)	Reactor I (30 h)	Reactor II (30 h)	Reactor III (20 h)	Reactor I (30 h)	Reactor II (30 h)	Reactor III (20 h)
0	-327	-371	-376	19	19	19
2	-306	-360	-367	20	20	20
4	-326	-370	-386	22	22	21
6	-328	-371	-332	19	19	19
8	-329	-374	-341	20	20	20
10	-331	-374	-343	19	19	19
13	-341	-380	-345	19	19	19
15	-337	-377	-329	19	19	19
17	-350	-381	-353	21	21	21
20	-355	-383	-351	18	19	18
average last 3 values	-347	-380	-345	20	19	19

**Table B.28 Analyses along Reactors' Height
During Steady State. Set 2**

Sulfide (mg/L)			
Sampling Port	Reactor I (30 h)	Reactor II (30 h)	Reactor III (20 h)
effluent	222	185	177
3rd port	198	197	168
2nd port	183	183	149
1st port	197	187	141
VSS (mg/L)			
Sampling Port	Reactor I (30 h)	Reactor II (30 h)	Reactor III (20 h)
effluent	38	17	21
3rd port	40	23	22
2nd port	36	20	33
1st port	31	22	36
pH			
Sampling Port	Reactor I (30 h)	Reactor II (30 h)	Reactor III (20 h)
effluent	6.3	6.7	6.7
3rd port	6.3	6.8	6.6
2nd port	6.3	6.7	6.7
1st port	6.2	6.6	6.5
ORP (mV)			
T (°C)			
Sampling Port	Reactor I (30 h)	Reactor II (30 h)	Reactor III (20 h)
effluent	-354	-391	-377
	19	19	18
3rd port	-349	-385	-366
	19	20	20
2nd port	-353	-383	-361
	20	20	19
1st port	-355	-383	-351
	18	19	18

Table B.29 Effluent and First Port Sulfide Concentrations. Set 3

SULFIDES (mg/L): EFFLUENT				SULFIDES (mg/L): FIRST PORT			
Time (d)	Reactor I (20 h)	Reactor II (20 h)	Reactor III (10 h)	Time (d)	Reactor I (20 h)	Reactor II (20 h)	Reactor III (10 h)
0	196	187	116	0	181	180	125
2	183	183	108	2	181	149	53
4	193	172	82	4	177	160	73
6	192	175	82	6	187	141	66
9	191	182	75	9	161	150	47
11	197	185	75	11	181	160	67
13	197	177	73	13	190	171	79
average last 3 values	195	182	74	average last 3 values	177	160	64

Table B.30 Effluent and First Port VSS Concentrations. Set 3

VSS (mg/L): EFFLUENT				VSS (mg/L): FIRST PORT			
Time (d)	Reactor I (20 h)	Reactor II (20 h)	Reactor III (10 h)	Time (d)	Reactor I (20 h)	Reactor II (20 h)	Reactor III (10 h)
0	43	46	38	0	154	26	41
2	38	20	20	2	38	34	10
4	24	13	23	4	21	16	41
6	23	16	18	6	15	30	24
9	16	10	16	9	26	18	11
11	16	10	19	11	24	36	21
13	17	12	20	13	25	35	20
average last 3 values	16	11	18	average last 3 values	25	30	17

Table B.31 Effluent and First Port pH Values. Set 3

pH: EFFLUENT				pH: FIRST PORT			
Time (d)	Reactor I (20 h)	Reactor II (20 h)	Reactor III (10 h)	Time (d)	Reactor I (20 h)	Reactor II (20 h)	Reactor III (10 h)
0	6.4	6.7	6.7	0	6.2	6.6	6.5
2	6.3	6.8	6.9	2	6.3	6.7	6.8
4	6.7	6.9	6.9	4	6.7	6.9	6.7
6	6.7	6.8	6.8	6	6.7	6.8	6.7
9	6.8	6.9	6.9	9	6.7	6.8	6.8
11	6.8	6.7	6.8	11	6.7	6.7	6.9
13	6.8	6.7	6.8	13	6.7	6.8	6.9
average last 3 values	6.8	6.8	6.8	average last 3 values	6.7	6.8	6.9

Table B.32 Effluent ORP Values. Set 3

ORP (mV): EFFLUENT				TEMPERATURE (°C)		
Time (d)	Reactor I (20 h)	Reactor II (20 h)	Reactor III (10 h)	Reactor I (20 h)	Reactor II (20 h)	Reactor III (10 h)
0	-355	-393	-377	20	20	20
2	-343	-381	-360	22	20	20
4	-359	-381	-350	19	19	19
6	-379	-383	-364	20	20	20
9	-378	-382	-360	20	20	20
11	-376	-381	-353	19	19	19
13	-378	-382	-354	20	19	18
average last 3 values	-377	-382	-356	20	19	19

Table B.33 First Port ORP Values. Set 3

ORP (mV): FIRST PORT				TEMPERATURE (°C)		
Time (d)	Reactor I (20 h)	Reactor II (20 h)	Reactor III (10 h)	Reactor I (20 h)	Reactor II (20 h)	Reactor III (10 h)
0	-355	-389	-374	20	20	20
2	-353	-371	-339	20	20	20
4	-357	-365	-347	19	19	19
6	-369	-368	-331	20	20	20
9	-378	-356	-332	20	20	20
11	-375	-370	-332	19	19	20
13	-376	-359	-330	20	19	20
average last 3 values	-376	-362	-331	20	19	20

**Table B.34 Analyses along Reactors' Height
During Steady State. Set 3**

Sulfide (mg/L)			
Sampling Port	Reactor I (20 h)	Reactor II (20 h)	Reactor III (10 h)
effluent	197	177	73
3rd port	197	171	74
2nd port	181	166	71
1st port	190	171	79
VSS (mg/L)			
Sampling Port	Reactor I (20 h)	Reactor II (20 h)	Reactor III (10 h)
effluent	16	10	19
3rd port	15	12	15
2nd port	17	12	18
1st port	24	36	21
pH			
Sampling Port	Reactor I (20 h)	Reactor II (20 h)	Reactor III (10 h)
effluent	6.7	6.7	6.8
3rd port	6.8	6.6	6.8
2nd port	6.7	6.7	6.7
1st port	6.7	6.7	6.8
ORP (mV)			
T (°C)			
Sampling Port	Reactor I (20 h)	Reactor II (20 h)	Reactor III (10 h)
effluent	-367	-375	-360
	20	20	19
3rd port	-369	-377	-335
	20	20	19
2nd port	-370	-360	-332
	20	20	20
1st port	-372	-367	-337
	20	20	20

Table B.35 Effluent and First Port Sulfide Concentrations. Set 4

SULFIDES (mg/L): EFFLUENT			SULFIDES (mg/L): FIRST PORT		
Time (d)	Reactor II (10 h)	Reactor III (5 h)	Time (d)	Reactor II (10 h)	Reactor III (5 h)
	12 kg/d/m ³	6 kg/d/m ³		12 kg/d/m ³	6 kg/d/m ³
0	159	58	0	82	52
2	53	26	2	12	4
4	160		4	64	
6	170		6	58	
8	165		8	56	
10	162		10	47	
average last 3 values	165		average last 3 values	53	

Table B.36 Effluent and First Port VSS Concentrations. Set 4

VSS (mg/L): EFFLUENT			VSS (mg/L): FIRST PORT		
Time (d)	Reactor II (10 h)	Reactor III (5 h)	Time (d)	Reactor II (10 h)	Reactor III (5 h)
	12 kg/d/m ³	6 kg/d/m ³		12 kg/d/m ³	6 kg/d/m ³
0	22	21	0	34	20
2	19	3	2	24	6
4	30		4	30	
6	29		6	45	
8	30		8	65	
10	29		10	51	
average last 3 values	29		average last 3 values	54	

Table B.37 Effluent and First Port pH Values. Set 4

pH: EFFLUENT			pH: FIRST PORT		
Time (d)	Reactor II (10 h)	Reactor III (5 h)	Time (d)	Reactor II (10 h)	Reactor III (5 h)
	12 kg/d/m ³	6 kg/d/m ³		12 kg/d/m ³	6 kg/d/m ³
0	6.5	6.5	0	6.3	6.2
2	7.1	7.2	2	7.0	7.4
4	7.0		4	6.9	
6	7.1		6	7.1	
8	7.0		8	6.9	
10	6.9		10	6.8	
average last 3 values	7.0		average last 3 values	6.9	

Table B.38 Effluent ORP Values. Set 4

ORP (mV): EFFLUENT			TEMPERATURE (°C)	
Time (d)	Reactor II (10 h)	Reactor III (5 h)	Reactor II (10 h)	Reactor III (5 h)
	12 kg/d/m ³	6 kg/d/m ³	12 kg/d/m ³	6 kg/d/m ³
0	-382	-342	19	20
2	-341	-348	20	20
4	-367		20	
6	-362		19	
8	-367		20	
10	-363		20	
average last 3 values	-364		20	

Table B.39 First Port ORP Values. Set 4

ORP (mV): FIRST PORT			TEMPERATURE (°C)	
Time (d)	Reactor II (10 h)	Reactor III (5 h)	Reactor II (10 h)	Reactor III (5 h)
	12 kg/d/m ³	6 kg/d/m ³	12 kg/d/m ³	6 kg/d/m ³
0	-381	-349	20	19
2	-290	83	20	19
4	-341		21	
6	-326		20	
8	-341		21	
10	-303		20	
average last 3 values	-323		20	

**Table B.40 Analyses along Reactors' Height
During Steady State. Set 4**

Sulfide (mg/L)			
Sampling Port	Reactor I	Reactor II (10 h)	Reactor III
effluent	NR	162	NR
3rd port	NR	146	NR
2nd port	NR	127	NR
1st port	NR	47	NR
VSS (mg/L)			
Sampling Port	Reactor I	Reactor II (10 h)	Reactor III
effluent	NR	29	NR
3rd port	NR	35	NR
2nd port	NR	41	NR
1st port	NR	51	NR
pH			
Sampling Port	Reactor I	Reactor II (10 h)	Reactor III
effluent	NR	6.9	NR
3rd port	NR	6.8	NR
2nd port	NR	6.8	NR
1st port	NR	6.8	NR
ORP (mV)			
T (°C)			
Sampling Port	Reactor I	Reactor II (10 h)	Reactor III
effluent	NR	-363	NR
		20	
3rd port	NR	-356	NR
		21	
2nd port	NR	-345	NR
		21	
1st port	NR	-303	NR
		20	

*NR= Not Recorded

Table B.41 Effluent and First Port Sulfide Concentrations.
Set 5

SULFIDES (mg/L): EFFLUENT			SULFIDES (mg/L): FIRST PORT		
Time (d)	Reactor II (12 h)	Reactor II (7.5 h)	Time (d)	Reactor II (12 h)	Reactor II (7.5 h)
	12 kg/d/m ³	12 kg/d/m ³		12 kg/d/m ³	12 kg/d/m ³
0	163	187	0	87	140
2	197	125	2	131	42
4	189	138	4	104	50
7	194	152	7	127	76
average last 3 values	193	138	average last 3 values	121	56

Table B.42 Effluent and First Port VSS Concentrations.
Set 5

VSS (mg/L): EFFLUENT			VSS (mg/L): FIRST PORT		
Time (d)	Reactor II (12 h)	Reactor II (7.5 h)	Time (d)	Reactor II (12 h)	Reactor II (7.5 h)
	12 kg/d/m ³	12 kg/d/m ³		12 kg/d/m ³	12 kg/d/m ³
0	28	20	0	50	33
2	19	26	2	37	20
4	30	18	4	47	16
7	29	14	7	52	14
average last 3 values	26	19	average last 3 values	45	17

Table B.43 Effluent and First Port pH Values.
Set 5

pH: EFFLUENT			pH: FIRST PORT		
Time (d)	Reactor II (12 h)	Reactor II (7.5 h)	Time (d)	Reactor II (12 h)	Reactor II (7.5 h)
	12 kg/d/m ³	12 kg/d/m ³		12 kg/d/m ³	12 kg/d/m ³
0	6.9	7.0	0	6.8	6.9
2	6.7	7.0	2	6.9	7.0
4	6.8	7.1	4	6.8	7.1
7	6.9	7.1	7	6.8	7.0
average last 3 values	6.8	7.1	average last 3 values	6.8	7.0

Table B.44 Effluent ORP Values. Set 5

ORP (mV): EFFLUENT			TEMPERATURE (°C)	
Time (d)	Reactor II (12 h)	Reactor II (7.5 h)	Reactor II (12 h)	Reactor II (7.5 h)
	12 kg/d/m ³	12 kg/d/m ³	12 kg/d/m ³	12 kg/d/m ³
0	-363	-369	20	20
2	-373	-361	20	20
4	-373	-364	22	20
7	-365	-363	20	19
average last 3 values	-370	-363	21	20

Table B.45 First Port ORP Values. Set 5

ORP (mV): FIRST PORT			TEMPERATURE (°C)	
Time (d)	Reactor II (12 h)	Reactor II (7.5 h)	Reactor II (12 h)	Reactor II (7.5 h)
	12 kg/d/m ³	12 kg/d/m ³	12 kg/d/m ³	12 kg/d/m ³
0	-303	-356	20	20
2	-355	-320	21	21
4	-345	-326	22	21
7	-329	-335	20	20
average last 3 values	-343	-327	21	20

**Table B.46 Analyses along Reactors' Height
During Steady State. Set 5**

Sulfide (mg/L)			
Sampling Port	Reactor I	Reactor II (12 h)	Reactor II (7.5 h)
effluent	NR	194	152
3rd port	NR	179	94
2nd port	NR	149	98
1st port	NR	69	76
VSS (mg/L)			
Sampling Port	Reactor I	Reactor II (12 h)	Reactor II (7.5 h)
effluent	NR	29	14
3rd port	NR	52	24
2nd port	NR	50	24
1st port	NR	52	14
pH			
Sampling Port	Reactor I	Reactor II (12 h)	Reactor II (7.5 h)
effluent	NR	6.9	7.1
3rd port	NR	6.9	7.0
2nd port	NR	6.8	7.0
1st port	NR	6.8	7.0
ORP (mV)			
T (°C)			
Sampling Port	Reactor I	Reactor II (12 h)	Reactor II (7.5 h)
effluent	NR	-365	-363
		20	19
3rd port	NR	-357	-357
		21	20
2nd port	NR	-345	-350
		20	20
1st port	NR	-329	-335
		20	20

*NR= Not Recorded

APPENDIX C

STATISTICS

The following statistical calculations were carried out during this study.

Under steady state conditions in the reactor, six different samples were withdrawn from the same port within a period of 15 minutes to determine the confidence in analyses.

C.1 Sulfide Determination

Measurement	mg S/L
1	215
2	207
3	196
4	210
5	200
6	224
n=6	$\bar{x} = 209$

$$\bar{x} = \frac{\sum x}{n}$$

$$s = \left[\frac{\sum (x - \bar{x})^2}{(n - 1)} \right]^{-1/2}$$

where,

s = standard deviation

x = result of each measurement

n = number of measurements

$\bar{x}$ = mean value

It is sufficiently accurate to state that 95% of the values are within $\pm \frac{t \cdot s}{\sqrt{n}}$ where $t = 2.8$

for $n=6$. The use of t compensates for the tendency of a small number of values to underestimate uncertainty (APHA, 1995).

It was determined that the standard deviation value was ± 9.98 mg/L and the 95% confidence interval was ± 11 mg/L of the mean value. Thus, if the difference in results falls within $\pm 5\%$ of the mean value, the variation is considered to be statistical.

C.2 VSS Determination

Measurement	mg VSS/L
1	48.
2	49
3	53
4	45
5	50
6	45
n=6	$\bar{x} = 48$

$$\bar{x} = \frac{\sum x}{n}$$

$$s = \left[\frac{\sum (x - \bar{x})^2}{(n - 1)} \right]^{1/2}$$

where,

s = standard deviation

x = result of each measurement

n = number of measurements

$\bar{x}$ = mean value

It is sufficiently accurate to state that 95% of the values are within $\pm \frac{t^*s}{\sqrt{n}}$ where t= 2.8

for n=6. The use of t compensates for the tendency of a small number of values to underestimate uncertainty (APHA, 1995).

It was determined that the standard deviation value was ± 3.1 mg/L and the 95% confidence interval was ± 3.5 mg/L of the mean value. Thus, if the difference in results falls within $\pm 7\%$ of the mean value, the variation is considered to be statistical.

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