DESIGN OF A BIOREACTOR FOR REDUCING SULPHATE IN CATTLE DRINKING WATER

by

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Abstract

A 5 litre laboratory-scale upflow anaerobic sludge blanket (UASB) bioreactor was constructed and operated for approximately one year to reduce sulphate in water using an agricultural byproduct, silage, as carbon source. The purpose of this water treatment system was to test the suitability of the UASB design to treat simulated ground water with high sulphate concentrations destined to be used as cattle drinking water. The UASB reactor design was selected after performing an extensive literature review of all available sulphate-reduction processes. A previous MASc project (Amber Brown, 2007) demonstrated the suitability of silage as a carbon source for sulphate reducing bacteria and, furthermore, in this thesis, fate of the organic compounds in the silage leachate during sulphate-reduction was determined. Six particular tests were performed in order to quantify the type of organics in the feed and effluent: chemical oxygen demand (COD), total organic carbon (TOC), total carbohydrates, total alcohols, total phenols, and selected organic and volatile fatty acids (VFA). The reactor ran continuously for approximately one year with a constant silage leachate feed COD concentration of 10,000 mg L^{-1}, and sulphate feed concentrations varying from 2,000 to 3,200 mg L^{-1}. The flow rates for each feed stream were maintained at ~0.5 mL min^{-1} for silage leachate and ~1 mL min^{-1} for sulphate feed for most of the experiment. The sulphate reduction rates (SRR) ranged from 368 to 845 mg L^{-1} d^{-1} and the amount of organics consumed was between 80-90%. Sulphide levels in the UASB bioreactor were consistently high for most of the experiment, ranging from 600-800 mg L^{-1}. When the sulphate feed concentration was increased to a maximum of 3,282 (± 27.22) mg L^{-1}, the sulphide concentration within the bioreactor reached a maximum of 1,273 (± 473.5) mg L^{-1}. A sulphide stripping column was introduced midway through the experiment in an attempt to reduce the sulphide concentration in the system. Short-term results were promising, however, prolonged sulphide removal in the system could not be maintained due to operational problems. Interestingly, during the last month of operation, despite the high sulphide levels, the SRR was at its highest with an upward trend.

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List of Symbols and Abbreviations

1+1	equal volumes
ABR	anaerobic baffled reactor
AGLR	anaerobic gas-lift reactor
Ag_2SO_4	silver sulphate
APHA	American public health association
ASBR	anaerobic sequencing batch reactor
Ba^{2+}	barium ion
BaCl	barium chloride
$BaSO_4$	barium sulphate (Barite)
B.C.	British Columbia
$CaCO_3$	calcium carbonate
$CaSO_4$	calcium sulphate (gypsum)
CH_3COOH	acetic acid
$CH_3COONa \cdot 3H_2O$	sodium acetate
Cl^-	chlorine ion
CO_2	carbon dioxide
CO_3^{2-}	carbonate ion
COD	chemical oxygen demand
DO	dissolved oxygen
DM	dry matter
dH_2O	distilled water
Fe^{3+}	iron ion
$FeCl_3$	ferric chloride
FeS	iron sulphide
$FeSO_4 \cdot 7H_2O$	iron sulphate heptahydrate
FSBR	falling sludge bed reactor
GSS	gas solid separation
H^+	proton
HCO_3^-	bicarbonate ion
HDPE	high density polyethylene
HPLC	high performance liquid chromatography
HS^-	hydrogen sulphide ion
H_2O	water
H_2S	hydrogen sulphide
H_2SO_4	sulphuric acid
ID	inner diameter
$K_2Cr_2O_7$	potassium dichromate
KHP	potassium phthalate
KH_2PO_4	potassium phosphate
KNO_3	potassium nitrate
M^+	metal ion
$MgCl_2 \cdot 6H_2O$	magnesium chloride hexahydrate
Mn^{4+}	manganese ion

MS	metal sulphide
N_2	nitrogen gas
Na^+	sodium ion
NaOH	sodium hydroxide
$Na_2S \cdot 9H_2O$	sodium sulphide nonahydrate
Na_2SO_4	sodium sulphate
NO_3^-	nitrate ion
NO_3^--N	ammonia-nitrogen
NPOC	non-purgeable organic carbon
O_2	oxygen
O.D.	optical density
PO_4^{3-}	phosphate
PFR	plug flow reactor
PSD	particle size distribution
S^o	elemental sulphur
S^{2-}	sulphide ion
sCOD	soluble chemical oxygen demand
SO_2	sulphur dioxide
SO_3^{2-}	sulphite
SO_4^{2-}	sulphate
$S_2O_3^{2-}$	thiosulphate
SOB	sulphur oxidizing bacteria
SRB	sulphate reducing bacteria
SRR	sulphate reduction rate
T	time (days)
TEA	terminal electron acceptor
TS	total sulphide
UASB	upflow anaerobic sludge blanket
ZnS	zinc sulphide
±	standard deviation of 3 or more measurements (or difference between two points if specified)

List of Units

^{o}C	degrees Celsius
cm	centimeters
cm d^{-1}	centimeters per day
d	day
g	grams
g L^{-1}	grams per liter
g $L^{-1}d^{-1}$	grams per liter per day
hr	hour
kg	kilograms
kg d^{-1}	kilograms per day
kg d^{-1} m^{-3}	kilograms per day per cubic meter
L	liters
L hr^{-1}	liters per hour
m	meters
m^2	square meters
m s^{-1}	meters per second
mg $d^{-1}g^{-1}$	milligram per day per gram
mg L^{-1}	milligram per liter
mg $L^{-1}d^{-1}$	milligram per liter per day
mg $L^{-1}d^{-1}g^{-1}$	milligram per liter per day per gram
mL min^{-1}	milliliter per minute
mm	millimeter
µm	micrometer
mol	mole
mmol L^{-1}	millimoles per liter
mmol L^{-1} a^{-1}	millimoles per liter per annum
mV	millivolt
N	normal
nm	nanometer
ppm	parts per million

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Chapter 1 Introduction

The availability of suitable drinking water has always been a major concern for livestock farmers. For these farmers, drinking water sources come from either surface lakes or ponds or from underground aquifers through the use of water pumps. As many of these water sources are relatively stagnant, they tend to have higher than normal salinity levels as a result of water evaporation. Sources for increased salinity include highly soluble sulphate salts naturally present in soils, or oxidation of mineral sulphides within rocks and soils, which can be accelerated due to mining activities, for example, leading to very high sulphate concentrations (Baldwin 2003). Recent tests conducted by Agriculture and Agri-Food Canada found sulphate levels higher than 6,000 mg L^{-1} in some surface ponds located in the dry Okanagan region of British Columbia (Baldwin 2003). These were natural ponds subject to very high evaporation rates.

Good quality drinking water is essential for healthy livestock. Although cattle are known to tolerate sulphate levels as high as 3,000 mg L^{-1}, their health declines if they consume too much water of this salinity (Hibbs 1983). Therefore, the government recommends that sulphate concentrations in cattle drinking water be less than 1,000 mg L^{-1}. Despite this, most of the surface as well as underground aquifers in the BC interior as well as in the Prairies that the farmers use as cattle drinking water sources have sulphate levels much higher than this (Brown 2007). As these aquifers are the only viable water source farmers have for their livestock, a suitable sulphate removal treatment process is needed to meet the demand for a healthy and productive herd.

For a typical cattle farm in the BC interior or the Prairies, daily livestock water consumption averages 1,000 imperial gallons (Baldwin 2003) per day. Currently all sulphate removal technologies can be divided into two main groups: chemical and biological processes. Chemical treatment processes for sulphate reduction can be very efficient, however, the high initial equipment cost as well as high operating costs mean that they are unsuitable for cost-conscious small-scale farming operations (Baldwin 2003). By contrast, biological treatment processes have the potential to be more

economical, with lower energy consumption, as well as flexible operating temperatures. The type of carbon source used and its rate of consumption determine the economics of biological sulphate-reduction processes. A previous MASc student, Amber Brown, identified silage as a very suitable carbon source for sulphate-reducing bacteria that farmers would have access to. She tested a trough-based passive treatment system for sulphate reduction, but the slow rates meant that reactor volumes would be too large to be practical (Brown 2007). Therefore, the overall objective of this thesis was to design a more effective active bioreactor to increase sulphate-reduction rates and to decrease the footprint of the reactor vessel so that it can be built and accommodated on a typical farm site. An additional objective was to determine the nature and fate of the organic compounds that compose silage leachate and to calculate their rates of their consumption.

As the target users are farmers, the new treatment system must meet the following requirements:

- low cost [below $3 per 1,000 Igal. (~4,500 L) of water]
- low maintenance
- easily constructed using materials that most farmers would have at their farm
- utilize a cheap and readily available nutrient source
- must meet water quality guidelines for livestock for sulphate, sulphide and nutrients (N, P and C)

The primary focus of this project was to test the first stage in the water treatment process: sulphate reduction. However, sulphate is reduced to sulphide, which still needs to be removed since it is also toxic. Therefore, some additional preliminary experiments were performed to remove sulphide from the effluent to produce low sulphate and low sulphide water.

1.1 Overall Objective

The overall project objective was to develop and operate a cost-effective process to remove sulphate from cattle drinking water. According to the Canadian Water Quality Guidelines, 1,000 mg L^{-1} sulphate is the maximum allowable concentration for livestock drinking water. In addition, the treatment process must also meet the other Canadian Water Quality Guidelines' recommended minimal levels of sulphides (≤0.05 mg L^{-1}) (Health_Canada 1987), total organic carbon (TOC) (4 mg L^{-1}) (Ministry-of-Environment 2001), other nutrients (C, N(13 mg L^{-1}), and P(100 µg L^{-1})) (Environment-Canada 2005)and a pH between 6.0 and 8.5.

1.1.1 Sub-Objectives

- Perform a review of all reactor types used for sulphate-reduction so as to determine the most suitable design specification and configuration for this project
- Construct and operate a bioreactor at various sulphate loading rates to determine its maximum sulphate-reduction capability.
- Analyze samples collected from the system in order to profile the nature of the organics present in the silage leachate feed, reactor sludge and the effluent.
- Compare the efficiency of this lab-scale system to other treatment systems of similar or different designs, and make necessary adjustments and suggestions for field implementation**.**

Chapter 2 Background and Literature Review

Availability of sufficient drinkable water for cattle herds has always been a major concern for farm operators. This concern is escalating in inland regions of British Columbia and in Saskatchewan where access to fresh water sources (either from rainfall or river systems) is limited. One solution many farmers turn to is to tap into large natural aquifers that exist underground. These underground aquifers are essentially storage ponds of water from a variety of sources. Leaching of ions from the surrounding rock and soil can negatively impact the water quality in these aquifers. In some locations of the Okanagan, due to the local mineralogy, surface water ponds within cattle range areas have very high sulphate concentrations (from 100 – 6,000 mg L^{-1}) [Baldwin: personal communication]. Consumption of water high in sulphate by the cattle can lead to serious health risks (AAFC_Drought_Watch 2008). Although studies have shown that some cattle can tolerate sulphate levels as high as 3,000 mg L^{-1} in their drinking water, treated potable water with sulphate concentrations below the government recommended level of 1,000 mg L^{-1} for livestock can significantly reduce these health risks, leading to a more healthy and productive cattle herd (AAFC_Drought_Watch 2008).

This need to treat groundwater with high sulphate concentrations down to the government recommended level of 1,000 mg L^{-1} led to implementation of a pilot-scale passive biological treatment process in Saskatchewan, which was based on a previous MASc student's thesis (Brown 2007). However, this passive treatment process is slow (sulphate reduction rate = 10 mg L^{-1} d^{-1}) and therefore a very large volume reactor is needed to handle the quantity of water required for a typical cattle herd. Therefore, a more active (or high rate) treatment process is prefered due to space limitations on the farm.

This Chapter presents background information and literature that were used to select the new biological reactor configuration, which was built and tested for removal of sulphate from cattle drinking water.

2.1 Sulphur Cycle

Sulphur is one of the most interesting elements because of its chemical complexity, geochemical abundance, and biological importance (Lens 2001). As a result of more than 200 years of scientific research on sulphur and its compounds, a well-established biological sulphur cycle that encompasses all three aspects has been constructed and illustrated in many books and articles. Figure 2-1 below summarizes this information.

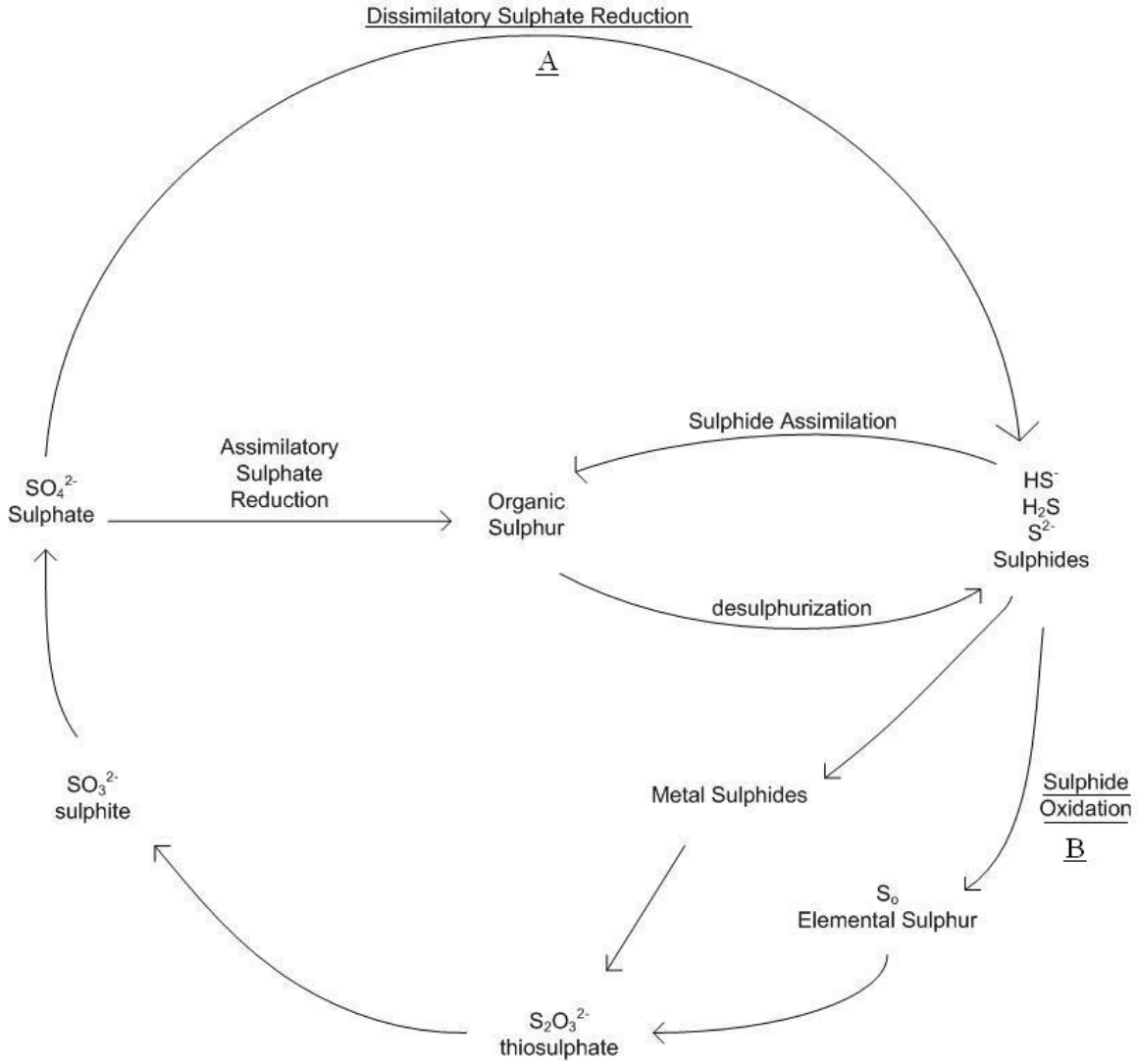

Figure 2-1 The sulphur cycle

Sulphur species of importance to this project are sulphate, sulphide and elemental sulphur. The two lines that connect these three species in this S-cycle are dissimilatory sulphate reduction (Figure 2-1, Line A) and sulphide oxidation (Figure 2-1, Line B), which are the two important steps necessary for a successful biological sulphate reduction

process. In the first step, dissimilatory sulphate reduction, a group of microorganisms called sulphate reducing bacteria (SRB) converts sulphate to sulphide for the purpose of respiration. The overall reactions of this step are given below:

$$CH_3CHOHCOO^- + 0.5\,SO_4^{2-} \rightarrow 0.5HS^- + HCO_3^- + CH_3COO^- + 0.5H^+ \quad \text{(Eqn. 2 -1)}$$

$$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^- \quad \text{(Eqn. 2 - 2)}$$

$$H_2S \Leftrightarrow HS^- + H^+ \quad \text{(Eqn. 2 - 3)}$$

The conversion of sulphate to sulphide occurs under an oxygen free environment; as such H_2S is always the end product of this reaction step (Lens 2001). However, the toxicity and corrosiveness of H_2S means that further reactions are needed to minimize exposure of H_2S to the environment. One method could be the addition of metals to form metal sulphides that will precipitate from the solution, as shown below:

$$HS^- + M^{2+} \rightarrow MS\downarrow + H^+ \quad \text{(Eqn. 2-4)}$$

These metal sulphides are sparingly soluble in water, but they can easily be converted back to sulphate once exposed to air and water. Hence another method of reducing sulphides would be preferred.

The second step in the biological sulphur cycle involves the oxidation of sulphides to elemental sulphur as shown below:

$$H_2S + 0.5O_2 \rightarrow S^0 + H_2O \quad \text{(Eqn. 2-5)}$$

This step can be carried out by using any of many different types of sulphur oxidizing bacteria (SOB) such as a colorless sulphide oxidizing bacteria identified as *Thiobacillus sp*. W5 (Visser 1997). One important fact to note here is that the supply of oxygen to this reaction must be limited to stoichiometric amounts, as excess oxygen will lead to complete oxidation of sulphides back to sulphates, which is not desired.

2.2 Sulphate Reducing Bacteria

The sulphate reducing bacteria (SRB) are a group of microorganisms found in the oxygen free (also known as anaerobic) zones of natural aquatic environments (Barnes 1992). SRB have also been found in the digestive systems of animals (Morvan 1996). According to Nagpal (2000), these anaerobic microorganisms consume low molecular weight organic compounds and "respire" using sulphate or another oxidized sulphur compound as the terminal electron acceptor. Some of the organic compounds used by SRB are lactate, ethanol, and propionate. Studies have shown that SRB can also use simple molecules like CO_2 and H_2 as well as higher molecular weight hydrocarbons (e.g. long chain alkanes, benzoate, phenol) as energy sources (Rueter 1994). SRB grow in the pH range of 5.5 – 9, with optimum growth at pH of 7.5 (Postgate 1984). They are typically found in moderate temperature environments in the range of 25 – 43ºC. However, specific SRB species have been found to exist also in thermophilic as well as psychrophilic regions, but with much lower specific growth rates (Nakagawa 2002, Knoblauch 1999).

2.3 Physical and Biological Treatment Systems for Sulphate Reduction

Sulphate can be removed from water either physically or biologically. Physical treatments are highly effective in removing sulphate, especially when large quantities of water need to be treated. On the other hand, biological treatments have a much lower operating cost. As such, there are many industrial applications that utilize either physical or biological treatments or a combination of both for sulphate removal. Some of these physical and biological treatment applications are discussed below; followed by a comparison between the two treatment types.

2.3.1 Physical Treatment

Physical sulphate removal methods have been in use for a long time. Some of the proven technologies include distillation, ion exchange and nanofiltration. A brief description of each of these technologies is outlined below.

In distillation, water is boiled and cooled until the steam condenses into a separate container of clean potable water. Sulphate is left in the residue. This treatment has the ability to achieve complete sulphate removal. However, considerable amounts of time and energy are required to produce only small amounts of potable water. Hence it is not feasible for treating large quantities of water

Ion exchange is used successfully for sulphate removal in factories, large farms and government facilities. In ion exchange, water passes through a packed bed column filled with an anion exchange resin. The anion in the resin leaves with the effluent water, while sulphate is removed by binding with the cation in the resin. An experiment conducted by Darbi (2003) used a high capacity, type 2 ionic resin comprised of $CaCO_3$ with a total exchange capacity of 1.4 eq L^{-1} and water content between 38 and 45%. Through his experiments, Darbi reported sulphate removal efficiency of 99% within 50 minutes of operation. Commercially available ion exchange units that can remove 2,500 mg L^{-1} of sulphate at a flow rate of 12 gpm typically cost in the range of $1,500 – $2,000 range (RainDance-Water-Systems 2009).

In nanofiltration, water is driven by pressure through a porous membrane with a theoretical pore size of 1nm (Darbi 2003). It performs similarly to reverse osmosis in that the sulphate ions are captured by the membrane as water passes through (Darbi 2003). Currently there are many off-the-shelf nanofiltration units available for industries of varying sizes as well as smaller units for home use. Darbi et al used a nanofiltration unit with Filmtec 2.5” nanofiltration elements from Waters Group in their experiment. Sulphate removal efficiencies of 91% or better were reported (Darbi 2003). Most of the cost associated with nanofiltration comes from membrane replacements as a result of membrane fouling due to heavy metals and scaling from calcium sulphate (Darbi 2003). Current estimates place the cost of treating 1,000 gallons of water for livestock use at $12.

2.3.2 Biological Treatment

Although physical processes are effective for sulphate removal, the initial upfront purchase price of the treatment unit, as well as the ongoing costs of maintenance and

chemicals mean that they are not suitable for small scale farming operations (Baldwin 2003). Biological treatment processes, on the other hand, have much lower operating costs. For example, to treat 4,500 l of water per day with 1,000 mg L^{-1} sulphate removal, a rough cost estimate by Baldwin (2003) puts the cost of nanofiltration at $12, while the patented Paques (http://www.paques.nl/ , The Netherlands) process would cost just $1-$2 for the same quantity.

The many different treatment processes available today for sulphate reduction can be divided into two main types: passive and active treatment. Passive treatment processes have a low capital cost and require minimal maintenance. However, system control difficulties and inconsistent effluent water quality along with the large surface area required because of the long residence times, makes them not ideal for sulphate reduction when the sulphate loading rate is high, as is the case in most cattle drinking water sources. On the other hand, active treatment processes need much shorter residence times and, therefore, have a smaller footprint. In addition, active treatment processes are much easier to control leading to consistent effluent water quality.

Passive treatment processes typically are large water-saturated, surface or sub-surface areas that are either natural or man-made, which rely on SRB growing on natural or waste organics to reduce sulphate. These low cost and low maintenance ponds and wetlands have been used extensively for treatment of industrial and agricultural wastewater. In some cases, surface and sub-surface water ponds have been used in the treatment of acid mine drainage. However, little information is available on sulphate reduction rates in these latter systems since their primary focus was metal removal (Baldwin 2003). Most of the SRB activity occurs in the sediments of these ponds where anaerobic conditions exist. Hence, sulphate reduction depends on water flowing through the anaerobic sediments. However, in surface water wetlands much of the water flows over the top of the sediments and diffusion is the only process by which sulphate is transferred to the location where sulphate-reduction takes place. As a result of this, steep vertical sulphate concentration gradients exist from the water column into the sediment (Brown 2007). Another concern in surface water treatment ponds is the adequate supply of carbon

sources for the SRB, which can be limited. Therefore, for these reasons, surface water ponds are not very successful at reducing sulphate to the extent needed to make cattle drinking water potable. Constructed wetlands are essentially human excavated ponds filled with submerged soil, sand, gravel and organic matter as the sediment mixture (Baldwin 2003). Plants such as cattails and sedges are added to contribute more organic matter, as well as provide neutralizing chemicals needed for metal removal. Addition of waste organic mixtures and seeding of specifically grown SRB enriched cultures should aid in increasing the performance of constructed wetlands. In one study sulphate was reduced by 3,500 mg L^{-1} in 70 days using just a manure and straw mixture (Baldwin 2003). In these types of systems, the water flows vertically, often from the bottom up, through the organic-rich matrix. Inclusion of fresh organics, such as silage and hay for example, contributes to high sulphate reduction rates (Brown 2007). However, steps must be taken to keep these systems anaerobic otherwise sulphide will re-oxidize back to sulphate (Brown 2007). Constructed wetlands can operate for a long time if there is some way of amending them with fresh organics once the original material has degraded completely.

In an active treatment process, a vessel is used to contain the SRB, which are either immobilized as a biofilm on a support material or the microbes stick to each other leading to formation and growth of biomass pellets known as granules. Since the biomass is retained in the reactor, the hydraulic retention time is not equal to the solids retention time, which is much longer. Therefore the reactor is capable of handling wastewater with much higher loading rates than passive treatment processes, which is the reason why active treatment processes are often referred to as “high rate” reactors by some authors. Currently there are a number of active treatment processes in use around the world with varying degrees of success in wastewater treatment.

Major advantages active treatment processes have over passive treatment processes include their ability to adapt to different water treatment quantity demands with simple adjustments of flow rates along with modular reactor designs. This coupled with the active treatment process’ ability to treat large quantities of water at much faster rates

compared to that of passive treatment processes makes them more appropriate for groundwater treatment on farms.

2.4 Modes of Operation

The most important factor during modular reactor design of biological sulphate reduction process is determining which of the three modes the reactor will operate: batch, plug-flow, or continuous. A brief discussion of each mode is listed below leading to the recommended mode of operation for this application.

2.4.1 Batch Reactors

In a batch operation, the reactor is filled with the water to be treated and SRB bacteria inoculum as well as the nutrients that they require are added. There is no flow entering or leaving the reactor (Metcalf and Eddy 2003). After an incubation period, which typically lasts one to two weeks, sulphate reduction takes place as a result of SRB growth (Baldwin 2003). Depending on the initial sulphate concentration, time to completion could be a month or more. Limitations of this type of operation include the need for fresh inoculum for each batch and down-time as a result from reactor cleaning at end of each batch operation (Baldwin 2003).

2.4.2 Plug Flow Reactors

Also known as trench or trough bioreactors, these types of reactors generally have a high length-to-width ratio, and water enters and leaves the reactor horizontally (Brown 2007). As such there is minimal or no axial mixing. If the reaction kinetics are directly proportional to the substrate (sulphate) concentration, then a plug flow configuration achieves greater conversions when compared to completely mixed reactors. However, the SRB must be immobilized inside the plug flow reactor on a stationary support matrix, or continuously added with the feed. Channeling inside a packed bed plug flow reactor may lead to short circuiting and plugging inside the reactor vessel, which reduces the residence time and causes operational problems. Thus, these plug flow reactors have not been used very successfully for sulphate-reduction.

2.4.3 Continuous Stirred Tank Reactors

For a perfectly mixed reactor, as the fluid enters the reactor, it is mixed instantaneously and uniformly with fluids already inside the reactor (Metcalf and Eddy 2003). This type of operation normally runs under steady state conditions, and the uniform mixing means the outflow stream has concentrations equal to those inside the reactor. For sulphate reduction, reactors operating in this mode are preferred as they can have a much smaller footprint, being taller than they are wide, and less downtime for maintenance (Baldwin 2003) due to fewer plugging problems. In addition, sulphate reduction efficiency can be increased or overall residence time reduced by using several reactors in series, which has an effect similar to plug flow without the plugging issues. For biological processes that are sensitive to changes in their environment, a CSTR operating under steady state conditions is advantageous since optimal pH, temperature and nutrient levels are maintained (Baldwin 2003). Therefore, I decided to use a continuous, mixed reactor configuration for this application.

2.5 Selection of the Biological Reactor Configuration

The first treatment process to be tested at the WBDC farm in Saskatchewan was a trough bioreactor with a 4:1 length to width ratio, which was an anaerobic pond; the simplest design for a biological sulphate-reduction process. Its limitation was low sulphate-reduction rate, so that in order to treat the amount of drinking water needed on a cattle farm reactor sizes would be too large to accommodate on site. Cows consume about 55 L $day^{-1}cow^{-1}$ (for beef cattle) and 160 L $day^{-1}cow^{-1}$ (for dairy cattle). Space limitations on a typical farm necessitate a treatment system with a small footprint that can handle higher water flow rates in order to meet the water demands. For this reason an active treatment process was chosen to replace the previous trough bioreactor because of higher sulphate-reduction rates.

A comprehensive literature review was conducted on all types of active sulphate-reduction treatment processes. Table 2-1 below lists examples of these reactor designs together with some performance data.

Table 2-1 Examples of different reactor configurations use for sulphate-reduction

Reactor Type	Source	Temperature (ºC)	pH	Carbon Source	HRT (hr)	Sulphate reduction rate ($g\ L^{-1}\ d^{-1}$)	COD removal (%)
Anaerobic filter	Henry 2000	19-25	7.4-8	Landfill leachate	0.5-2	0.13-2.44	50-93
Fluidized bed	Nagpal 2000	25	~7	Ethanol	5.1	6.33	
UASB	Scheeren 1991	20-38	6-8	Ethanol	4	6.84	
Sequencing batch	Krapivina 2000	35	6.5-8	Waste-water	60	1.39	50-70
Baffled	Fox 1996	35	7-7.25	Acetate	24	4.5	50
Gas lift	van Houten 1997	55	7	H_2/CO_2	4.5	7.5	

An anaerobic filter is a type of anaerobic digester where the biomass is immobilized inside the reactor vessel. A typical filter consists of a column filled with high porosity plastic rings (Henry 2000). This filter medium provides the anaerobic microorganisms with a support structure to grow on. In the study conducted by Henry (2000), their filter, which had a porosity of 88%, was used to treat landfill leachate spiked with sulphate. As shown in Table 2-2 above, the anaerobic filter was successful in removing sulphate while reducing COD at the same time; however, the authors did indicate that the higher reduction rates came from treatment of low-strength landfill leachate where the COD level was below 5,000 mg L^{-1}. When the COD/SO_4^{2-} ratio was below 1, the system achieved the highest sulphate reduction rate of 2.435 g L^{-1} d^{-1}, although at higher COD/SO_4^{2-} ratios, the SRR was much lower (~0.15 – 0.6 g L^{-1} d^{-1}). For high-strength landfill leachate with COD over 5,000 mg L^{-1}, the reduction efficiency was poor (Henry 2000). Since the authors did not report the sulphate concentration in their feed, it is not known what sulphate loading rates can be handled by this type of reactor.

The anaerobic sequencing batch reactor (ASBR) is a type of suspended growth process where the reaction and separation of solids from liquids occurs in the same container (Metcalf and Eddy 2003). Its operation is similar to that of the upflow anaerobic sludge

bed reactor where development of a good dense granulated sludge is key to its success (Metcalf and Eddy 2003). During the operation of the ASBR, wastewater is first fed into the reactor vessel containing the granulated sludge. The wastewater is allowed to react with the sludge with mechanical mixing. Once the reaction is complete, the solids are allowed to settle to the bottom of the vessel. Finally the treated water leaves the vessel through a port at the top of the container, leaving behind the granulated sludge, ready for the next batch of treatment. Krapivina (2007) used three ASBRs of varying sizes to treat yeast wastewater that contained 3.5 - 5.3 g SO_4^{2-} L^{-1} and 14.4 - 25.7 g COD L^{-1}. The 100-day operation period produced an average SRR of 1.39 g L^{-1} d^{-1}, with high sulphate removal efficiency and high COD removal rate. Effluent sulphide levels were consistently below 125 mg L^{-1}, which is much lower than inhibitory levels (Krapivina 2007). Sequencing batch reactors are more complicated to operate than continously operating reactors.

The anaerobic gas-lift reactor (AGLR) operates similar to fluidized bed bioreactors, with one major difference. The reactor is divided into a riser and a downcomer section, with the feed gas entering upwards only in the riser section (Beeftink 1986). In one case, liquid-adhesion sand was used as support material for aggregation of bacteria (Beeftink 1986). To minimize biomass (microbes) and sand washout, effluent leaves the reactor in an upward direction from a "relatively quiescent" section separated from the riser and downcomer section of the reactor (Beeftink 1986). Van Houten et al. (1994) used a gas mixture of 80% H_2 and 20% CO_2 as the energy and carbon source, respectively, in a AGLR. Their system obtained a maximum sulphate reduction rate of 30 g SO_4^{2-} L^{-1} d^{-1} under mesophilic conditions (van Houten 1994; van Houten 1997). Despite their superior performance, gas lift bioreactors are not suitable for on-farm use, since purchasing and transporting H_2 and CO_2 gases would be very expensive.

Therefore, reactor designs with liquid or low solids content feeds are more appropriate for farmers. Then they can use a farm-based organic, such as silage or hay, for a carbon and nutrient source. In particular, we decided to use silage leachate, since dissolved organic compounds are more readily available to bacteria than more recalcitrant

cellulosic solids, which need to be hydrolyzed: a kinetically slow process. In addition, excluding solids from entering the reactor will prevent plugging problems that were experienced with the trough bioreactor. Batch or sequencing batch reactors were excluded from our choice of appropriate designs due to their more complicated operation. Therefore, from the list of reactor configurations given in Table 2-1, the two deemed most appropriate for this application were the anaerobic baffled reactor (ABR) and the upflow anaerobic sludge blanket reactor (UASB). These two reactors were chosen because their designs allow improved mixing that needs no mechanical moving parts within the reactor and there is the potential for granular sludge formation in the reactor. The formation of granulated sludge, discussed later in this chapter, is the key to improved loading rates of these reactors.

2.5.1 Anaerobic Baffled Reactor

The anaerobic baffled reactor (ABR), first developed by McCarty at Stanford University in the late 1970s (Barber 1999), was based on the plug flow reactor design, with the addition of vertical baffles along the length of the reactor. In this modified plug flow reactor design, water flows under and over the baffles as it passes from the inlet to the outlet, as shown in Figure 2-2(A) below. Various attempts have been made to modify this original ABR reactor design in order to improve its performance. Better solids retention capacity, ability to treat difficult wastewaters, and capital cost reduction were some of the reasons for the modifications (Barber 1999). Figure 2-2(B) and 2-2(C) depict two modified ABR reactor designs.

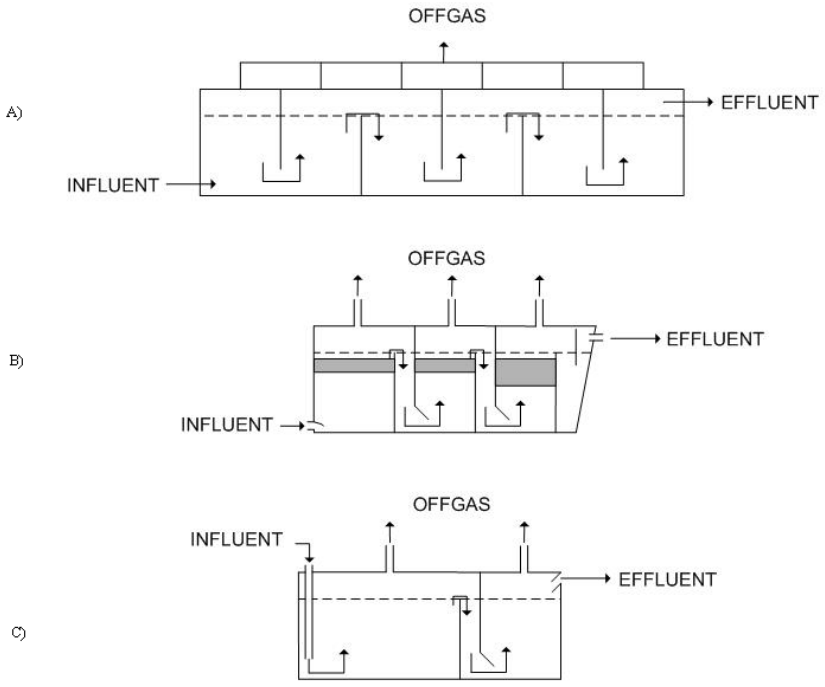

Figure 2-2 Different designs of the anaerobic baffled reactor.
A) Original Design, B) Hybrid, C) Enlarged first compartment.
Source: Barber 1999

According to Barber (1999), the ABR design has been used to treat various types of wastewater, albeit on a pilot-scale basis only. These include low-strength wastewaters such as greywater (Witthauer 1982), sucrose (Orozco 1988), and high-strength wastewaters such as swine and raw molasses (Boopathy 1992). The COD removal for treating these wastewaters varied depending on the hydraulic retention time and the operating temperature, summarized in Table 2-2.

Table 2-2 Anaerobic baffled reactor performance data

Wastewater type	Reactor volume	Number of chambers	COD removal (%)	HRT (hr)	Temperature (ºC)
Graywater	8	6	63 - 84	48 - 84	25 - 33
Sucrose	75	11	85-93	6-12	13-16
Swine	15	2-3	62-69	360	35
Raw molasses	150	3	40-88	24-144	37

Source: Barber 1999

Results shown above indicate the ABR design has the ability to treat a wide variety of wastewater types under mostly mesophilic conditions. However, there are few applications of this design for sulphate reduction. In the only one case that I found, Fox (1996) used an ABR to treat sulphate-containing pharmaceutical wastewater. They found that 1,900 mg L^{-1} of sulphate was reduced in one pass, which lead to an overall sulphate reduction efficiency of 95% and a COD removal efficiency of 50%. A sulphide oxidation step removed the sulphide as elemental sulphur. However, they reported that most of the sulphate was reduced in the first chamber. Likely, the vertical upflow of liquid in the first chamber was responsible for the improved rates of sulphate reduction over a horizontal flow system. The advantage of multiple chambers is to increase the residence time without increasing the reactor height, which would be needed for more recalcitrant water or at lower temperatures when the kinetics are slower.

Formation of granulated sludge has been observed in ABRs and is accompanied by improved kinetics (Metcalf and Eddy 2003). Although its presence is not necessary for optimal performance, the formation and growth of granules with in the reactor has been reported by various authors to improve COD removal efficiency and solids retention (Barber 1999). For example, Boopathy reported granule growth from 0.5 mm to 3.5 mm after the first three months of operation in a hybrid reactor. A higher methanogenic activity in the first compartment of the reactor corresponded with a larger granule size compared to subsequent compartments in the reactor. Orozco reported a change in granule size from 5.4 mm in the first compartment down to 1.5 mm in the last compartment of a reactor treating dilute carbohydrate waste. Production of sulphides can inhibit both methanogenic and SRB growth; however the SRB can outcompete the methanogens for essential nutrients in the reactor. Solutions to this problem include simultaneous sulphide removal and elevation of reactor pH (Fox 1996).

2.5.2 Upflow Anaerobic Sludge Blanket (UASB) Reactor

The upflow anaerobic sludge blanket reactor (UASB) was developed by Gatze Lettinga and his research group at Wageningen University, The Netherlands in the late 1970s (Metcalf and Eddy 2003). A typical UASB reactor is shown below in Figure 2-3. Four important elements are critical in the successful operation of this reactor design: the influent distribution system, existence of granular sludge, three phase separator, and the effluent withdrawal design (Metcalf and Eddy 2003).

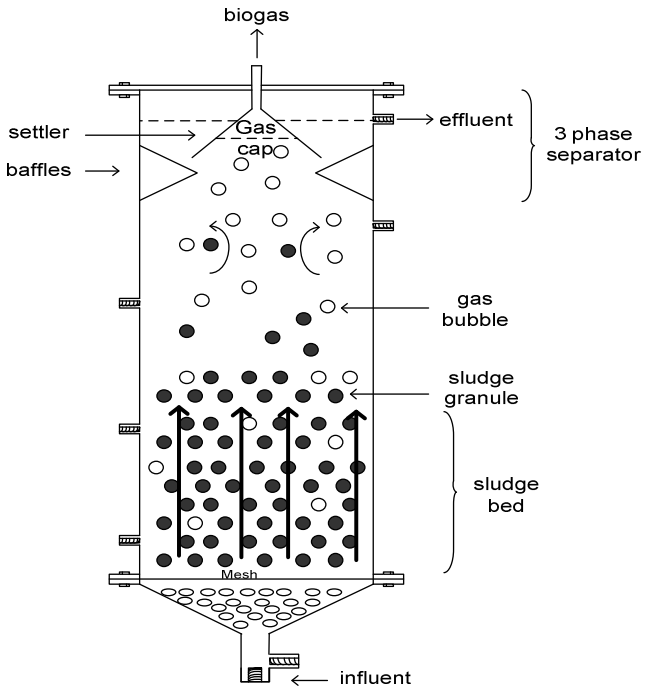

Figure 2-3 Diagram of UASB reactor

In this reactor design, the influent wastewater enters at the bottom of the reactor through appropriately spaced inlets and travels upwards through a granular sludge blanket bed where the bacterial community within the sludge bed comes in contact with the wastewater-substrate mixture. The granules in the sludge bed are essentially communities of microorganisms that use each other as support structures as they grow. The growth of these granules prevents solids washout, which is one major issue with anaerobic wastewater treatments. These granules can range in size from 0.5 to 2 mm in diameter. In the process of anaerobic degradation gas bubbles are formed containing CO_2, CH_4 and H_2S. As these gas bubbles rise, combined with the upflow of the incoming

wastewater, together with the sedimentation of the granules, they provide mixing without any mechanical parts, which encourages further sludge granule formation. The development of this dense granulated sludge is a key feature of this reactor design, as this allows for the use of high volumetric COD loadings compared to other anaerobic processes (Metcalf and Eddy 2003).

The three-phase separator system at the top of the reactor comprises an inverted V-shaped gas hood, which collects and separates the biogas formed in the reactor chamber from the liquid and solids. The gas then discharges through the top of the reactor. The funnel in the settling compartment reduces liquid turbulence created by biogas production and also allows for separation and settling of sludge particles by flocculation and sedimentation. In addition, the shape of the funnel limits expansion of the sludge bed, which reduces or prevents carry-over of sludge particles from the system. The final characteristic of the three-phase separator system is inclusion of baffles that are attached to the wall of the reactor. Their function is to reduce the cross sectional area of the reactor, allowing the upflow of gases to be deflected into the gas cap opening.

Since the introduction of the UASB design, it has been applied successfully to many types of wastewater both non-complex and complex (Lettinga 1991). There are numerous full-scale UASB plants treating wastewater such as domestic sewage, as well as effluents from chemical and petrochemical plants, the textile industry, sugar processing, paper and pulp plants, pharmaceutical facilities, and brewery wastes, with reactor volumes ranging from 200 – 50,000 m^3 (Lettinga 1991). Widespread application of this technology is due to much better COD removal as well as shorter HRT when compared to other anaerobic treatment processes, as shown below in Table 2-3.

Table 2-3 Examples of applications of UASB reactors worldwide.

Location	Volume (m^3)	Temperature (ºC)	HRT (hr)	COD removal (%)
Netherlands	0.030-205	<20	2-50	<75
South Africa	0.008	20	24	90
Thailand	0.030	30	3-12	90
Columbia	64	25	6-8	75-82
India	12000	18-32	8	51-63

Source: Seghezzo 1998

The industrial full scale applications of UASB reactors worldwide as reported by Seghezzo have reactor volumes that range from 0.008 to 12,000 m^3, with varying degrees of success in terms of COD removal efficiency (1997). For larger applications with reactor volume that exceeds 400 m^3 however, a modular design approach can be implemented to improve overall efficiency (Lettinga 1991). In this approach, several UASB reactors with smaller reactor volumes can be connected either in series in order to improve overall process performance, or in parallel to allow for easier start-up period and as well as easier maintenance process (Lettinga 1991).

Since the early 1990s, Lettinga and his group as well as many others have conducted pilot scale tests treating industrial wastewaters containing high sulphate concentrations using the UASB design. Since then a number of applications, both laboratory and commercial scale have been developed with sulphate reduction efficiencies greater than 90%. Table 2-4 below lists some of the UASB applications for sulphate reduction.

Table 2-4 Process conditions of UASB applications for sulphate reduction

Source	**Reactor Volume (L)**	**Temperature (C)**	**Sulphate Removal (%)**	**HRT (hr)**
Scheeren 1992	12,000	20	91.4	4
Dries et al., 1998	2.3	33	80-90	1.9-2.5
Muthumbi et al., 2001	2.3	32	>90	5-28
Vallero et al., 2003	0.92	55	95	7.28-7.73
Rose 2004	50,000	25	67	46
Rowley 1997	2,300	30	~99	48

There are several industrial applications of the UASB, or slightly modified sludge-bed versions, for treating high sulphate waters that are most notable: the Paques Thiopaq® process, the BioSURE process, and the Biosulphide process.

The patented Paques® Thiopaq process is a two step biological process in which sulphate is reduced to sulphide in the first step, followed by partial oxidation of sulphide to elemental sulphur in the second step (Scheeren 1992). The elemental sulphur is recovered and processed elsewhere to produce sulphuric acid. Several full-scale applications of this process have been in operation since the early 1990s, with most of them located in the Netherlands. One installation treats metal-contaminated groundwater at the Budelco zinc refinery (Scheeren 1992). Greater than 99% of zinc and cadmium is removed from 5,000 m^3 d^{-1} of groundwater with metal sulphides and sulphur returned to the smelter for metal recovery and sulphuric acid production. Clean water free of metals and sulphur is returned to the environment. It should be noted that this treatment system uses ethanol and methanol as electron donors. A variation of this process uses CO_2 and H_2 for carbon and energy sources (van Houten 1994). In this case, a gas-lift bioreactor was used and small particles of pumice or lava rock were used to immobilize the biomass.

The BioSURE process was developed at Rhodes University in South Africa. This process uses sewage sludge as the food source for SRB in the treatment of acid mine drainage (Rose 2000). A so-called falling sludge bed reactor (FSBR) is used in the BioSURE process. In the FSBR, large particles become hydrolyzed and breakup as they fall in the reactor, then after the smaller organic compounds are consumed in the sulphate reduction process, the residual solids settle and are recycled back to the inlet to go through the process again (Rose 2000). A pilot plant at the Grootvlei mine in South Africa was installed and in the 18 months of operation, it has been proven to be a reliable method for acid mine drainage treatment (Rose 2000).

The NTBC Research Cooperation in Canada developed the Biosulphide process. This biogenic sulphide system involves a biological stage followed by a chemical stage (Rowley 1997). The operation of this process provides several key advantages to

traditional sulphate reduction processes. First, only part of the influent water passes through the bioreactor to generate the necessary reagents (sulphides and alkalinity), this leads to lower amounts of dissolved metals and sulphides that can be toxic to the bacterial community. Second, the biological and chemical steps each operate at their respective optimal rates, and thus a greater degree of control on the extent of the reactions in each step is possible (Rowley 1997). The company Bioteq (Vancouver, Canada) adapted this particular process for the main purpose of selectively recovering metals from Industrial wastewater. Their sulphate-reduction unit operates similarly to the ones described above.

The common feature of all these reactors is the presence of a sludge bed that retains SRB in the reactor. The greater the density of the sludge, the shorter the HRT required for a desired sulphate reduction rate. Characteristics of the feed water and mixing inside the reactor determine the sludge density. For typical sludge particle sizes and densities, the recommended upflow velocity is 1 m hr^{-1} (Metcalf and Eddy 2003). To achieve this, the reactor cross-sectional area is calculated for the required volumetric flow rate. For low volumetric flow rates that require long residence times, this could result in very narrow and extremely tall reactor. Therefore, a recycle of the effluent back through the reactor is recommended in order to avoid this impractical design.

2.5.3 Comparison

The major advantage of the ABR is its simple design, which has no moving parts or special gas and solid separation devices. Thus, it is inexpensive to build and operate. Its improved efficiency results in lower hydraulic retention times and intermittent operation is possible. However, currently there are few full-scale applications and, thus, limited data to demonstrate reliability. Another disadvantage of the ABR design is that, due to the shape of the reactor, the influent is not distributed evenly and short-circuiting may occur. Another issue with the ABR is carryover of biomass from the first compartment into the ones downstream. Thus, solids recycle is often required or retention of the solids within the compartments is needed. Thus, the ABR design was eliminated from our choice of appropriate on-farm reactors.

The big advantage of the UASB reactor is that it is one of the most commonly used anaerobic bioreactors for treating a wide variety of wastewaters. Its high efficiency has lead to worldwide usage. Thus, much data and experience are available from both laboratory-scale and large-scale applications. The presence of granules means that packing materials can be eliminated – a cost-saving feature common to both reactor types. Disadvantages include higher energy consumption due to the recycle pump and a bit more complicated design compared to other anaerobic treatment processes. However, during scale-up, higher fluid velocities will reduce the need for liquid recirculation. Therefore, the UASB design was used in this thesis. A simple-to-make version was built for this project and operated for over 1 year. Its performance was compared with the previous simple plug flow trench reactor that was first installed at Lanigan. In addition, results were compared to those of other UASB reactors used for sulphate-reduction that have been reported in the literature. Some key aspects of the UASB were examined in greater detail. Firstly granulation, which is desired since it improves performance, was characterized. Always a difficult and crucial choice in design of sulphate-reduction bioreactors is the carbon and energy source. Thus, we measured the characteristics and degradation rates of carbon compounds in the silage leachate. Next is a description of factors that influence granulation.

2.6 Granulation

Granulation is the formation of aggregates through sticking together of smaller particles via agitation methods (Rhodes 1998). In anaerobic wastewater treatment, the granules are bacterial granules, comprised of different bacterial trophic groups that perform their respective roles in the degradation of wastewater (Tiwari 2006). Due to their large size, granules prevent washout of biomass from the reactor, which leads to improved performance of anaerobic wastewater treatment facilities. Compared to conventional flocs, the granulated biomass has the following advantages: it has a densely compact biofilm, high settle-ability of up to 80 m hr^{-1}, high mechanical strength, balanced microbial community (syntrophic partners are closely associated) and resistance to toxic shock (Tiwari 2006). An added benefit of the larger biomass granules is that the flow of water through the reactor helps to agitate the granules, which enhances mixing and further increases the kinetics. Factors affecting the development of granulated biomass are carbon compounds in the water to be treated, pH, liquid flow velocity and nutrient addition (Metcalf and Eddy 2003). Dense granule formation can be obtained when organic acids are present as electron donors. Soluble COD and the presence of very little solids in the feed water are preferred. The optimal pH for maximum granule growth should be neutral. The steady-state COD:N:P ratio should be 600:5:1 (start-up: 300:5:1) (Metcalf and Eddy 2003). High liquid upflow velocities are required (1-3 m h^{-1}) (Metcalf and Eddy 2003).

Granules are beneficial for growth of SRB, which in turn contributes to more granule formation. As granules, SRB are less sensitive to fluctuations in reactor conditions when compared with other organisms such as methanogens (Britz 2000). While contained in the granules they are protected from changes in pH, loading rate, and additions of toxic substances. Slower growing methanogens, also located inside the granules, use acetate that is produced by SRB and acetogens.

The most important factor in the successful operation of an anaerobic treatment process is the retention of the sludge biomass. The typical anaerobic treatment process has a large

footprint when compared to its height, whereas the UASB has a very small footprint when compared to its height. Since the UASB has superior sludge settling characteristics it is capable of handling much higher loading rates. Granule formation enhances sludge settling and retains even more biomass in the reactor further reducing the vessel size needed, which lowers the investment and operational costs of the process (Hulshoff Pol 2004). Another advantage is that the use of granulated seed sludge can reduce the UASB startup time from 2 – 8 months to a mere 30 days.

As granule growth is key to the successful operation of the UASB, characterization of granules during the experiment will be very important. However, only a few studies exist where granule growth was monitored in UASB operations (Yan 1997). Yan (1997) measured bioparticle size along with sludge methanogenic activity to show how granules contribute to biomass growth (Yan 1997). Another research group monitored the increase in sludge bed volume as basis for determining the success of the granulation process in their UASB experiment (Britz 2000).

2.7 Carbon Source Selection

SRB are known to use a variety of chemical compounds as energy sources for sulphate reduction. These compounds are mostly simple molecules such as carbon dioxide and hydrogen, or low molecular weight (MW) organic compounds such as VFAs and alcohols (Kousi 2009). Higher molecular weight organic hydrocarbons such as phenols and long chain alkanes were consumed by SRB in some studies (Rueter 1994). Studies from bench top experiments that use these nutrients have shown varying degrees of success in reducing sulphate. However, larger applications were successful only from a much more narrow range of organic materials. In fact, most of the industrial sulphate-reducing applications utilize man-made chemicals such as lactate and ethanol as the main nutrient source. But the recurring cost of these chemicals means that cost conscious cattle farmers need to look for a more economical alternative. Table 2-5 lists some of the compounds suitable for SRB in the sulphate reduction process.

Table 2-5 Compounds utilized by SRB

Compound Size	Compound Type	Compounds
Simple		Carbon Dioxide Hydrogen
Low MW organics	Organic acids	Lactate, pyruvate, formate, malate
	Fatty acids	acetate
	Alcohols	Methanol, ethanol, propanol, butanol
High MW organics	Alkanes	
	Phenols	

Source: Kousi 2009, Rueter 1994

With all these nutrient sources to choose from, SRB prefer low MW organic acids as the carbon source for sulphate reduction, with lactate as the substrate most often used for culturing SRB in the laboratory (Ren 2007). Utilization of lactate by SRB in the sulphate reduction process, when described as chemical equation, is a stepwise reaction mechanism, shown below.

$$3SO_4^{2-} + 2H^+ + 2CH_3CHOHCOO^- \xrightarrow{SRB} 3H_2S + 6HCO_3^- \quad \text{(Eqn 2-6)}$$

$$HCO_3^- + H^+ \longrightarrow CO_{2(g)} + H_2O \quad \text{(Eqn 2-7)}$$

Carbon source requirements can be calculated based on the number of electrons transferred. For example, in order to reduce one mole of sulphate to sulphide, eight moles of electrons are accepted. These electrons are obtained from oxidation of the organic substrate. Lactate is oxidized incompletely to acetate while donating 4 electrons. Some SRB can oxidize acetate, which donates another eight electrons. Therefore, in order to reduce one mole of sulphate two thirds of mole of lactate is required, assuming complete oxidation. However, SRB are not the only organisms growing in anaerobic bioreactors. There are many other bacterial groups that compete with SRB for carbon source. Some of the important ones are described below.

2.7.1 Anaerobic Bacterial Community

In an anaerobic system, four groups of bacteria are actively involved in the decomposition of organic compounds (Ren 2007). The four groups are identified as fermentative organisms, acetogenic bacteria, methanogenic bacteria, and sulphate reducing bacteria (Ren 2007). The first group of bacteria, fermentative organisms, breaks down higher MW compounds into simpler compounds, for which the other three bacteria groups compete (Ren 2007). SRBs can out-compete both methanogens and acetogens for available resources, especially at the preferred pH range of 7-8. In this pH range, sulphide concentrations resulting from sulphate reduction are high, which "poisons" the methanogens and halts their growth (primary inhibition); at the same time, the SRB population increases dramatically, which leads to secondary inhibition of other bacteria in the system (Ren 2007). Eventually very high sulphide concentrations inhibit SRB growth as well. When the pH is slightly acidic, the inhibitory sulphide concentration was found to be 350 mg L^{-1}. This was mainly due to the presence of H_2S. At higher pH values, however, SRB tolerate much higher sulphide concentrations. For example, at pH 7, the inhibitory sulphide concentration is 750 mg L^{-1}; and at pH 8, SRB can tolerate sulphide concentrations as high as 1,500 mg L^{-1} (Baldwin 2003). This is because at pHs above 7

sulphides are present in the dissociated forms HS^- and S^{2-} rather than as the much more toxic H_2S (Baldwin 2003).

Sulphate reducing bacteria are very diverse with many different species that can be broadly classified according to the preferred carbon or energy source (Table 2-6).

Table 2-6 Types of SRBs in an anaerobic environment

SRB Groups
Hydrogen-utilized SRB(HSRB)
Acetic acid-utilized SRB(ASRB)
Fatty acid-utilizing SRB (FSRB): Propionate-utilized SRB(p-SRB) Lactic acid-utilized SRB(l-SRB) Butyric acid-utilized SRB(b-SRB)

Source: Ren 2007

In an ecosystem, the HSRBs consume hydrogen produced by acidogenic bacteria; and the FSRBs consume fatty acids coming from fermentation to produce acetic acid, which in turn is consumed by both ASRBs and methanogens. This multi-stage feeding scheme is essential for the efficiency of the whole system (Ren 2007). Acetate can inhibit SRB growth and therefore presence of acetate consuming organisms together with acetate producing organisms prevents product inhibition, which would otherwise halt sulphate reduction.

There are other anaerobic bacteria that could be present and compete with SRB for carbon source. These include denitrifiers, present if the electron acceptor nitrate is available, and iron reducers. Both of these electron transfer reactions are more thermodynamically favourable than sulphate reduction. Therefore nitrate or iron may inhibit sulphate reduction.

With all the bacteria present in an anaerobic system only a small fraction of the carbon resources are used for sulphate reduction. Since carbon source is an expense, it is important to optimize reactor conditions that favour SRB so that as much carbon as possible is used for sulphate-reduction.

2.7.2 Silage Leachate as Carbon Source

Since SRB require low molecular weight carbon compounds, they need to coexist in a consortium with other microbes that can produce these compounds from an inexpensive complex organic material. In terms of economics, a variety of organic materials from both nature and industrial effluents can serve as a good carbon source for sulphate reduction with little or no cost to the farmers. Industrial effluent sources include landfill leachate (Henry 2000), cheese whey, and wine waste (Martins 2009). Natural organic sources can include molasses (Gonçalves 2005), mushroom compost (Hammack 1992), straw and hay (Vainshtein 2003), as well as leaf mulch, wood chips, sawdust, animal manure, and vegetable compost (Martins 2009). The organic chemical make-up of these carbon sources, be it natural or man-made, contains fatty acids, proteins, carbohydrates, alcohols, phenols and amines. Many of these can be utilized by SRB during sulphate reduction. Therefore, selection of carbon source used for the active treatment system on rural farms comes down to two important criteria: availability of a self-sustaining carbon source (Boshoff 2004) and the degradability of the organic substrate (Martins 2009).

For farmers, use of industrial effluents would not be beneficial as farms may be located in remote and isolated areas where transportation issues can be a problem (Boshoff 2004). Secondly the farmers would need to rely on industries to provide sufficient quantities for sulphate reduction (Boshoff 2004), which defeats the purpose of a self-sustaining treatment process. In addition to availability and transportation issues, man-made wastes typically do not contain the variety of different organics present in natural carbon sources. For example, proteins, lactose, and fats accounted for just over 5% w/v of cheese whey, while wine waste mainly consists of ethanol (Martins 2009). On the other hand, a natural organic source such as algal biomass and farm livestock feed normally contains a variety of VFAs, carbohydrates, alcohols, and amines.

All of the natural organic sources listed above are suitable for SRB growth, however, not all farms would have large quantities of mushroom and vegetable composts (good sources of alcohol and sugars) on hand. What they do have in abundance is hay, barley, molasses, and silage. Boshoff et al. (2004) used dried algal biomass as carbon source in a

UASB reactor. Their study showed a SRR of ~0.3 g L^{-1} d^{-1}. Cheese whey and wine waste as carbon sources for SRB were tested by Martins 2009). Results showed that minimal sulphate reduction occurred when cheese whey and wine waste alone were used; however, when a buffering and neutralizing agent was included – calcite tailing – 95% of the incoming sulphate was reduced for both cheese whey and wine wastes (Martins 2009).

Silage is an agricultural bioproduct, produced as a result of hay fermentation. During this fermentation process lactic acid bacteria (LAB) ferment the hay and produce lactic acid as well as a number of other organic acids. In silage making, lactic acid produced by the bacteria causes the pH to decrease to around 4, which inhibits the growth of other organisms, including the lactic acid bacteria themselves. This serves to prevent further decomposition of the silage and preserve it as a future feed source for livestock. In the UASB, where the pH is above 7, lactic acid is neutralized and will not inhibit bacterial growth, instead the lactate serves as a readily available electron donor for the SRB. Other organic acids such as propionate, butyrate and fumerate are also used by SRB as electron donors. Tests performed by various research groups showed that silage is composed of both low MW and high MW organic compounds, many of which are identical to the ones listed above in Table 2-5. Table 2-7 summarizes some of the chemicals identified by these research groups.

Table 2-7 Chemical composition of silage

Compound Size	Compound Type	Compounds	Quantity ($g\ Kg^{-1}$ dry matter)
Low MW organics	Organic acids	Lactate	49.3
		Formate	2.6
		Acetate	28.6
		Propionate	1.0
		Butyrate	6.0
	Alcohols	Ethanol	6.8
High MW organics	Water soluble carbohydrate		33.0
	Non-protein nitrogen		605
	True soluble protein		37.0
	Total amines		4.82

Source: Krizsan 2007

Many of the organic compounds in Table 2-7 are known to be ideal low molecular weight carbon sources for SRB growth. This was confirmed in the previous study where silage leachate achieved higher sulphate-reduction rates than the other agricultural wastes that were tested (Brown 2007). In my study I chose to monitor the composition of silage more closely to see what specific compounds are present and how they are consumed in the process.

Chapter 3: Materials and Methods

Based on the work of Amber Brown (Brown 2007), a pilot-scale biological sulphate reduction process was built at the Lanigan experimental farm in Saskatchewan. For the first part of my thesis, I assessed the performance of this treatment system by analyzing water samples that were sent to the UBC laboratory. Subsequently, an upflow anaerobic sludge blanket bioreactor was designed based on the previously described literature review. This reactor was first operated in batch mode for one month, and then continuously with increasing sulphate concentrations in the feed. These reactors' designs, their modes of operation are described below. The analytical methods used can be found in the Appendix.

3.1 1st Lanigan Trough Bioreactor

3.1.1 Design Specification

The Lanigan trough bioreactor was a passive biological sulphate-reduction process designed to treat 50 L d^{-1} groundwater with a sulphate concentration of 1,400 mg L^{-1}. This pilot-scale process was built at the Western Beef Development Centre in Humboldt, Saskatchewan. A diagram of the system with numbered sample ports is shown below in Figure 3-1.

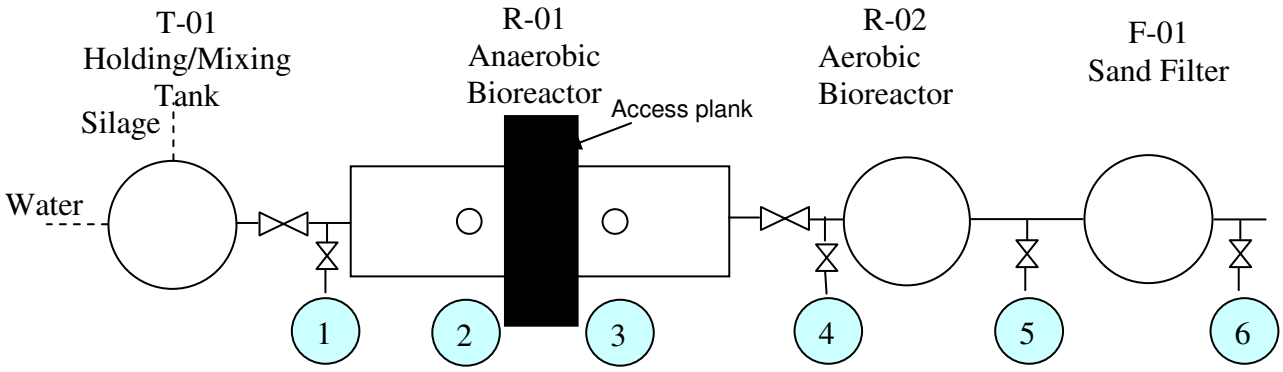

Figure 3-1 Diagram of the Lanigan trough bioreactor.

Two bioreactor systems were built and operated on the farm. Reactor A was built indoors and Reactor B was built outdoors. Both reactors have the same components as shown above, as well as identical dimensions. Reactor A was built in October 2005, while Reactor B was built in May 2006.

There are four main components in this process. The first is the holding/mixing tank. The purpose of this tank is to wash soluble organic compounds off fresh silage material by mixing the silage with the water to be treated. The holding tank holds enough water for a treatment period of 24 hours. Fifty liters of the water to be treated is mixed with 0.5 kg of wet silage, which is contained in a permeable bag to prevent silage solids from clogging downstream pipes. Water plus silage leachate is fed at a rate of 2.1 L h^{-1} through the anaerobic bioreactor (the 2nd component in the system). This anaerobic bioreactor is a rectangular-shaped wooden box (1.4 m L x 1 m W x 1 m H) lined with high-density polyethylene and filled with a 1:1 hay and silage mixture. This hay/silage mixture provides solid support surfaces for the sulphate reducing bacteria growing in the reactor. The purpose of the anaerobic bioreactor is to reduce the sulphate concentration in the feed water by 1,000 mg L^{-1}.

The third component is the aerobic sulphide oxidizing bioreactor. Since the effluent water that leaves the anaerobic bioreactor contains higher than allowable levels of sulphide, this will need to be removed from the water in order to meet cattle drinking water guidelines. To achieve the desired sulphide removal, effluent from the anaerobic bioreactor is sent into a 4 L holding tank where aeration stones at the bottom of the tank inject the right amount of air to maintain the dissolved oxygen concentration below 1 mg L^{-1}. This will oxidize the sulphide to elemental sulphur, with limited oxidation all the way back to sulphate. Elemental sulphur accumulates in the tank and is removed by skimming from the surface or by draining any sludge that settles. The last component of the system is a sand filter. The purpose of this final step is to remove the suspended solids including bacteria, precipitates and other organic materials carried over from the bioreactors. The sand filter, which consists of sands with an effective size of 0.2 – 0.3 mm, is cleaned, by back flushing, periodically to avoid clogging.

3.1.2 Start-up

To start the anaerobic bioreactor, a 30 cm layer of a fifty-fifty mixture of loose hay and silage was placed on the bottom of the bioreactor. This organic material mixture provides a solid substrate for the bacteria to grow on and provides some fermentation byproducts as part of the carbon source requirements. Hay is readily available and a mixture of hay and silage was shown in a previous study (Brown 2007) to be the most effective combination in sulphate reduction experiments. As the reactor was filled with groundwater, an inoculum of sulphate-reducing bacteria, obtained from natural sediment (Lac DuBois near Kamloops, B.C.) known to contain highly active sulphate-reducing bacteria, and a load of organic material, in the form of silage leachate, were added to the bioreactor. The bacteria in the reactor can tolerate a pH range of 6.5 – 9. However, optimum SRB growth has a narrower pH range of 7 – 8. In order to achieve this optimum growth, pH within the reactor needs to be monitored regularly and adjusted with sodium hydroxide if it drops below 6.0. The bioreactor was allowed to sit for about one month to allow anaerobic conditions to establish and the bacteria to acclimate and multiply before continuous feed water was added.

3.1.3 Sampling Method

In order to monitor the status of the treatment system and to assist with troubleshooting, samples were taken from six ports located at various positions within the system. These ports are labeled 1 though 6 in Figure 3-1. The samples were taken once every two weeks. As the volume of the anaerobic bioreactor is quite large, samples from different locations (Ports 2 and 3) and at three different depths within the bioreactor, surface, middle, and bottom, were taken in order to assess the distribution of activity in the reactor. Other sample ports were located before and after the anaerobic and aerobic bioreactor, as well as after the sand filter to determine the final effluent chemical makeup. Samples were analyzed for sulphate, sulphide and COD concentrations.

3.2 UBC Up-flow Anaerobic Sludge Blanket (UASB) Bioreactor Treatment System

3.2.1 Design Specification and Construction

The UBC UASB treatment system was a laboratory-based active treatment system with a continuous mixed feed of simulated groundwater with a sulphate concentration of 2,000 mg L^{-1} and silage leachate with COD concentration of 10,000 mg L^{-1}. This laboratory-scale system was constructed and operated at the UBC Department of Chemical and Biological Engineering. A flow diagram of the system is shown below in Figure 3-2. A complete schematic of the treatment system, including safety measures and system monitoring components, can be found in Appendix A-1.

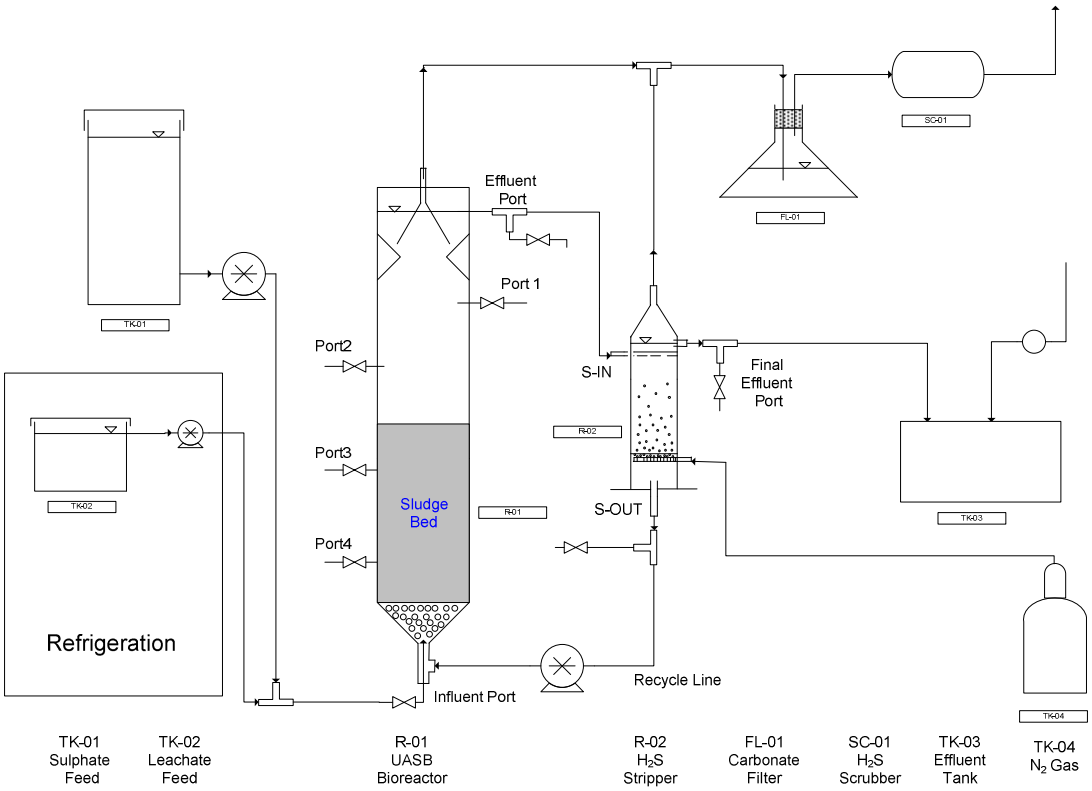

Figure 3-2 Diagram of the laboratory UASB bioreactor treatment process.

3.2.1.1 Feed Tanks and Mixing of the two Feed Streams

Two feed tanks were used in this system. A 20 L Nalgene HDPE tank housed the simulated groundwater with high levels of sulphate. The simulated groundwater was a mixture of four sulphate salts that were premixed before being added to the sulphate feed tank. The cations and their mass ratios (obtained from analysis of Saskatchewan groundwater by WBDC) in the simulated ground water are listed in Table 3-1. The simulated groundwater feed tank contains a lid to minimize evaporative water loss. The sulphate feed line to the reactor was Cole-Parmer Masterflex Microbore Tygon LFL tubing with an inner diameter of 1/16". A Cole-Parmer Masterflex C/L series peristaltic pump delivered 1 mL min^{-1} of simulated ground water to the reactor.

Table 3-1 Simulated ground water sulphate salt composition.

Cation	Mass Concentration (mg L^{-1})
Sodium	35
Potassium	10
Calcium	215
Magnesium	105

Like the Lanigan treatment system, the carbon sources for the bacteria come from the silage leachate. Unlike the Lanigan system, the making of silage leachate is a pre-treatment process. Pre-weighed silage is soaked in water for about 30 min (silage/water ratio = 40.36 g-wet weight per 0.273 L dH_2O). After this the water is twice drained through fine mesh cloth before being sent to the autoclave. The autoclaved silage leachate is then filtered and stored at 4^oC to minimize bacterial activity. In early versions of the feed setup, the silage leachate was not autoclaved, this coupled with the low flow rate in the narrow feed line lead to microbial growth resulting in feed line plugging. Also, exposure of the silage leachate to air is not desired since this would result in biological degradation of organic compounds in the leachate by aerobic microbes. So-called spoilage of silage is known to occur, which is why it needs to be stored wet under anaerobic conditions. Therefore the autoclaved and filtered silage leachate was placed in a 4 L beaker with a floating lid to exclude air. To further inhibit microbial activity in the silage leachate feedline, the leachate feed beaker and pump were housed in a small refrigerator at 4^oC. Also, the silage feed line to the reactor was kept cool by wrapping it

with a water line attached to a chiller that maintains a temperature of 4ºC. The point of mixing between the simulated groundwater and silage leachate is a simple T-connector located as close to the inlet of the UASB bioreactor as possible. The leachate feed line was Cole-Parmer Masterflex Microbore Tygon LFL tubing with an inner diameter of 0.16 cm (1/16"). The tubing line went through a Cole-Parmer Masterflex C/L series peristaltic pump delivering leachate feed at a rate of 0.5 mL min^{-1}.

3.2.1.2 UASB Bioreactor

The sulphate feed line combines with the leachate feed line and enters the main component of this system - an upflow anaerobic sludge blanket (UASB) bioreactor. A detailed mechanical drawing of this bioreactor can be found in Appendix A-2. The design parameters, as listed previously in Chapter 2, were obtained from *Wastewater Engineering: Treatment and Reuse* (Metcalf and Eddy 2003). The empirical formulas, as outlined on p.1010 in Metcalf and Eddy (2003), are listed below.

$$A = \frac{Q}{v} \qquad \text{Eqn 3-1}$$

where A = cross sectional area, m^2

Q = influent flowrate, $m^3\ hr^{-1}$

v = design upflow superficial velocity, $m\ hr^{-1}$

$$V = \frac{Q \times \Delta S}{SRR} \qquad \text{Eqn 3-2}$$

where V = the required working reactor volume, m^3

ΔS = the desired reduction in sulphate concentration, $kg\ m^{-3}$

SRR = sulphate reduction rate (the kinetics) kg COD $m^{-3}\ d^{-1}$

The first Equation calculates the reactor cross-sectional area required to maintain the ideal upflow velocity for granule formation and mixing. The second Equation determines the working volume needed to achieve the desired amount of sulphate reduction in water fed through the reactor. The *SRR* depends on the nature of the carbon source as well as

the composition and amount of biomass. A one meter section of a pre-manufactured column was used for the UASB (Figure 3-3).

Figure 3-3 Picture of UASB column with sample ports.

This Plexiglas cylinder had an 8.26 cm ID (3.25") with a wall thickness of 0.64 cm (1/4"). Since this was a pre-manufactured column, the total volume and cross sectional area for the reactor were pre-determined. From Table 10-14 of Metcalf and Eddy (2003), the lowest upflow velocity for a soluble COD feed was 1 m hr^{-1}, which results in an influent flowrate of 84 mL min^{-1}. However, based on sulphate reduction kinetics from the batch mode operation, as well as previous work from Amber Brown (Brown 2007), the maximum influent flowrate was determined to be 1 mL min^{-1}(Appendix B-2 for detailed calculations). This problem was solved by the use of a recycle stream, as shown in Figure 3-2, which maintains the upflow velocity at 1 m hr^{-1}. Sampling ports were attached with off-the-shelf tubing adaptors. To complete the bioreactor, the gas-solid separation (GSS) device and the inlet assembly were made separately following recommendations set out on pages 1010-1011 of Metcalf and Eddy (2003). The GSS device and inlet assembly were made from 0.64 cm (1/4") Plexiglas. Diagrams of both devices are shown below. Please refer to Appendix A-3 and A-4 for detailed mechanical diagrams of both devices.

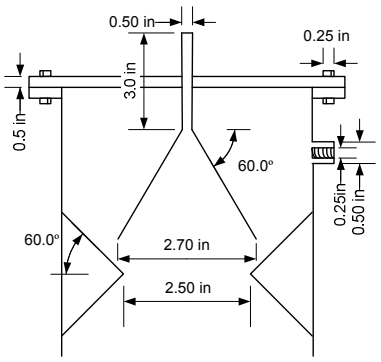

Figure 3-4 GSS device

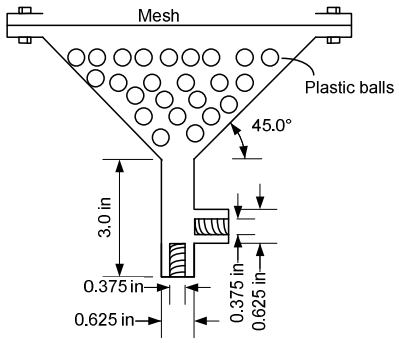

Figure 3-5 Inlet assembly

The GSS device was comprised of an inverted Plexiglas funnel and a Plexiglas ring with a tapered top and bottom angled at 60° and annulus diameter of 8.26 cm (3.25”). Two rubber gaskets secured the ring to the column wall. The effluent port was on the top right above the ring and approximately half way up the funnel (Fig. 3-2). The inverted funnel collected the gases that were produced in the reactor. These gases exited out the top and went through the scrubbing train. The inlet assembly, in Figure 3-5, is conically shaped with two ports at the bottom to accept the influent and the recycle streams, respectively. Glass marbles filled the cone area and were covered with mesh. This was done in order to evenly distribute the influent radially. With the exception of the influent feed line, which was 0.16 cm ID (1/16”) Cole-Parmer Masterflex Microbore C/L series Tygon tubing, all lines entering and exiting the bioreactor were Cole Parmer Masterflex L/S series Tygon tubing with an inner diameter of 0.32 cm (1/8”).

3.2.1.4 Final Effluent Tank

The treatment system was first operated without the H_2S stripper column, R-02. Thus, the UASB reactor effluent leaving the top of the column flowed directly into the final effluent tank (TK-03). An aquarium aeration pump sent air into this tank so as to oxidize the sulphide into elemental sulphur.

3.2.1.5 Gas Scrubbing

Gases passed through two scrubbing units. One was an upflow bubble column containing a solution of 2M sodium hydroxide for removal of carbon dioxide. A second column containing soda lime pellets removed any hydrogen sulphide gas (Weijma 2000). Final effluent gases and any fugitive gases from the whole system were removed through an overhead, ventilated hood.

3.2.2 Start-Up

One major difference between the UASB and the Lanigan trough reactor was the organic support structure that was used to immobilize the bacteria inside the reactor. As discussed earlier, the granulation process plays a key role in promoting bacteria growth and immobilizing the bacteria cells inside the reactor, thus increasing their concentration and consequently the reaction kinetics. Formation of these granules inside the UASB can take some time, however the start-up period can be shortened greatly by inoculating with granules from another operating UASB. With this in mind, the UASB bioreactor was seeded with 1 L of granulated sludge from an existing UASB bioreactor currently operated by Fleischmann's Yeast in Calgary. The sludge was added to the UASB along with 1 L of SRB inoculum, obtained from a natural sediment (Lac DuBois, Kamloops, B.C.) known to contain highly active SRB, as well as 1.7 L of silage leachate, and 700 mL of sulphate water. The recycle pump was then started to maintain an upflow velocity of 1 m hr^{-1} inside the UASB column. The bioreactor was filled to the top with distilled water so as to provide a total of 5 L of reactor contents. Initial samples were taken from the effluent port and analyzed for pH, dissolved oxygen (DO), sulphate, sulphide, chemical oxygen demand (COD), total organic carbon (TOC), and organic acids. The bioreactor was allowed to sit for about one month to allow anaerobic conditions to establish so that the SRB could acclimate and enter exponential growth before continuous feed water was added.

3.2.3 Sampling Method

There are a total of 8 sample ports in this UASB treatment system. Six ports are located on the UASB bioreactor and two ports are on the sulphide stripper column.
Ports on the UASB bioreactor are labeled Effluent Port, Port 1, Port 2, Port 3, Port 4, and Influent Port from top to bottom (Figure 3-2). Initially the height of the sludge bed was just above Port 4, therefore liquid samples for concentration analyses were taken from the Effluent Port, Port 1, Port 3, and the Influent Port. As the sludge bed grew, it eventually covered Port 3 and then samples for concentration analyses were taken from only the Effluent Port, Port 2, and the Influent Port. With the addition of the sulphide stripper column, effluent concentration profiles came from samples taken from Final Effluent Port. These samples were taken at various intervals ranging from a few hours during the batch start-up stage, to a week during the stable continuous stage. About once a month samples of granules were taken from Port 4 and analyzed for particle size distribution.
Ports on the sulphide stripper column are labeled S-IN and S-OUT. S-IN is essentially the Effluent Port from the UASB bioreactor and it is located on the top of the stripper column. S-OUT is located at the bottom of the stripper column where liquid stream with reduced sulphide concentration is pumped back into the UASB bioreactor. Samples from these two ports were taken at the same time as samples from the UASB bioreactor is taken, but only sulphide concentrations were analyzed.

3.2.4 Sulphide Removal

For most of the experiment, the recycle system consisted only of a peristaltic pump that recycled fluid back through the UASB column at a rate of 84 mL min^{-1}. This was done so as to maintain the required upflow velocity. However, as the influent sulphate concentration was increased, consistent sulphate-reduction increased also the sulphide concentration within the bioreactor. As high sulphide concentrations are known to inhibit SRB activity, a sulphide stripper column was added during the last stage of the experiment to determine its effectiveness in reducing the sulphide concentration within the bioreactor. Our hypothesis was that if sulphide concentrations inside the UASB could be reduced, then the process would be able to achieve the high extents of sulphate reduction needed when treating water high in sulphate.

3.2.4.1 Stripper Column Design Specification

Design considerations for the sulphide stripper column were taken from journal articles published by Yamaguchi (1999) and Gangagni Rao (2003). Values provided by Gangagni Rao (2003) indicated a stripper to bioreactor ratio of 1:35. For simplification purposes a ratio of 1:10 was used to size the stripper column. Again to avoid construction wait times, a 500 mL graduated cylinder made of polypropylene was used. A laboratory funnel was attached to the top of the cylinder using silicone. Four holes were drilled to accommodate the inlet, recycle return, gas feed, and effluent outlet. Purge gas for the stripper was industrial grade N_2 gas from Praxair, supplied and monitored with an inline flow meter. A diagram of the stripper column is shown below. A more detailed mechanical drawing can be found in Appendix A-6.

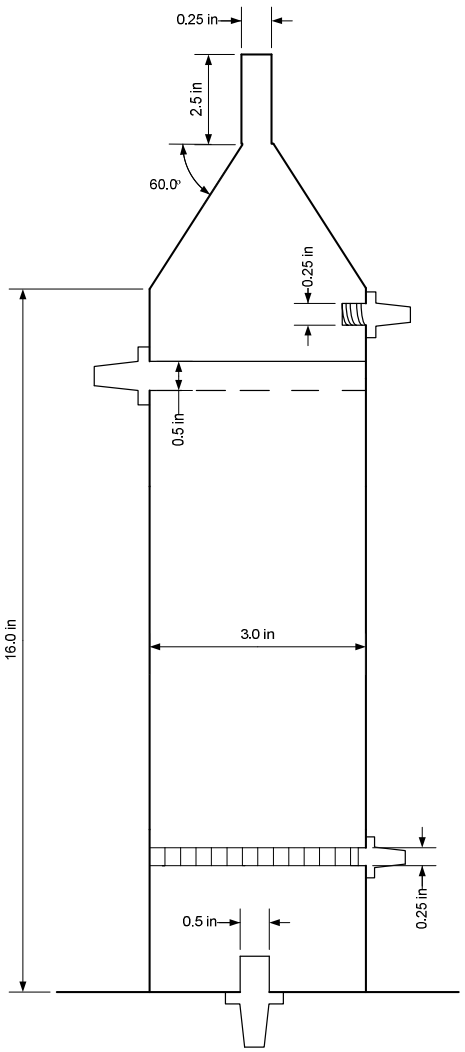

Figure 3-6 Sulphide stripper column.

Like the UASB bioreactor, all lines entering and leaving the stripper column were Cole Parmer Masterflex L/S series Tygon tubing with an inner diameter of 0.32 cm (1/8”), with the exception of the N_2 gas line, which was Cole Parmer Masterflex L/S series Tygon tubing with an inner diameter of 0.32 cm (1/8”) and a wall thickness of 0.08 cm (1/32”).

3.2.4.2 Packing

It is well known that filling the column with packing in a gas-liquid operation increases the surface area of the liquid that comes in contact with the gas, thus leading to better mass transfer efficiencies. In the case of this experiment, this could theoretically lead to

lower sulphide concentration in the recycle line, thus further reducing inhibition of SRB activity. With this in mind, initially the counter-current stripper column was built with Jaeger Tri-Packs® 1" hollow spherical column packing balls filling the middle section of the column (Figure 3-7).

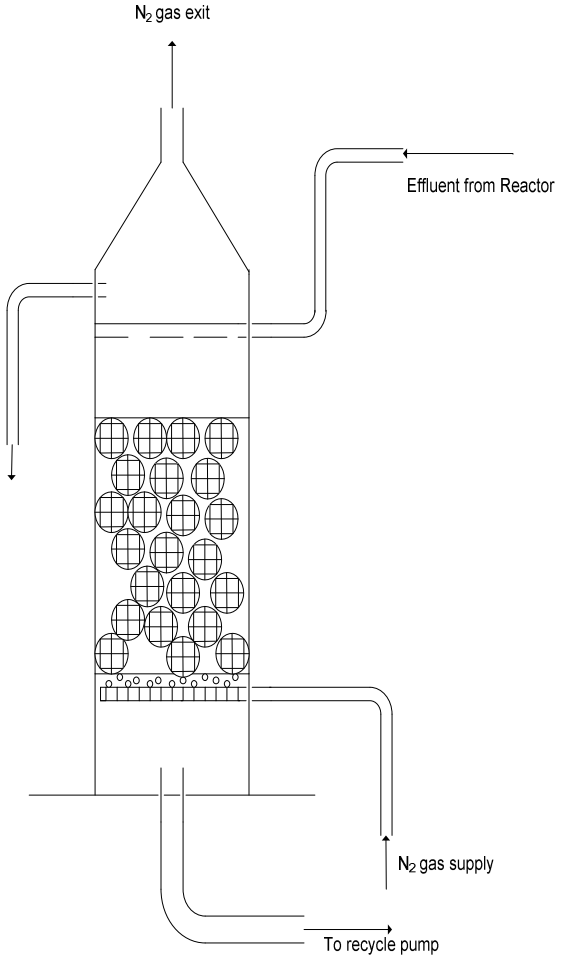

Figure 3-7 Stripper column with packing

This stripper column with the Jaeger Tri-Packs® packing was in operation for a period of five days beginning on Day 331. Then due to operational problems, discussed in the Results Chapter, the packing was removed for the rest of the experiment.

Chapter 4: Results

The goal of this project was to design and operate a lab-scale bioreactor to reduce sulphate in cattle drinking water using silage as the carbon source. Use of silage in sulphate reduction is new and innovative, and this application was intended to demonstrate silage's potential as a carbon source by determining the sulphate reduction rates attainable and to simultaneously measure rates of consumption of dissolved organic compounds in the silage leachate.

First this Chapter begins with results from the two pilot-scale trough reactors, which were in operation at the Lanigan farm in Saskatchewan. Following this, results for the lab-based UASB reactor are presented. Ten tests were carried out to characterize performance of this reactor: sulphate, sulphide, COD, TOC, carbohydrates, organic acids (HPLC), phenols, alcohols, granule size distribution and pH. All tests were conducted either in triplicate or duplicate. For all tests the standard deviations were within 5% of the average values. Error bars were not included on the plots since these analytical errors were small.

4.1 The Lanigan Bioreactors: Reactor A (indoor) and Reactor B (outdoor)

Based on Amber Brown's work (Brown 2007), two pilot-scale bioreactors were built at the research farm in Lanigan SK. These were box-shaped horizontal flow (Figure 4-1) systems: both Reactor A and Reactor B had the same dimensions (1.4m length x 1m width x 1m height) with Reactor A located indoors and Reactor B located outdoors. For the first part of my thesis work, I analyzed performance of these reactors by measuring sulphate, sulphide and COD values in samples sent from Lanigan to UBC.

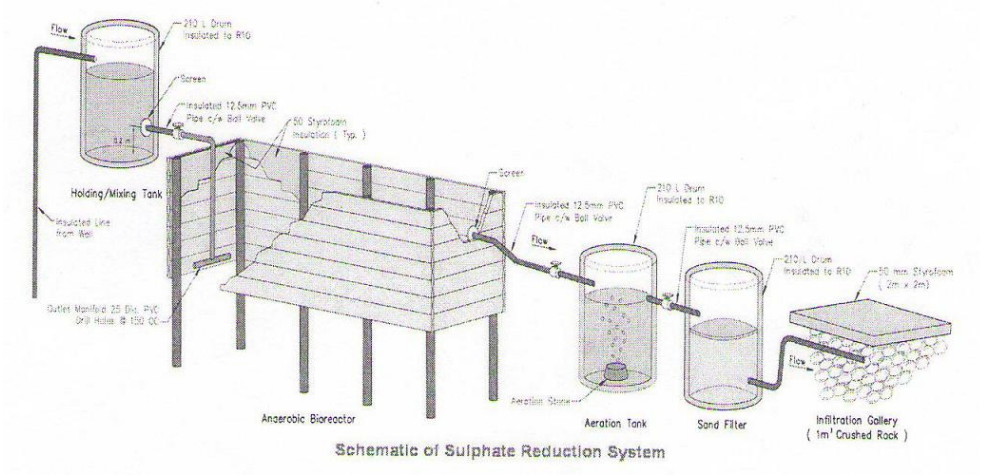

Figure 4-1 Lanigan bioreactor treatment system diagram

In October 2005, Reactor A began operating in batch mode. The inoculum was obtained from sediment removed from Lac du Bois Lake, which is near Kamloops, B.C. This sediment was shown in lab tests to contain active SRB. Silage available on the Lanigan farm was used as substrate. High sulphate water (1,500 mg SO_4^{2-} L^{-1} as $NaSO_4$) was added to the reactor together with a 30 cm layer of 1:1 hay/silage mixture and the closed system was given time to allow the bacteria in the inoculum to acclimate to the silage leachate and the sulphate water.

After three months of batch operation, 1,400 mg L^{-1} sulphate water (as $NaSO_4$) was fed at a rate of 2.1 L hr^{-1} into a tank where it mixed with 0.5 kg d^{-1} of wet silage. The sulphate water and the silage leachate then flowed into the bioreactor as shown in Figure 4-1. Selected samples taken during this continuous stage were sent to UBC to test for reactor performance. Summary of the tests conducted at UBC are listed below in Table 4-1. The first four sample sets were from Reactor A and the last sample set was from Reactor B.

Table 4-1 Tests performed on samples shipped from Lanigan research farm.

Sample Set	Reactor	Tests Performed
Jan. 05/2006	A	Sulphate, Sulphide
Jan. 24/2006	A	Sulphate, Sulphide
Mar. 02/2006	A	Sulphate, Sulphide
Apr. 12/2006	A	Sulphate, Sulphide, COD, TOC, HPLC, phosphate, nitrate
May 16/2006	B	Sulphate, Sulphide, COD, TOC, HPLC, phosphate, nitrate

Sulphate and sulphide test results for this five-month period are shown in Figures 4-2 and 4-3, respectively, below. For the January and March sample sets, only samples from Ports 2 and 3 were analyzed. These two ports are located within the anaerobic bioreactor. Samples from all ports in the system were received in the April 12 and May 16, 2006 shipments. Please refer to Figure 3-1 for locations of the various ports.

4.1.1 Sulphate Profile

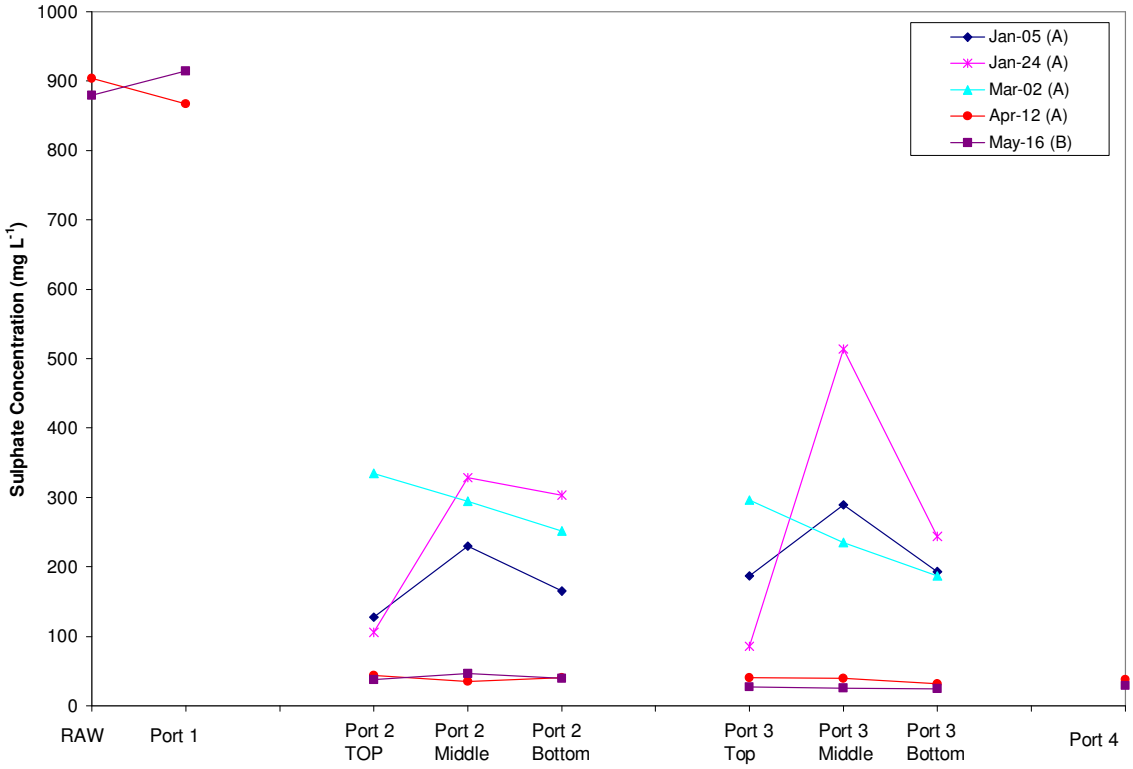

Figure 4-2 Sulphate concentrations at various ports for reactor A and reactor B.

As the January and March sample sets did not contain samples from the Raw feed port, data from the on-site sample analysis conducted by WBDC at the Lanigan Farm showed that the sulphate concentrations in the raw feed water averaged at 1,345 mg L^{-1}. Figure 4-2 above shows that for the three months of continuous operation between January and March 2006, sulphate was reduced within the reactor by an average of 1,100 mg L^{-1}, which meets the design specification. The overall effectiveness of the treatment system can be seen from the April 12 samples for the indoor system (Reactor A), and from the May 16 samples for the outdoor system (Reactor B). Both sample sets showed a sulphate removal efficiency of at least 95% between the Raw feed port and Port 4, an indication that the outdoor system performance is identical to that of the indoor system.

4.1.2 Sulphide Profile

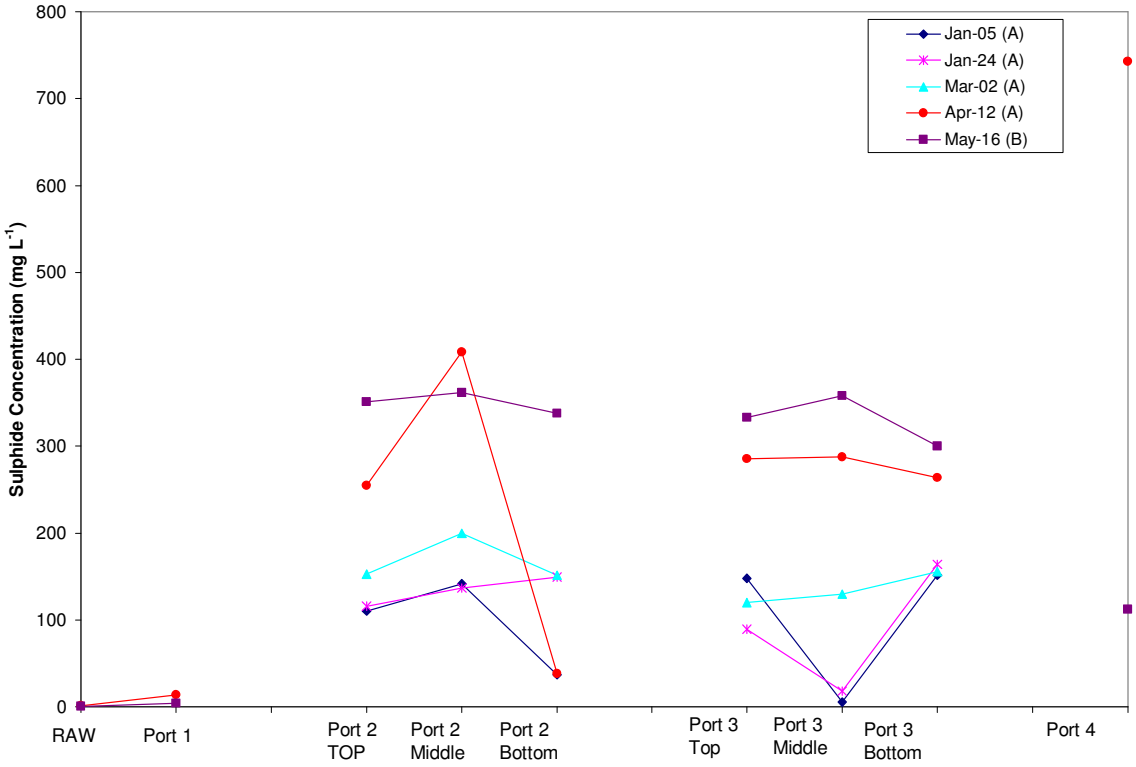

Figure 4-3 Sulphide concentrations at various ports for reactor A and reactor B.

From January to March, 2006 the sulphide concentrations within the anaerobic bioreactor averaged below 200 mg L^{-1}, which is less than the theoretical sulphide concentration of 367 mg L^{-1} produced from 1,100 mg L^{-1} sulphate reduced, though it is possible that sulphide escaped with the off-gas or iron sulphide precipitates accounted for this difference.

Over time, sulphide concentrations within the bioreactors continued to increase along with the improved sulphate reduction. The April 12 samples showed that the indoor system was operating, for the most part, with a sulphide concentration below 300 mg L^{-1}. On the other hand, the May 16 samples showed that the outdoor system was operating with a reactor sulphide concentration of over 300 mg L^{-1}, although both experienced the same sulphate reduction.

4.2 UBC Upflow Anaerobic Sludge Blanket (UASB) Bioreactor Treatment System

On June 11th, 2007, the UBC UASB treatment system began its operation in batch mode. Thirty three days later, on July 14th, 2007, the treatment system switched to continuous operation. Initially with 360 mL d^{-1} of 2,000 mg L^{-1} $NaSO_4$ solution and 115.2 mL d^{-1} 10,000 mg L^{-1} COD silage leachate entering the bioreactor. The treatment system operated in continuous mode for 358 days. For most of the experiment the sulphate feed flow rate was at ~1 mL min^{-1} and the silage leachate feed flow rate was at ~0.5 mL min^{-1}. During the experiment, samples were collected periodically from various ports and tested in order to monitor the system's effectiveness. Table 4-2 below outlines the tests performed for each port during the two operating modes. Important dates with changes pertinent to the experiment are labelled on all figures and their captions listed in Tables 4-2 (Batch mode) and 4-3 (Continuous mode) below.

Table 4-2 Tests performed on samples from various ports during the experiment period.

Test	Port	Operation Mode
Sulphate/Sulphide	Effluent, Port1, Port2, Port3, Influent, Feed	Batch, Continuous
COD/TOC	Effluent, Port1, Port2, Port3, Influent, Feed	Batch, Continuous
Organic Acids	Effluent, Influent, Feed	Continuous
Carbohydrate	Effluent, Influent	Continuous
Phenols/Alcohols	Effluent, Feed	Continuous
Granule Particle Size Distribution	Port4	Continuous

Note: Please refer to Figure 3-2 for locations of the various ports.

4.2.1 Batch Stage Sulphate/Sulphide and COD/TOC Profile

For the Start up/Batch stage, samples were taken from the Effluent port located at the top of the UASB bioreactor as indicated on Figure 3-2. Figures 4-4 and 4-5 below provide the sulphate, sulphide, COD, and TOC concentration profiles during the start-up/batch stage.

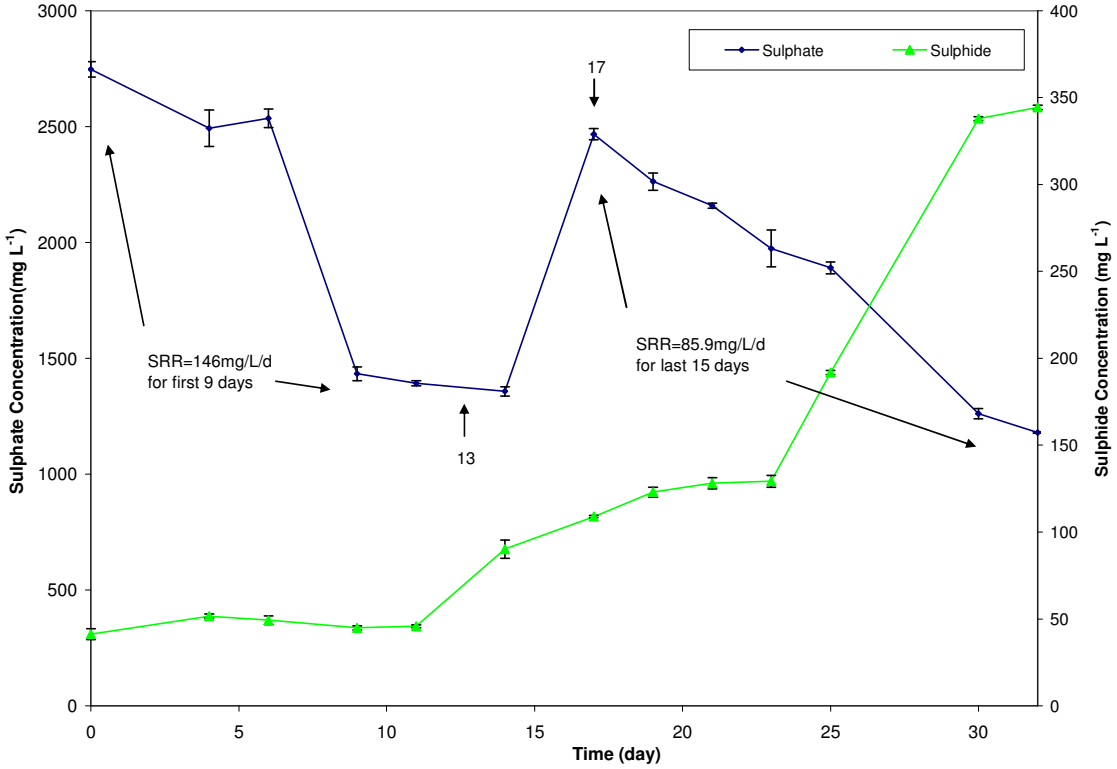

Figure 4-4 Start-up/batch stage sulphate and sulphide concentrations within the reactor

Table 4-3 Start-up/batch stage important dates

Time (Day)	Changes
13	Added 25.7mL Lactic Acid
17	Added 35mL NaOH and 10mL H_2SO_4

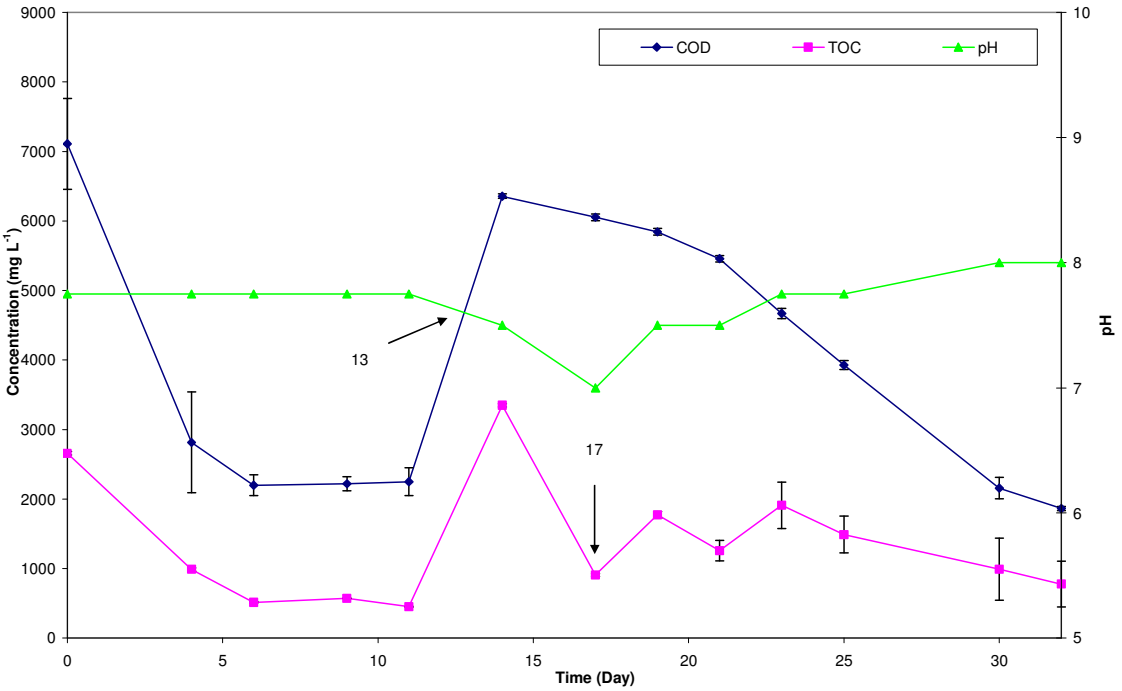

Figure 4-5 Start-up/batch stage COD and TOC concentrations within the reactor

Within the first 10 days of Batch mode operation the sulphate concentration in the bioreactor dropped to 1,433 ± 30.07 mg L^{-1}. This corresponds to a sulphate reduction rate of 146 mg L^{-1} d^{-1}. For the next 10 days of the acclimation period, changes made to the system tested its ability to adjust. On day 13, due to lower levels of COD and TOC (as seen above in Figure 4-5), additional nutrients were added in the form of lactic acid. As a result of this addition, the pH of the reactor decreased to 7 on day 17 (Figure 4-5). An addition of sodium hydroxide was necessary to bring the reactor back to the optimum SRB growth pH of 7.5 to 8. However, due to a miscalculation, excess sodium hydroxide was added on day 17, leading to an increase in the reactor pH. To compensate for the increase in reactor pH, sulphuric acid was added, leading to an increase in the sulphate concentration, as seen on Figure 4-4.

The normal procedure for pH adjustment calls for the use of 1N NaOH and/or 1N HCl. Use of H_2SO_4 here was a mistake, luckily the sulphate concentration in the reactor did not increase above what it was at the beginning of the experiment, and as such this increase was treated as another sulphate addition to the system. Within 24 hours the system had

adjusted to all the additions and the reactor pH was back to 7.5 and maintained the optimum SRB growth pH of 7.5 to 8 for the remaining batch mode operation period.

The sulphate reduction rate for the last 15 days of batch mode operation was 85.9 mg L^{-1} d^{-1}, shown in Figure 4-4. This SRR was 42% less than the SRR for the first 9 days of operation, but this sulphate reduction appears to be more linear than the first 9 days' SRR.

As the bacteria reduced more sulphate, sulphide concentrations rose accordingly (please see Eqn 1 and 2 in Chapter 2). Figure 4-4 above showed that for the first 11 days the sulphide concentrations within the reactor remained low at ~50 mg L^{-1}. This was followed by a moderate increase over the next 12 days to 129.2 mg L^{-1} on day 23. Then for the last 10 days of batch mode operation, sulphide production increased sharply, to 344.3 mg L^{-1} on day 32, the last day of batch mode operation.

In addition to sulphate reduction and sulphide production, another important factor in determining the bacteria's ability to adapt to the new reactor is the amount of organic carbon consumed by the bacterial community as indicated by the COD and TOC levels - within the bioreactor. The addition of lactic acid on Day 13 can be treated as a nutrient addition to the system. As such two COD and TOC consumption values can be calculated. For the first 10 days of operation, the SRR of 146 g L^{-1} d^{-1} consumed COD at a rate of 543 mg L^{-1} d^{-1} and TOC at a rate of 232 mg L^{-1} d^{-1}. After the sulphate and COD additions, the SRR of 85.9 mg L^{-1} d^{-1} consumed COD at a rate of 263 mg L^{-1} d^{-1} and TOC at a rate of 147 mg L-1 d^{-1}.

4.2.2 Continuous Stage Sulphate Profile

Sulphate concentrations in the feed, influent, and effluent (sample ports Feed, Influent, and Effluent in Figure 3-2) are plotted versus time in Figure 4-6. "Feed" refers to the sulphate solution in the feed tank. Influent into the bioreactor consists of both sulphate water and silage leachate. Therefore, sulphate concentrations in the influent that enters the bioreactor directly are diluted by addition of the silage leachate water.

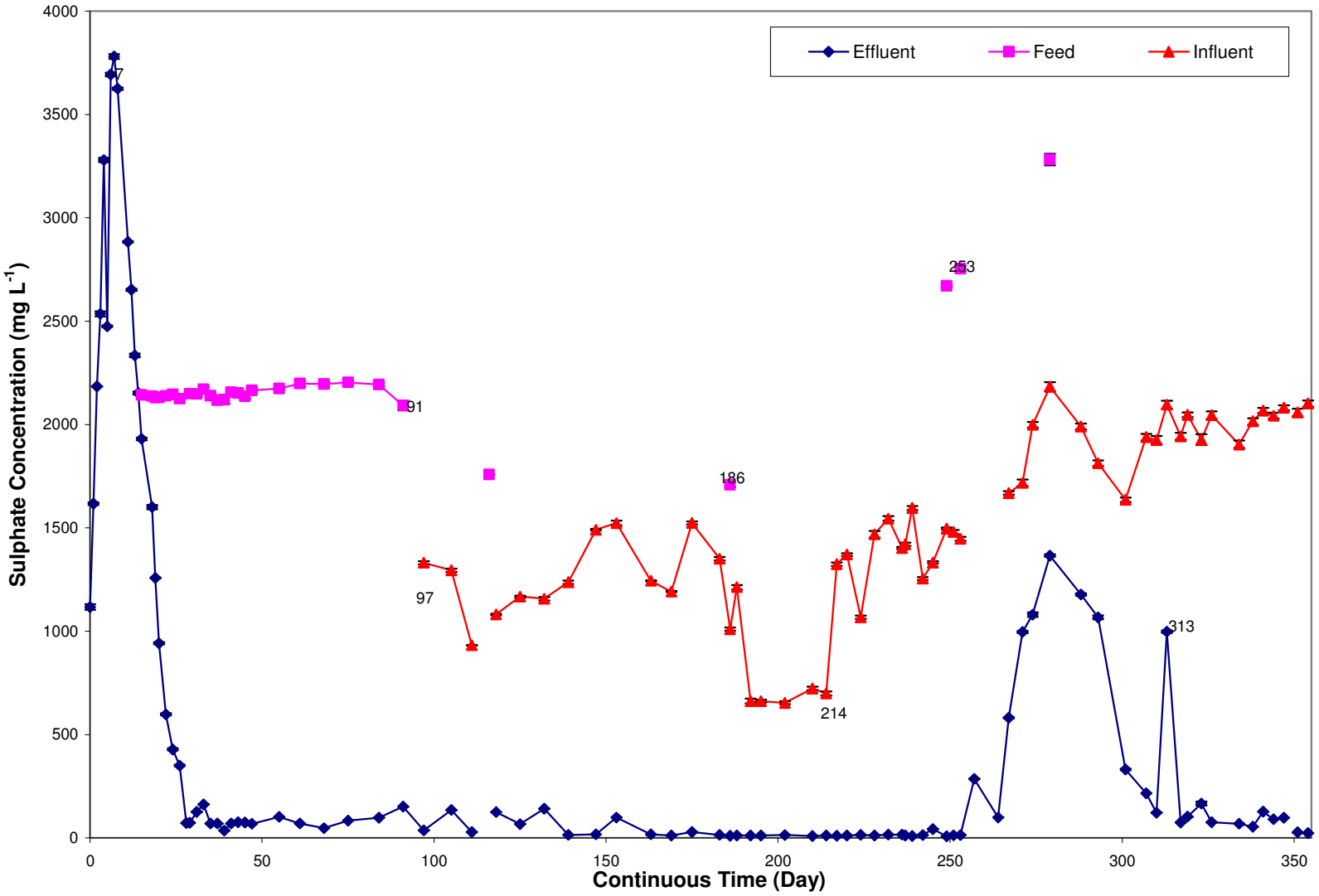

Figure 4-6 Continuous stage sulphate concentrations of the effluent, feed and influent streams

Table 4-4 Continuous stage important dates

Continuous Day	Comments
	Start continuous feed, leachate flow @ 0.08mL/min, sulphate flow @ 0.25mL/min, pumped into mixing tank before entering reactor
1	changed leachate flow to 0.1mL/min
6	stopped sulphate flow
7	removed mixing tank, only leachate flow @ 0.3mL/min
0	new feed system with leachate and sulphate combined flow @ 0.5mL/min
26	introduced small refrigerator for leachate cooling, also added air bubbling to effluent solution
29	changed leachate flow to 0.17mL/min, sulphate flow to 0.69mL/min
34	added ice water bath to cool leachate line
55	changed to 3L beaker for leachate, changed to larger refrigerator, changed to chiller to cool leachate line
72	sulphate flow @ 0.8125mL/min, leachate flow @ 0.625mL/min
75	sulphate flow @ 0.7mL/min, leachate flow @ 0.333mL/min
91	removed air bubbling from effluent solution
93	changed to larger sulphate feed tank
117	changed to larger effluent tank
157	leachate flow @ 0.43mL/min, sulphate flow @ 1.15mL/min
186	changed to simulated ground water with sulphate concentration of 2,600mg/L
214	re-calculated sulphate makeup, sulphate concentration at 2,600mg/L
236	addition of sulphide stripping column - co-current flow, N2 flow @ 10L/min
238	N_2 flow @ 1L/min
242	N_2 flow @ 65mL/min
249	leachate flow @ 0.58mL/min, sulphate flow @ 1.05mL/min
253	N_2 flow @ 75mL/min, sulphate concentration at 3200mg/L
301	leachate flow @ 0.52mL/min, sulphate flow @ 1.01mL/min
306	N_2 flow @ 200mL/min
308	removed N_2 gas flowmeter
310	re-attached flow meter, N2 flow @ 0.6L/min
311	N_2 flow @ 0.3L/min
312	N_2 flow @ 0.4L/min
313	N_2 flow @ 0.4L/min, leachate flow had stopped for ~43 hours
314	N_2 flow @ 0.1L/min,
326	leachate flow @ 0.6mL/min, sulphate flow @ 1mL/min
327	changed to counter-flow, N2 flow @ 0.1L/min
331	changed to new stripper with packing, N2 flow @ 0.5L/min
336	changed to stripper with no packing, counter flow, N2 flow @ 15mL/min
337	N_2 flow @ 25mL/min
338	N_2 flow @ 150mL/min
339	N_2 flow @ 140mL/min
340	N_2 flow @ 120mL/min
343	N_2 flow @ 110mL/min
344	N_2 flow @ 100mL/min
349	N_2 flow @ 150mL/min
352	N_2 flow @ 100mL/min
358	all experiments stopped

At the beginning of the continuous stage, a mixing tank upstream of the influent port combined the sulphate and the leachate feed streams before they entered the bioreactor. However, solids build-up in the influent line tubing entering the bioreactor made it difficult to achieve the desired flow rates during the first 7 days of operation. Removing the mixing tank from the system solved this problem. Alternately, the two feed lines were joined by a T-connector very close to the bioreactor influent port in order to minimize contact time between sulphate-water and silage leachate outside of the reactor. If these two flow streams mix before the bioreactor, then bacterial growth in the feed lines will occur resulting in plugging. Plugging of the feed lines and difficulty in maintaining consistent flows was still a problem for the next 19 days. Thereafter, silage leachate was kept chilled (4ºC) in a floating-lid tank to minimize contact with air and prevent contamination of the leachate with any aerobic organisms that would consume COD. The feed line from the silage leachate feed tank to the bioreactor was also kept cool by lagging with tubing through which water from a chiller flowed.

For the first 91 days of continuous operation, the sulphate concentrations entering the bioreactor were estimated from the sulphate feed tank concentration and the sulphate feed rate. Since flow rates were very low and difficult to control and measure accurately, after Day 97, I decided to measure sulphate concentrations in the combined influent just downstream of the T-connector in order to provide more accurate results. Consequently, the sulphate feed flow rate remained constant at about 1 mL min^{-1} for the duration of the experiment.

From Day 97 to Day 186 the UASB operation was very stable. The average sulphate concentration entering the reactor from the bottom was 1,253 mg L^{-1} and the average effluent concentration measured at the top of the bioreactor was 53.36 mg L^{-1}, showing that 300 mg sulphate was being removed per liter per day.

Up until that time, the sulphate feed water was made from $NaSO_4$. However, cattle drinking water in Saskatchewan comes from underground aquifers where the dissolved solids consist of other cations as well as Na^{2+}. As such, use of a simulated groundwater

with a chemical makeup similar to that of the ground water used in Saskatchewan would provide valuable insights into how those chemicals affect the treatment process. For example, cations can influence the size of the UASB granules (Tiwari 2006). Therefore, using a chemical analysis of groundwater provided by WBDC [Braul, personal communication, January 14, 2008], fresh simulated sulphate feed water was pre-mixed using a combination of Ca, K, Na, and Mg as outlined in Table 3-1 with a total concentration of 2,600 mg L^{-1} and added to the system on Day 186. Due to a calculation error, initial sulphate concentration of the feed from this simulated ground water feed was at 1,709 mg L^{-1}. On Day 214, the feed concentration was increased back to 2,600 mg L^{-1}. From Day 214 to Day 250 UASB operation was stable and an average of 1,400 mg L^{-1} in the influent was reduced to 14.77 mg L^{-1} (average) in the effluent (SRR=450 mg SO_4^{2-} L^{-1} d^{-1}).

Some of the ground water to be made potable for cattle contains very high sulphates (Table 4-5). Therefore, to further test the system, on Day 253, the simulated ground water sulphate concentration was increased to 3,200 mg L^{-1}.

Table 4-5 Saline groundwater concentrations in different regions as reported by Amber Brown.

Location	**Author(s)**	**Concentration**
Alberta	Beke & Hironaka, 1991	3,875 mg L-1
Navada	Weeth & Capps, 1972	2,500 mg L-1

Source: Amber Brown Masters Thesis, 2007.

At first, for some reason, sulphate concentrations in the effluent increased between Days 253 and 310. However, eventually, the system settled down and from Day 310 to the final day of operation an average of 2,020 mg L^{-1} of sulphate in the influent was reduced to 82.55 mg L^{-1} of sulphate in the effluent (SRR=538 mg SO_4^{2-} L^{-1} d^{-1}). On Day 313 the effluent sulphate concentration increased briefly due to the silage leachate pump accidentally turning off.

4.2.2 Continuous Stage Sulphide Profile

In the UASB, bacteria reduce sulphate to produce sulphide (Eqn. 2-1). However, this sulphide can be toxic to the cattle and must also be removed from the water. Thus, initially, for the first 50 days of continuous operation, the UASB effluent solution flowed into another downstream tank (TK-03, Appendix A-1), in which an aquarium pump (at a rate of 150 cm^3 min^{-1}) aerated the effluent water for the purpose of oxidizing the sulphides into elemental sulphur. Air was pumped into the effluent tank via tubing that was inserted into the solution. However, initial experiments showed that this setup was problematic as the tubing would not stay underwater, leading to no oxidation of the sulphides. A solution was found by using a floating lid, similar to that used in the silage leachate feed tank, which held the air line in place. As the effluent solution rises, the air line would rise with the solution, while staying just below the surface of the solution. The new setup was introduced on Day 28, and from Figure 4-7 below, the effluent sulphide level stayed below 10 mg L^{-1}, with just a few exceptions, which are likely due to analysis inaccuracies rather than the reactor performance. There was no need to seed the reactor with sulphide-oxidizing bacteria (SOB). In previous laboratory experiments done by a COOP student we found SOB such as *Beggiatoa* spp. and *Thiotrix* spp. in lightly aerated effluent from silage leachate-treated high-sulphate water.

The air-pumping rate had to be adjusted so that only stoichiometric amounts of oxygen are delivered to the water (Eqn. 2-4 – the stoichiometric equation for sulphide plus oxygen to give elemental sulphur) so as to avoid complete oxidation of sulphide back to sulphate, which would negate the purpose of the treatment system. This sulphide-oxidation was only operated for the first 97 days as proof of concept for this step.

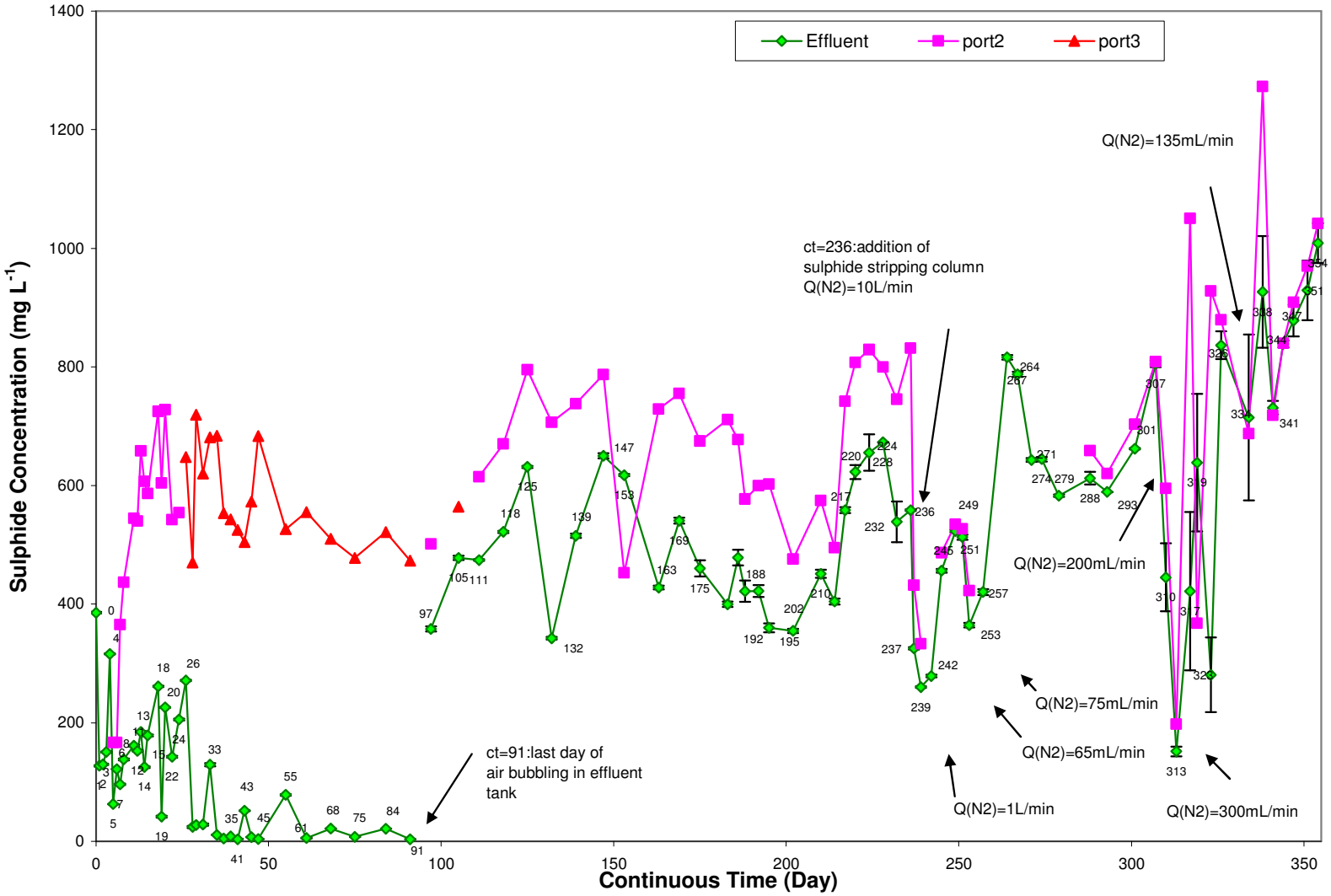

Figure 4-7 Continuous-stage sulphide concentrations of the Effluent Port, Port2 and Port3

In the UBC UASB the pH was above 7, where sulphide is present mostly as HS^-. Nevertheless, sulphide levels in the UASB were monitored, beginning on Day 97, by taking samples from the Effluent Port located at the top of the UASB bioreactor (Figure 3-2). The effluent sulphide concentration for Day 97 was determined to be 358.0±4 mg L^{-1}, which was less than the expected sulphide concentration of 431.7 mg L^{-1} produced from 1,294 mg L^{-1} of sulphate reduced on Day 97. However, between Day 97 and 169, the effluent sulphide concentration showed significant fluctuations between 342 and 650 mg L^{-1}. Examination of the influent sulphate profile from Figure 4-6 above showed fluctuations in the influent sulphate concentration during this period. However, further investigation into the chemical oxygen demand profile (Figure 4-9) showed influent COD fluctuations consistent with those from the sulphide profile shown above for the same period. On Day 214, the sulphate feed concentration was adjusted to 2,600 mg L^{-1}. As a result, the influent sulphate concentration increased to ~1,400 mg L^{-1}. From this, an effluent sulphide concentration of 467 mg L^{-1} was expected. However, samples taken after this increase in influent sulphate concentration revealed a much higher effluent sulphide concentration than expected, reaching a maximum effluent sulphide concentration of 672.8 mg L^{-1} on Day 228. However, a sample collected 4 days later, on Day 232, showed that the system responded quickly as the effluent sulphide concentration was 538.6±34.44 mg L^{-1}. This 4-day period coincides with the hydraulic retention time of the system of 3.47 days.

To prevent sulphide inhibition when treating high sulphate concentration water, a N_2-stripper was installed in the recirculation line (Figure 3-2) on Day 236. The purge gas used was commercial grade nitrogen gas as there was not enough biogas produced from the UASB bioreactor (recommended by Yamaguchi 1999) and air would have introduced unwanted oxygen into the bioreactor (used by Gangagni Rao 2003). Initial purge gas flow rate was set at 10 L min^{-1}, but this was reduced to 1 L min^{-1} the next day since gas carried over into the reactor leading to loss of biomass in the reactor effluent. The addition of this sulphide-stripping column had an immediate impact on the effluent sulphide level. Sample analysis of Day 239 sample showed effluent sulphide level at 260.2 mg L^{-1}, a significant decrease from Day 236's sulphide level of 558.6 mg L^{-1}.

However, the gas entrainment problem continued to persist, hence the purge rate was reduced to 100 mL min^{-1}. This solved the gas entrainment problem, but the effluent sulphide concentration increased to above 500 mg L^{-1}.

On Day 253, the sulphate feed concentration was increased further to 2,755 mg L^{-1}, which lead to an increase in the influent sulphate concentration for the following 2 weeks, reaching 2,000 mg L^{-1} by Day 274. The N_2 purge rate maintained a steady flow of 75 mL min^{-1} for the next fifty days. During this period of operation, effluent sulphide concentration increased rapidly for the first two weeks, peaking out at 816.2 mg L^{-1} on Day 264. Since then, the effluent sulphide level dropped to ~600 mg L^{-1} within a week and maintained at this level for the next month of operation. On Day 279, the sulphate feed concentration was increased again to 3,282 mg L^{-1}, which lead to an increase in influent sulphate concentration to 2,183 mg L^{-1}. This resulted in an increase in the effluent sulphide concentration to 805.2 ± 5.635 mg L^{-1} on Day 307. To counter the increase in sulphide levels, the purge rate increased to 200 mL min^{-1} on Day 308. However, this caused an overflow of solids from the reactor to the stripping column. Various gas flow rates were experimented over the next five days (Table 4-3), which resulted in fluctuations in the effluent sulphide concentrations (Figure 4-7). To alleviate the problem, the stripping column was modified to allow for counter-flow operation on Day 327. With the N_2 purge rate maintained at ~100 mL min^{-1}, overflow of solids stopped and the reactor operation returned to normal.

In order to test the feasibility of using packing to enhance sulphide stripping, a newly modified stripping column with packing was inserted into the system on Day 331. Initial sample test results three days after installation showed a decrease in sulphide levels, 714.7 mg L^{-1} on Day 334 compared to 836.6 mg L^{-1} on Day 326, which is a good indication that the packing in the stripping column is working properly. However, just two days later – five days after installation – the system was shut down due to solids build-up within the stripper column. A picture of the packed column with solids is shown in Figure 4-8.

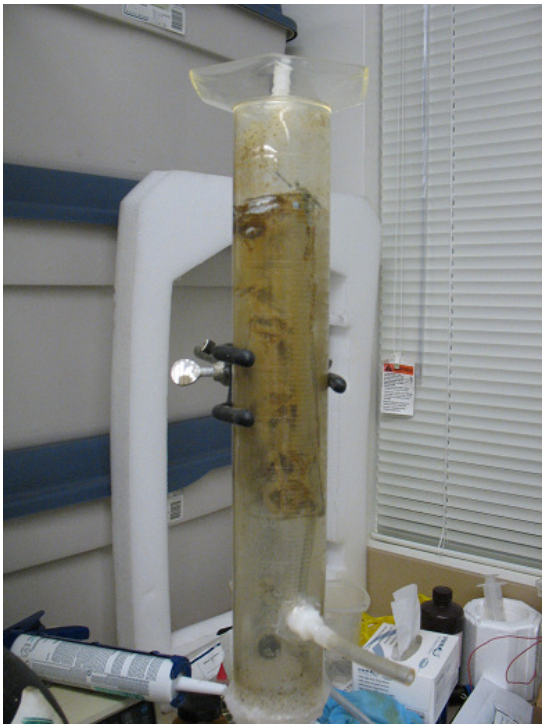

Figure 4-8 Stripper with solids build-up.

On Day 336, the original stripping column with no packing was inserted with the N_2 purging operating in counter-flow operation at 15 mL min^{-1}. The flowrate was increased to ~100 mL min^{-1} on Day 338 and remained consistent for the remainder of the experiment. During this period of operation, sulphate reduction in the system held steady at 573.6 mg SO_4^{2-} L^{-1} d^{-1}, however, effluent sulphide concentration fluctuated between 150 and 1000 mg L^{-1}, on an upward trend (Figure 4-7). Changes made to the sulphide-stripping column during this period meant that it was ineffective towards reducing sulphide levels in the effluent. In an effort to further study the stripping column, samples were taken from the top and bottom of the stripping column: S-IN and S-OUT ports (Figure 3-2) for sulphide analysis. Samples were collected for the last two weeks of the experiment and the results are shown below in Table 4-6.

Table 4-6. Sulphide concentrations from the final Effluent and S-IN and S-OUT ports for the last two weeks of experiment.

Day	Final Effluent	S-IN	S-OUT
344	838.7 ± 5.896	374.1 ± 7.531	382.6 ± 12.09
347	878.2 ± 26.40	410.4 ± 2.556	404.8 ± 8.600
351	929.3 ± 50.29	390.9 ± 19.96	408.3 ± 3.993
354	1,009 ± 33.66	417.5 ± 12.41	394.5 ± 1.353

Note: units = mg L^{-1}.

Looking at Figure 3-2, the S-IN port is essentially the effluent port from the UASB bioreactor; hence the S-IN port sulphide concentration would be identical to the effluent sulphide concentration from the UASB bioreactor. The S-OUT port is the recycle stream that returns back to the UASB bioreactor. Therefore the difference between the two ports would be indicative of the efficiency of the sulphide-stripping column. Results shown in Table 4-6 clearly indicate that the sulphide-stripping column did not reduce sulphide levels in the recycle stream.

4.2.3 Carbon Compound Testing

Silage leachate was chosen to be the carbon source, which provides the nutrients required for bacterial activity and growth in the bioreactor (Brown 2007). Silage leachate is comprised of many different types of organic compounds including lactate, acetate, carbohydrates, alcohols and phenols (Krizsan 2007). Based on calculations presented in Appendix B-1, the influent stream flowrate was set at 1 mL min^{-1}. Since plugging is possible at this low flowrate, extra care was taken to maintain delivery of the silage leachate to the UASB bioreactor. During the first stage of the continuous mode operation, the two influents were pre-mixed in an ambient temperature tank before being pumped into the UASB bioreactor. Lines quickly became clogged as a result of bacterial activity at room temperature both in the mixing tank and in the delivery tubes. Sample analysis showed a 40% decrease in influent sulphate and COD concentration before entering the UASB bioreactor. In order to minimize the bacterial activity, the leachate was autoclaved for 2 hours to kill off as much bacteria as possible. The autoclaved leachate was stored in 4^{o}C refrigerator as a further attempt to keep bacterial growth to a minimum. All leachate feeds were tested for COD and TOC prior to addition to the system to ensure that they meet minimum requirements. As mentioned previously, the mixing tank was removed and the sulphate and leachate lines were joined, just prior to entering the bioreactor, using a T-connector. The silage leachate tank and the leachate feed line were kept at 4^{o}C in order to minimize bacterial activity.

In order to know how much silage leachate to add to the reactor, the amount of carbon source required for the desired amount of sulphate to be reduced needed to be determined. Bulk carbon content of the feed was estimated with chemical oxygen demand (COD), which is often used to quantify the strength of organics in wastewater (Metcalf and Eddy 2003). Since silage leachate comprises many different carbon compounds, in an attempt to measure the rate at which different types of carbon compounds are used up, total organic compounds available in the feed source were tested with the total organic carbon (TOC) test, while organic acids were quantified with HPLC and total carbohydrate measured with the Anthrone assay.

4.2.3.1 Chemical Oxygen Demand

The soluble chemical oxygen demand (sCOD) test was performed on all fresh silage leachate before addition to the feed tank. This was done to ensure a constant sCOD concentration of 10,000 mg O_2 eq. L^{-1} in the silage feed tank. In addition, samples were taken from the feed tank regularly to check if any bacterial activity in the feed tank was degrading the COD. In Figure 4-9 sCOD concentrations in the silage leachate feed tank and UASB influent and effluent streams are plotted over time.

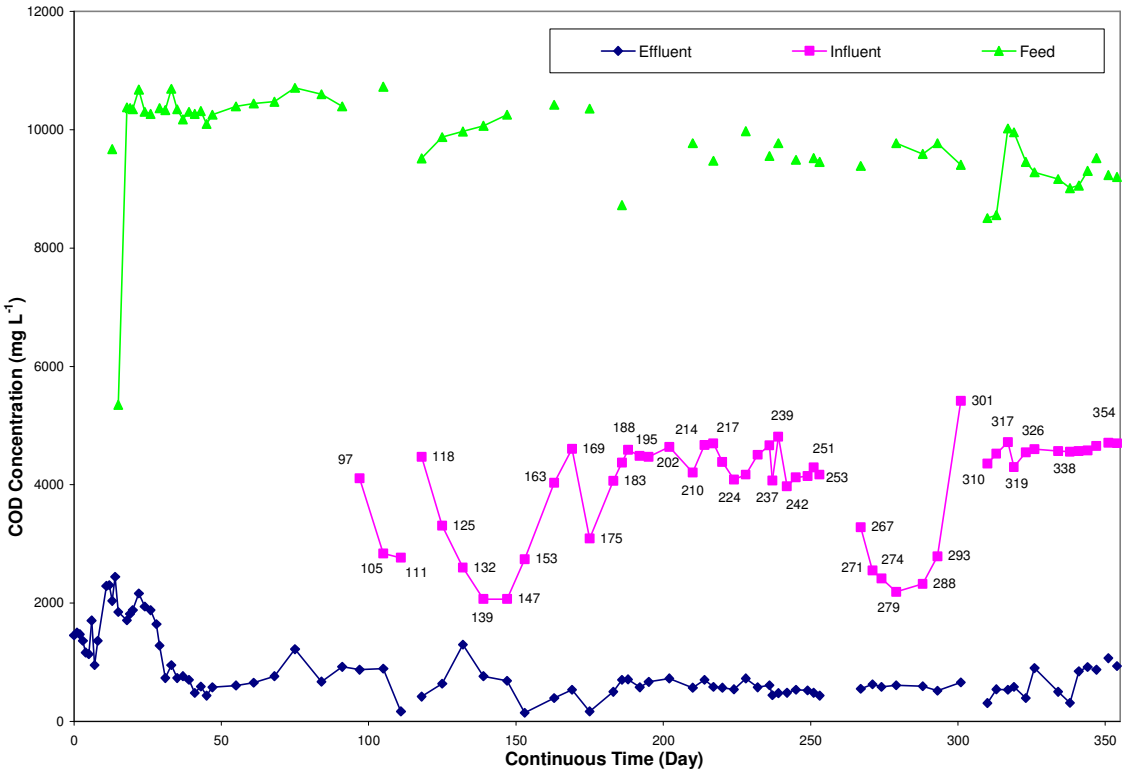

Figure 4-9 Continuous stage sCOD concentrations of the effluent, influent and feed streams

Throughout the experiment the feed COD concentration remained constant at ~10,000 mg O_2 eq. L^{-1}. In the initial days of influent COD measurement, the values fluctuated, varying between 2,000 mg O_2 eq. L^{-1} and 4,500 mg O_2 eq. L^{-1}. Once the system setup was changed to minimize biological activity in the feed line, the influent COD concentration stabilized at ~4,500 mg O_2 eq. L^{-1}. An upset occurred for about one month

starting on Day 267, when the influent sCOD dropped down to 2,190 mg O_2 eq. L^{-1}. This reduction in nutrient supply to the reactor coincided with poor performance with respect to sulphate reduction as seen in Figure 4-6. On Day 301, the influent sCOD concentration was restored to 4,500 (± 60) mg O_2 eq. L^{-1} where it remained for the duration of the experiment. The final effluent sCOD concentration averaged around 600 mg L^{-1} for the first part of the experiment, when sulphate concentration in the feed was approximately 1,400 mg L^{-1}. Interestingly, the sCOD effluent concentration was higher at the end of the experiment (~925 mg L^{-1}) despite the increase in feed sulphate concentration to ~2,000 mg L^{-1}.

4.2.3.2 Total Organic Carbon

The total organic carbon test was performed on samples collected from the Influent and Effluent Ports of the UASB bioreactor. As this test provides a quantitative measure of organic carbon present in the samples, the difference in TOC concentrations from the Ports can provide an estimate of the amount of organic carbon utilized by the bacterial community in their process of reducing sulphate. Influent and Effluent Results from the total organic carbon (TOC) test are summarized in Figure 4-10.

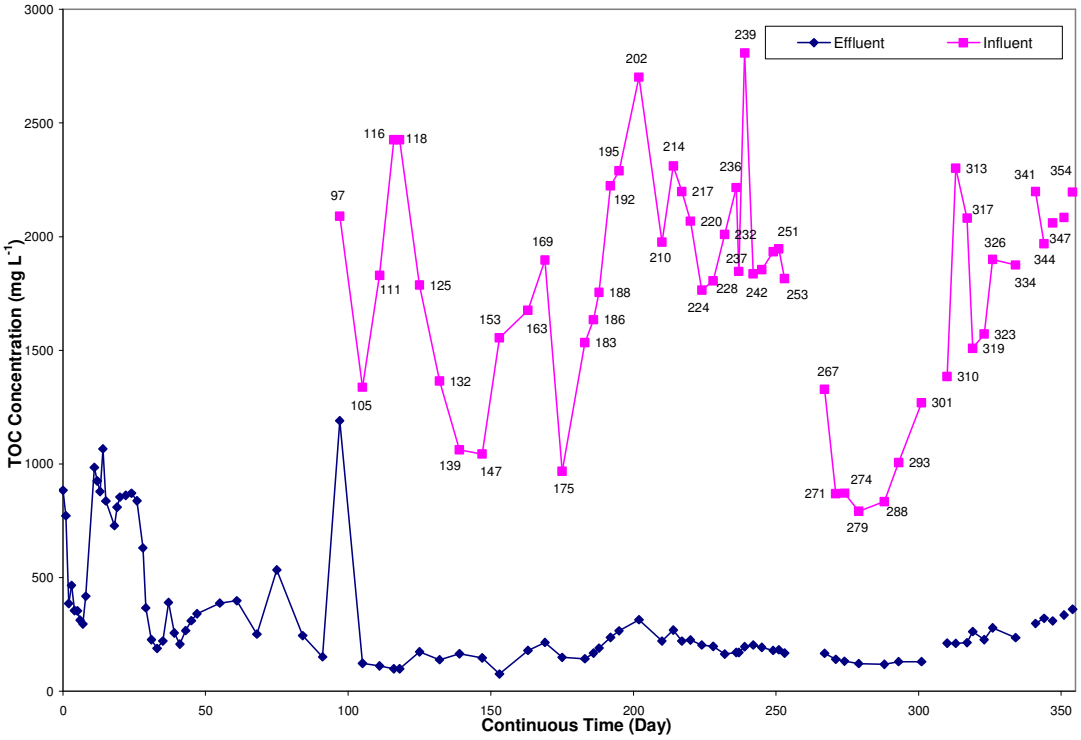

Figure 4-10 Continuous stage TOC concentrations of the effluent and influent streams

The TOC test was conducted only on effluent samples for the first 91 days of operation. However, in order to determine the amount of organic carbon used by the bacteria, both influent and effluent samples were tested for TOC beginning Day 97. The variation in TOC concentrations mirrored that of sCOD fluctuations. After fixing the influent line clogging problems, the TOC concentration of the entering flow stream averaged at about 2000 mg L^{-1} (except for during the one month upset). Effluent TOC concentrations were approximately 250 mg L^{-1}, rising slightly towards the end of the experiment to around 325 mg L^{-1}.

4.2.3.3 Carbohydrates

Total carbohydrate concentrations in the influent and effluent ports over the duration of the experiment are presented in Figure 4-11.

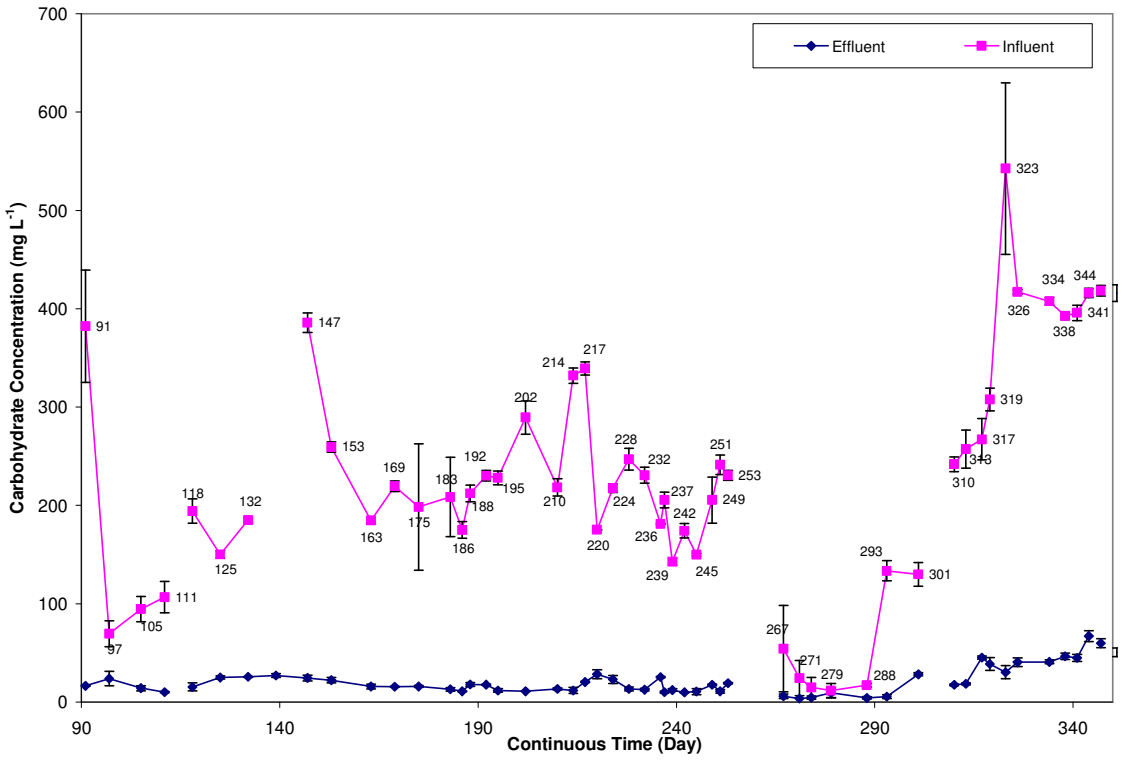

Figure 4-11 Continuous-stage carbohydrate concentrations (mg-glucose equivalents L^{-1}) of the effluent and influent ports

The carbohydrate analysis for the period from Day 91 to 163 cannot be relied on due to an incorrect methodology in the analysis. Disregarding those data, the trend in carbohydrate concentration was similar as those for sCOD and TOC (Figures 4-9 and 4-10). For the most part, carbohydrate consumption correlated more closely with that of TOC than COD, except for the last 50 days of operation. In this period, consumption of carbohydrates increased from ~220 mg L^{-1} to ~360 mg L^{-1}.

4.2.3.4 Organic Acids Analysis

As mentioned in the literature review, organic acids are products of silage making. I determined the organic acids present in two different sources of silage. One type of silage was used for the Lanigan reactor and the organics acids present in the influent and effluent are shown in Figures 4-12 and 4-13, respectively.

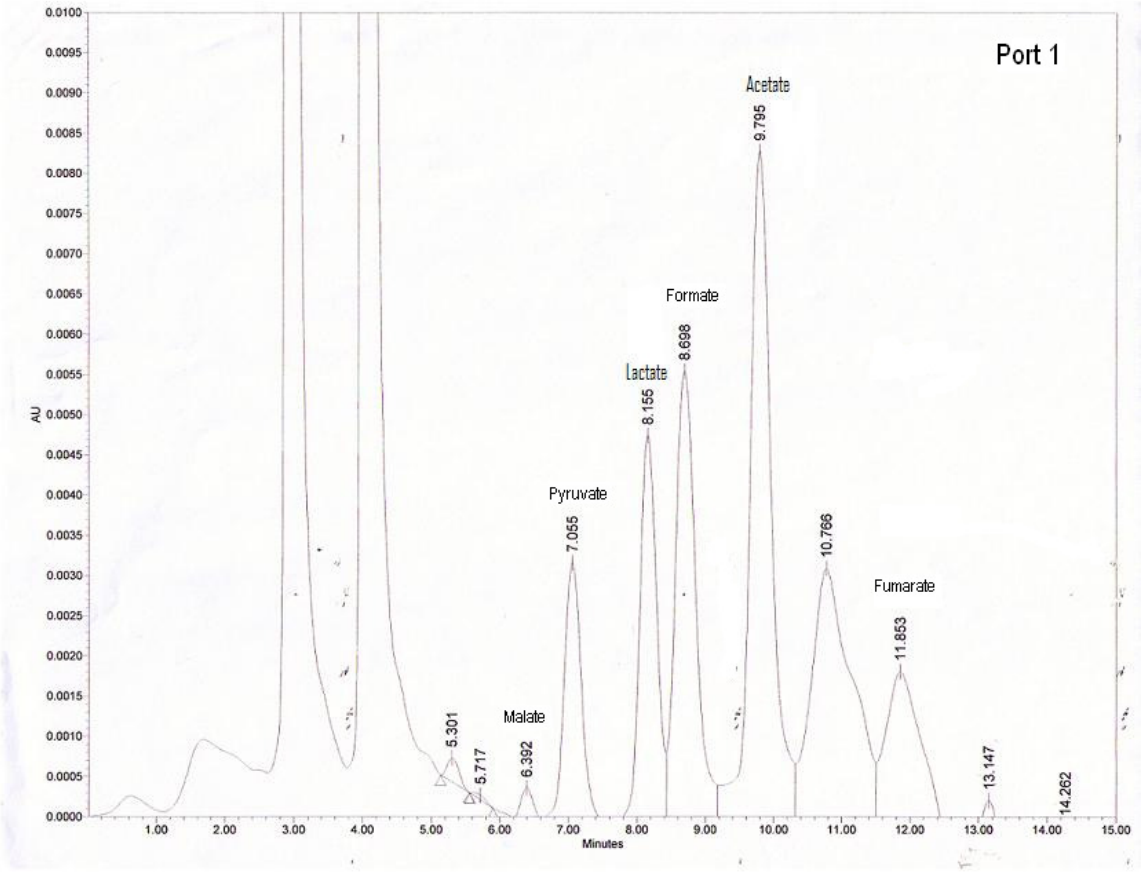

Figure 4-12 Organic acid composition of Port 1 (influent) sample from Lanigan farm.

Of the eight significant peaks in Figure 4-12, six were identified as being due to the presence of malate, pyruvate, lactate, formate, acetate, and fumarate.

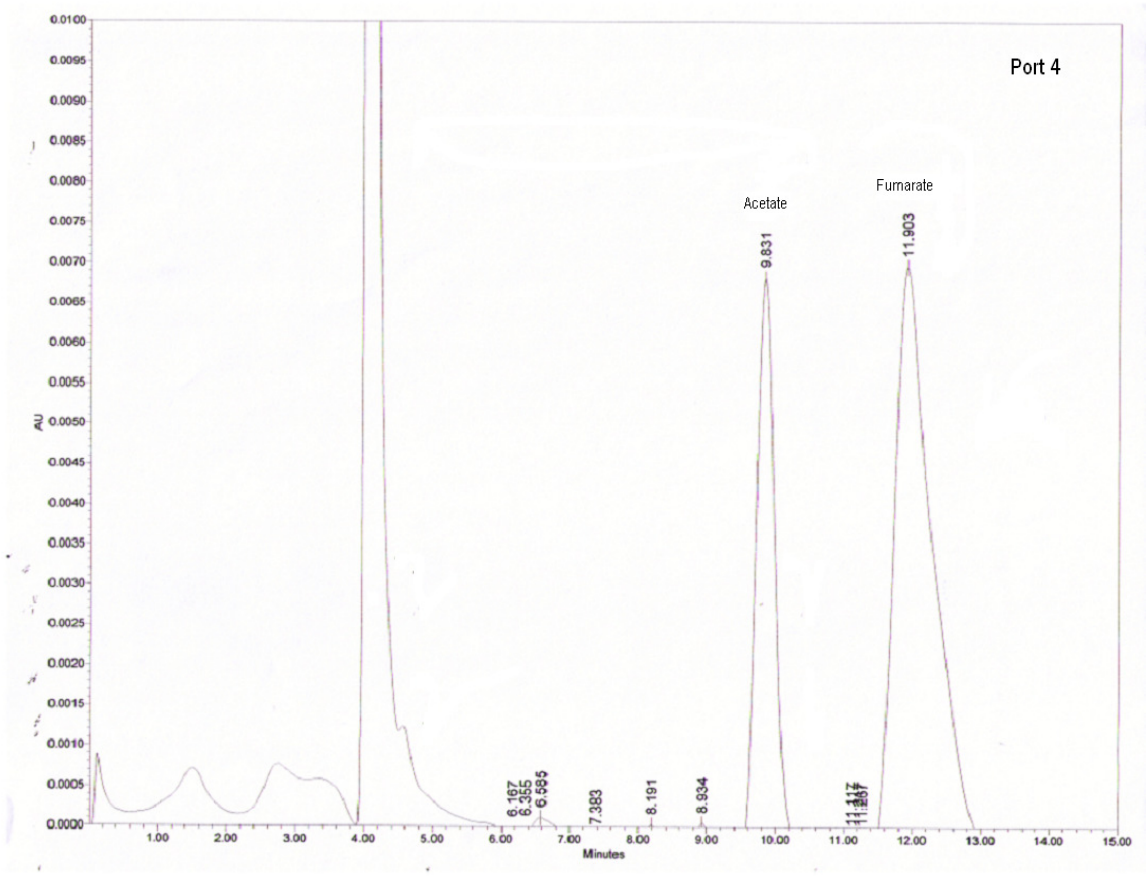

Figure 4-13 Fatty acid composition of Port 4 (effluent) sample from Lanigan farm.

Figure 4-13 shows that only acetate and fumarate remain in the effluent. All of the lactate along with malate, pyruvate and formate are consumed in the reactor. Acetate may accumulate if more is formed, by acetogenesis and incomplete oxidation by SRB, than is consumed, by ASRB and methongens, if present. Fumerate appears to be another byproduct of metabolic reactions in the process as its concentration is greater in the effluent than in the influent.

A different silage was used in the UBC UASB treatment system, which was obtained from the UBC Dairy Education and Research Centre, located in Agassiz, BC . While the silage from the Agassiz farm was similar to that from Lanigan in terms of physical appearance, its chemical make-up with respect to organic acids was different. Figure 4-14 indicates that lactate is by far the predominant organic acid in Agassiz farm silage.

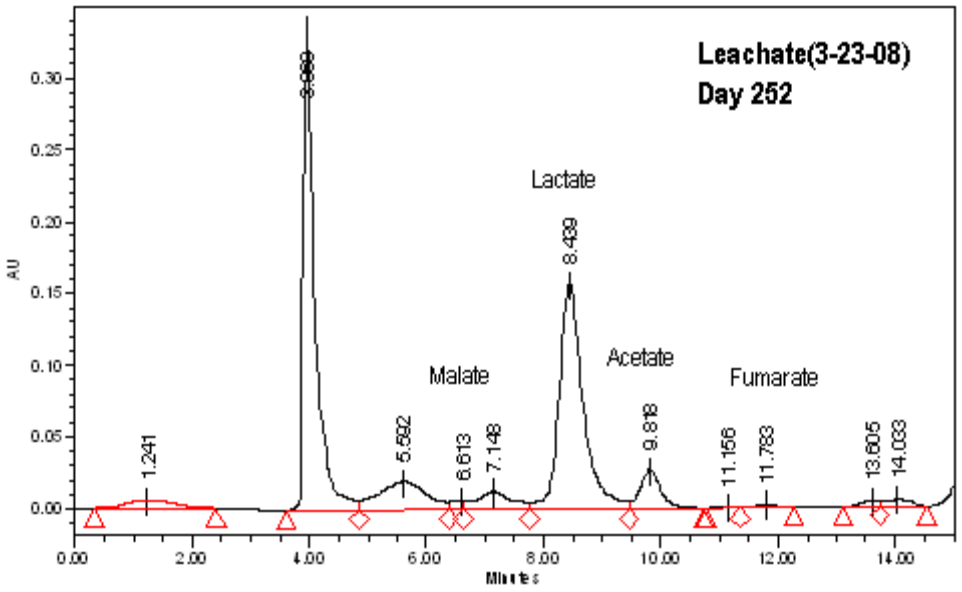

Figure 4-14 HPLC diagram of silage leachate from Agassiz farm.

An additional analysis of the influent organic acids on another day confirmed this (Figure 4-15).

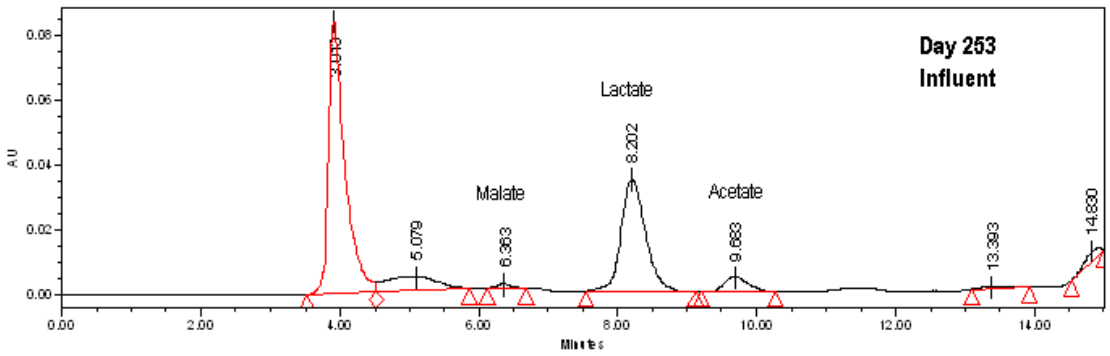

Figure 4-15 Fatty acid composition of the influent port of the UBC UASB bioreactor.

Therefore, only lactate and acetate concentrations in the influent and effluent were measured (Figure 4-16).

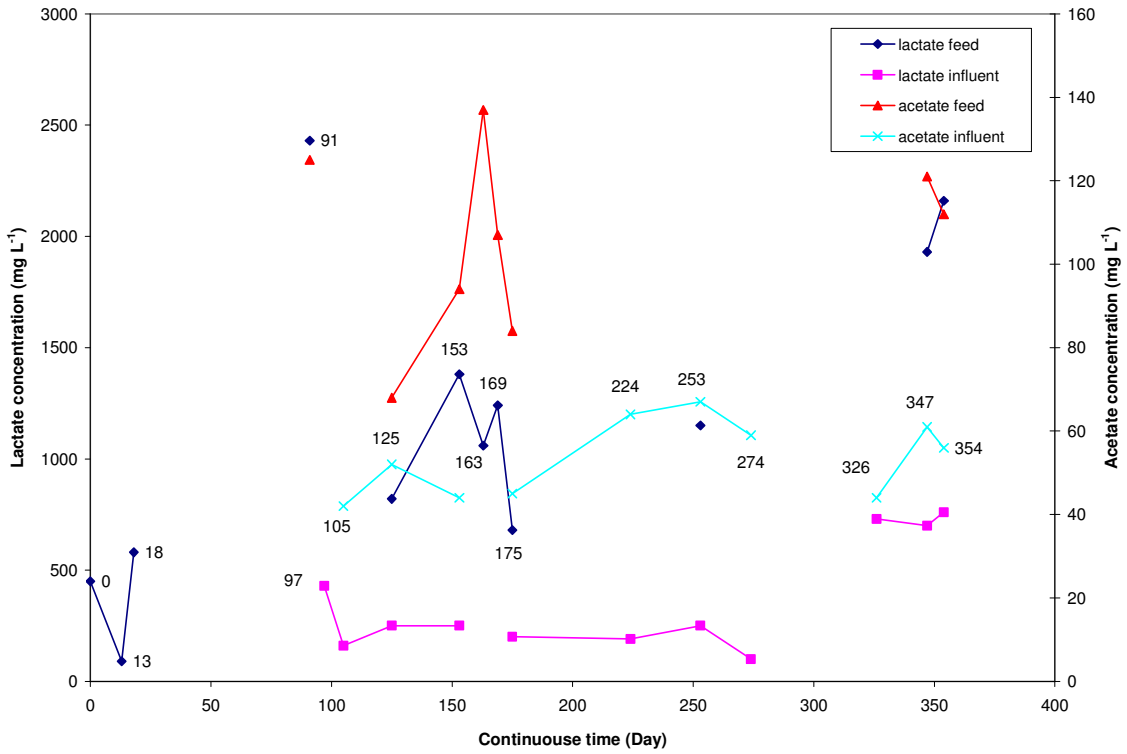

Figure 4-16 VFA concentration in the feed and influent streams for selected dates.

Organic acid analysis was performed for a few samples from the start-up, middle and end phases of operation (Figure 4-18). For the samples from the start-up and middle phases of operation, lactate and acetate concentrations in the influent remained constant at about 228 mg L^{-1} and 53.3 mg L^{-1}, respectively. For the last 4 weeks of the experiment, the influent contained a higher concentration of lactate, 730 mg L^{-1}, while the influent acetate concentration was about the same as before, 53.6 mg L^{-1}. In all of the effluent port samples tested, no organic acids were detected with the exception of two samples. These are from Day 19 with an acetate concentration of 95 mg L^{-1}; and Day 97 with an acetate concentration of 43 mg L^{-1}.

4.2.3.5 Alcohols and Phenols

Silage is known to contain also alcohols and phenols; therefore the fate of these in the UASB was monitored. Only two sets of samples were sent out to Bodycote Testing in Calgary, Alberta for analysis due to the expense of these tests. The first sample set consisted of two silage leachate samples and one reactor effluent sample, collected on

September 18th, 2007. The second sample set consisted of one silage leachate sample and two reactor effluent samples collected in March and May of 2008. Tables 4-7 and 4-8 below summarize findings from the Bodycote Analysis. A copy of the analysis report for the two sample sets can be found in Appendix C-1.

Table 4-7 Alcohols and phenols present in the reactor influent and effluent taken on Day 66 (September 18, 2007)

	Units	**Leachate**	**Influent**	**Effluent**
Alcohols:				
Methanol	mg L^{-1}	46	20	8
Phenol	mg L^{-1}	5.5	2.4	0.69

Note: Alcohol detection limit = 5mg/L, Phenol detection limit = 0.001mg/L.
All other alcohols were below detection limit. See Appendix for complete listing.

Table 4-8 Alcohols and phenols present in the reactor influent and effluent taken on Day 234, 310 and 338

	Units	**Leachate (Day 338) (June 17, 2008)**	**Influent (Day 338)**	**Effluent (Day 234) (March 5, 2008)**	**Effluent (Day 310) (May 20, 2008)**
Alcohols:					
Methanol	mg L^{-1}	19	6.4	<5	<5
Ethanol	mg L^{-1}	266	90.4	<5	<5
2-Propanol	mg L^{-1}	<5	<5	<5	<5
1-Propanol	mg L^{-1}	20	6.8	<5	<5
1-Butanol	mg L^{-1}	20	6.8	<5	<5
1-Pentanol	mg L^{-1}	<5	<5	<5	<5

Note: Alcohol detection limit = 5mg/L

There are alcohols present in the silage leachate, most notably ethanol. All of the alcohols were completely consumed by the bacteria in the UBC UASB, supported by the low effluent concentrations in Table 4-8.

4.2.4 Characterization of Biomass Granules

Formation of bacteria granules inside the UASB is desired since this increases the amount of biomass retained in the reactor, which enhances the rate of sulphate-reduction. This is one of the advantages of the UASB reactor configuration over other reactor types. To decrease the time needed for formation of granules, we used, as inoculum for our UASB, sludge from an existing UASB bioreactor (Fleishman's Yeast, Alberta), which contained mature granules. Characteristics of the sludge bed that were monitored over time included the total volume of sludge and the granule size distribution inside the sludge. Sludge bed volume was calculated based on sludge bed height measured at various times during the experiment, as shown below in Figure 4-17.

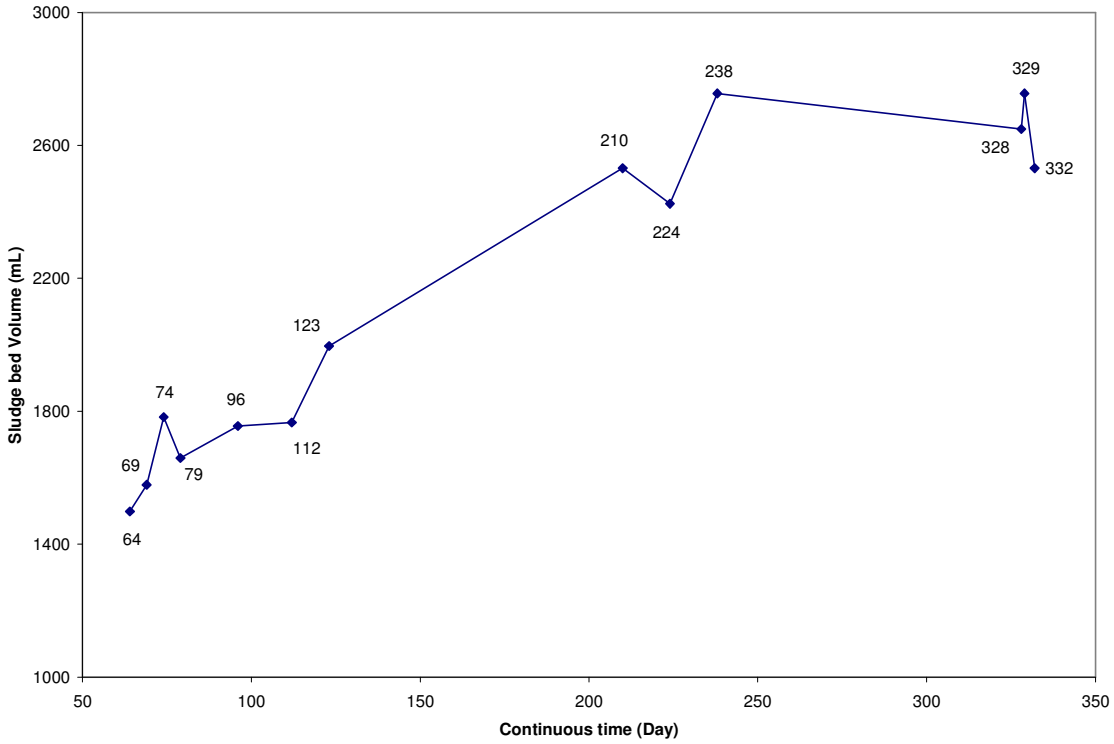

Figure 4-17 Sludge bed volume growth during the experiment.

Three stages of sludge growth are well defined during the experiment. Between Day 64 and Day 112, the sludge bed growth is slow, indicating the beginning stage of granular sludge growth in the reactor. Between Day 112 and Day 238, rapid growth of granular sludge occurred, evidenced by the rapid increase in sludge volume. After Day 238, the

sludge bed volume remained constant indicating that there was either no more biomass growth, or that new bacteria were leaving the bioreactor and not accumulating in the sludge.

Granule sizes were monitored using image analysis as shown in Table 4-9. The full set of data and results from image and statistical analysis, as well as all pictures of the granules used for the distribution analysis are in Appendix D.

Table 4-9 Granule size distribution summary

Day	N	Max size	Mean size	Median size	% > 1mm	n > 1mm
0	59	1.06	0.43	0.38	3.49	2
97	420	2.24	0.57	0.48	19.58	82
125	156	1.92	0.78	0.80	38.19	60
143	136	3.03	0.69	0.49	26.28	36
220	2256	3.38	0.47	0.31	12.93	292
236	258	2.64	0.74	0.71	25.98	67
288	1099	4.29	0.45	0.14	16.95	186
331	1398	4.92	0.52	0.12	20.91	292

These granule size distributions were obtained from random samples of sludge taken from port 4 of the UASB bioreactor on the day they were collected. This test was conducted to show the growth of the granules throughout the experimental period. Example pictures of four granule samples are shown below in Figures 4-18 to 4-21. These images are used to produce the distributions listed in the Appendix, as well as Table 4-9.

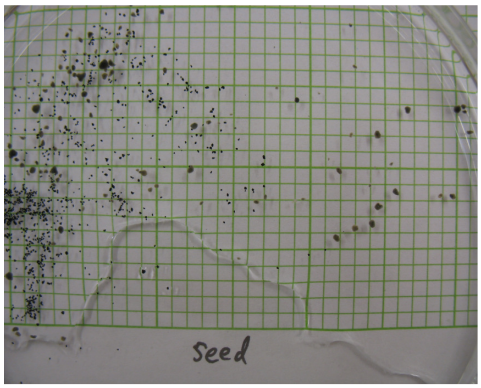

Figure 4-18 Seed granules

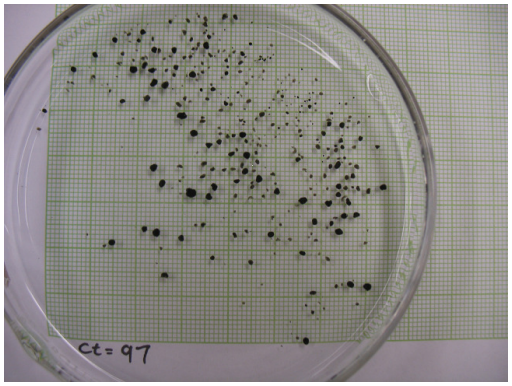

Figure 4-19 Ct=97 granules

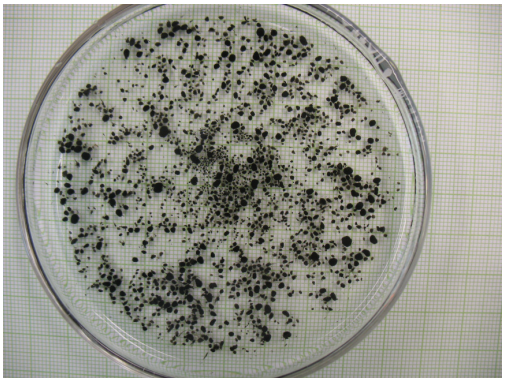

Figure 4-20 Ct=220 granules

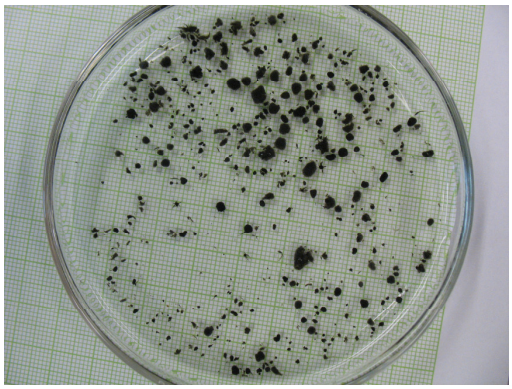

Figure 4-21 Ct=288 granules

From Table 4-9 and the four images above, a clear trend emerges. The granules have grown considerably during one year of operation. For example, on Day 97, the maximum granule size was 2.24 mm. Almost 20% of the granules were greater than 1 mm in diameter. By Day 288 the maximum granule size had increased to 4.29 mm. This sample population had a similar percentage greater than 1 mm when compared to the Day 97 sample; but the 16.95% represented 186 granules in the sample. The fraction of total granules analyzed that were greater than 1 mm in diameter varied from 13 – almost 40% and there was no clear trend over time. Early on, most granules were almost spherical in shape, as shown in Figure 4-18, for the seed sludge. During the growth stage, granules began to cluster together (Figure 4-22) and this probably accounts for the increase in maximum granule size seen over time. Additional microscope pictures can be found on the accompanying disc.

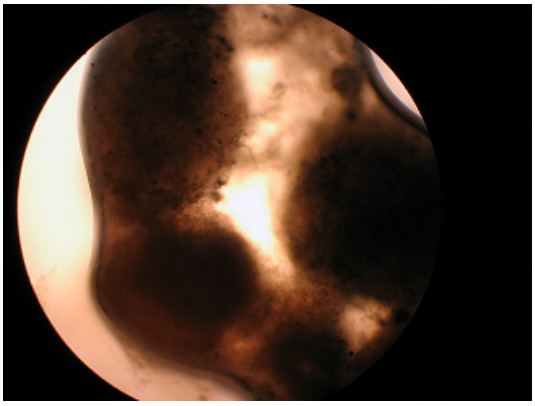

Figure 4-22 Day 80 microscope image showing granule growth, 10x magnification.

Chapter 5 Discussion

In this project, an UASB bioreactor was successfully operated to remove 845 mg of sulphate per liter of water per day (calculation based on change in sulphate concengtration against hydraulic retention time) using silage leachate as a carbon source. The treatment system ran continuously with few upsets for 13 months between June 2007 and July 2008. The unique feature that sets this laboratory-scaled system apart from others in the literature (some of which were described earlier in Chapter 2) is the use of silage leachate as a carbon source.

This chapter begins with a discussion of the performance of the treatment system by looking at the most important factors that influence the rate of sulphate reduction: organic carbon availability, biomass concentration in the bioreactor, presence of toxic compounds including byproducts that could inhibit sulphate-reduction and pH. Operating difficulties such as maintaining feed flow rates and sulphide removal using the stripper are also discussed. Then the performance of this UASB is compared with other sulphate-reduction treatment systems of similar or different designs to determine its effectiveness in treating sulphate rich waters. The suitability of silage as carbon source is discussed next and the chapter ends with recommendations to the cattle farming community as to the application of this technology.

5.1 Treatment System Performance

During the 13 months of operation, the ability of this laboratory-scale water treatment system to reduce sulphate from simulated ground water was tested by ramping up the sulphate concentrations from ~1,300 mg L^{-1} to a maximum of ~2,000 mg L^{-1}. The sulphate-reduction rates that were calculated for each phase of operation are shown in Table 5-1.

Table 5-1 Sulphate-reduction rates calculated for different phases of operation.

Day	Operation Mode	System Change	HRT (days)	SRR ($g\ L^{-1}\ d^{-1}$)	Sulphate Removal (%)	COD Consumption Rate ($g\ L^{-1}\ d^{-1}$)
0-17	Batch	System Start		0.146		
17-33	Batch	Sulphate addition		0.086		
33 Ct=0	Batch Continuous	Operation mode switch				
Ct=97-186	Continuous	Influent=1253 mg L^{-1}	3	0.425	95	1.029
Ct=186-214	Continuous	Influent=803.3 mg L^{-1}	2.2	0.368	98	1.741
Ct=214-250	Continuous	Influent=1400 mg L^{-1}	2.2	0.601	99	1.724
Ct=251-307	Continuous	Influent=1787 mg L^{-1}	2.1	0.481	59	1.256
Ct=310-354	Continuous	Influent=2014 mg L^{-1}	2.2	0.845	96	1.765

Note: Ct=continuous time, Influent=influent sulphate concentration, SRR=sulphate reduction rate.

When averaged over the entire 354 days of continuous operation, the UASB treatment system achieved a sulphate reduction rate of 375.7 mg L^{-1} d^{-1}. Sulphate removal efficiency was over 90% for most of the continuous operation mode, with the exception of Day 267 to 307, where the removal efficiency dropped to a low of 37.42% on Day 279. The main reason for the decline in sulphate removal efficiency during this period comes from the decrease in influent COD concentration entering the treatment system for this same period. Between Day 267 and 301, the influent COD concentration decreased to a low of 2,190 mg L^{-1} on Day 279. This decrease in COD strength in the influent meant that the bacteria were starved for nutrients, thus leading to a decrease in sulphate removal. As soon as the feed COD concentration was restored to ~4,500 mg L^{-1}, the sulphate-reduction improved. For the five periods of different influent sulphate concentrations (as shown in Table 5-1), the system experienced different sulphate reduction rates. So as to understand the factors that affect SRR in this system, several correlations were attempted. There were no strong correlations between SRR and

consumption rates of COD, TOC or carbohydrates. The only positive correlation was between SRR and the concentration of sulphate in the influent (Figure 5-1).

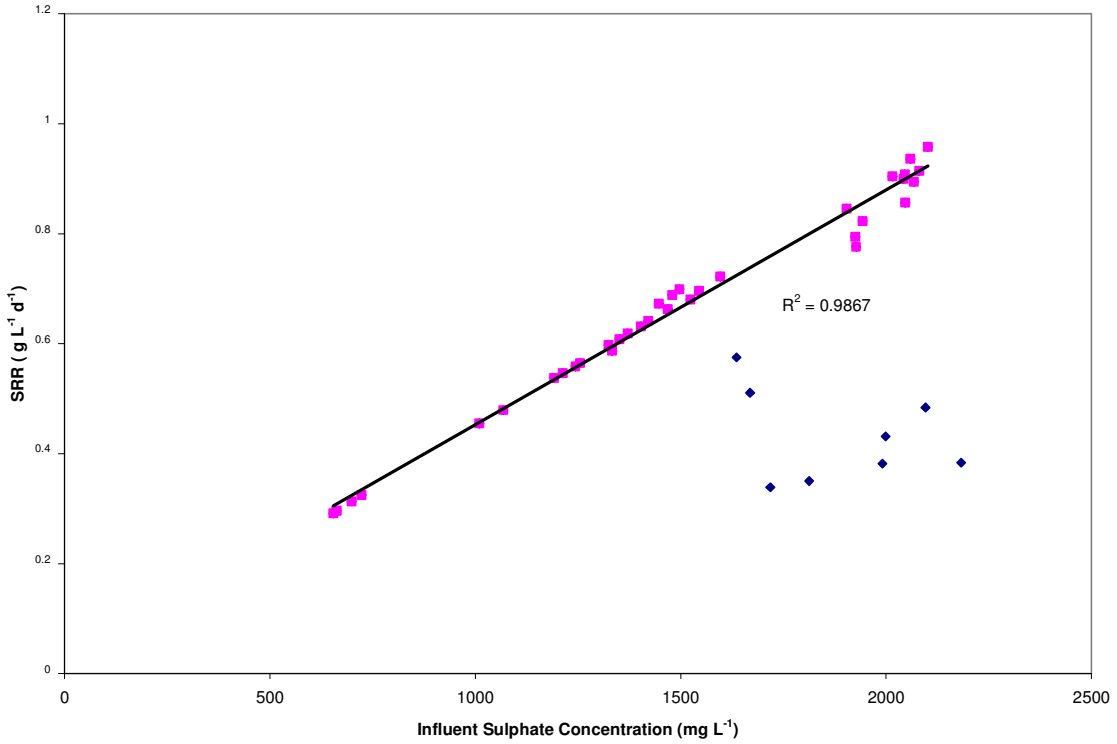

Figure 5-1 Correlation between SRR and influent sulphate concentration.

The outliers (blue points) were excluded from the trend as they were from Day 267 to 301, when the system experienced nutrient source depletion. Clearly SRR increased because the concentration of sulphate in the influent to the reactor was ramped up. Higher sulphate concentrations stimulate more SRB activity. Since COD and TOC consumption rates remained more or less the same (Table 5-1), this means that increasing sulphate loading did not result in more overall bacterial activity, but that the SRB must constitute a greater portion of the bacterial community when sulphate concentrations are high.

5.1.1 Organic Carbon Utilization and SRR

The data were also explored for any relationships between carbon source consumption rate and sulphate-reduction rate. The plot in Figure 5-2 compares the change in sulphate

concentration to changes in COD, TOC and carbohydrate concentrations, between the influent and effluent ports.

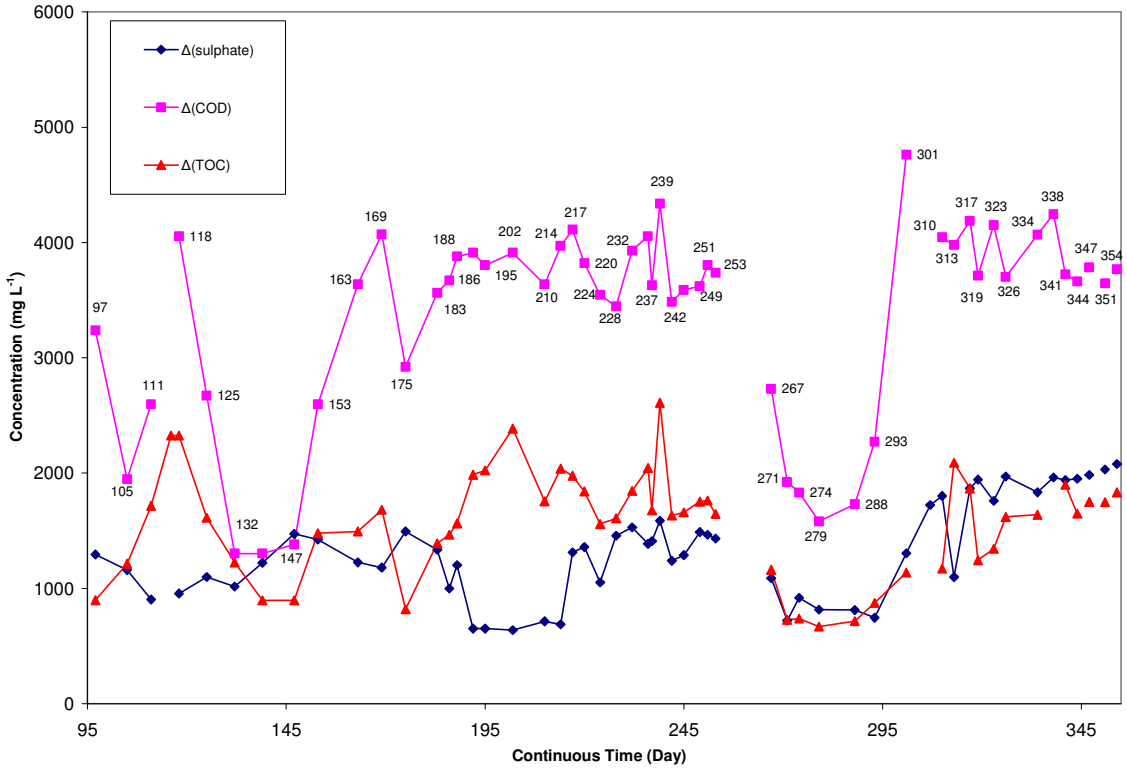

Figure 5-2 Correlation between sulphate reduction and carbon consumption by the bacterial consortium.
Δ(SO4)=sulphate concentration difference between influent and effluent ports
Δ(COD)=COD concentration difference between influent and effluent ports
Δ(TOC)=TOC concentration difference between influent and effluent ports

As COD and TOC measure the same bulk organics, changes in their consumption should be similar. Support for this is clearly reflected in Figure 5-2, where changes in TOC followed closely with changes in COD for the entire experiment. We expected sulphate reduction through the reactor to positively correlate with COD and TOC consumption. However, at some times during the first 215 days of operation sulphate reduction seemed to be inversely related to COD and TOC consumption. Nevertheless, after Day 215 increase in sulphate reduction did correlate with increase in COD and TOC consumption.

Several correlations similar to Figure 5-1 were performed to find out what factors in this process contributed to SRR. When SRR was plotted against COD or TOC consumption

rate there were no discernable trends. This is because, while the SRR increased over time in the reactor, the COD and TOC consumption remained more or less the same. COD consumption in the bioreactor is not affected by sulphate concentration and sulphate reduction does not result in an increase in COD consumption. Please refer to Appendix F for these correlations.

In order to run a bioreactor of this configuration in the field operators will need to know how much COD and TOC to add in order to achieve a particular sulphate-reduction in the water. Therefore we investigated the relationship between COD and TOC consumption and sulphate reduction rates (Figure 5-3).

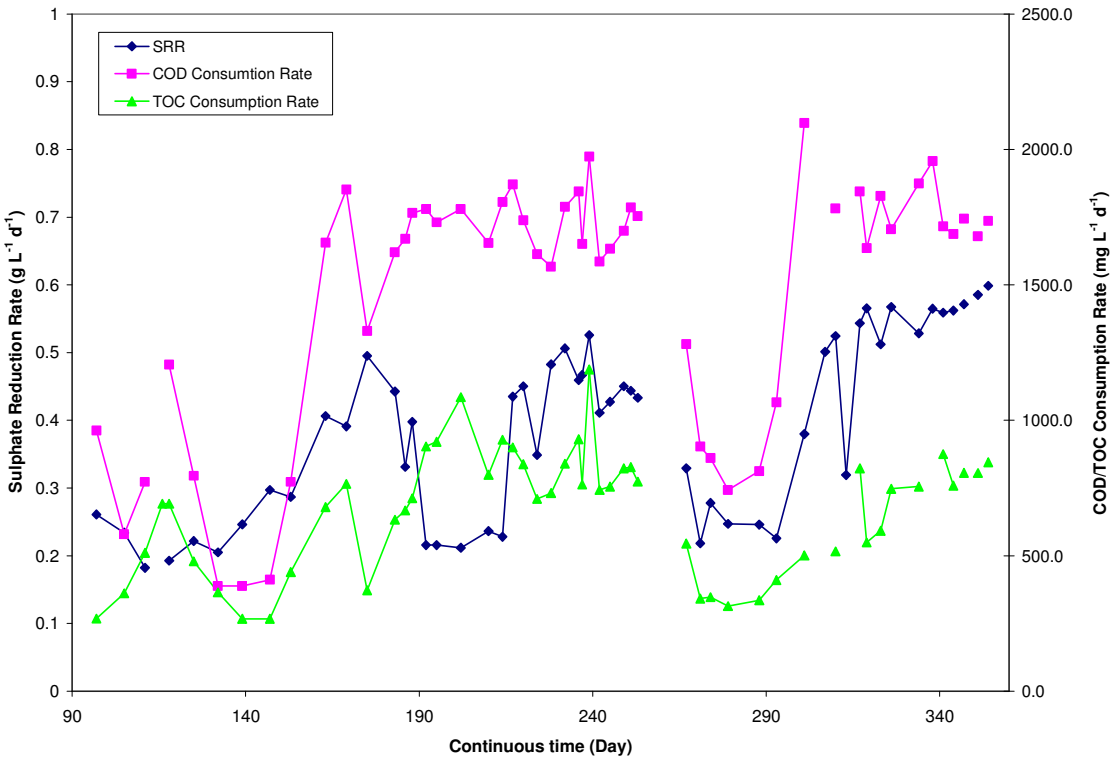

Figure 5-3 Correlation between COD consumption rate and SRR.
COD Consumption Rate = $(COD_{in} - COD_{out})/HRT$
TOC Consumption Rate = $(TOC_{in} - TOC_{out})/HRT$

When the COD and TOC consumption rates and sulphate reduction rates versus time are plotted on the same set of axes, for the most part, they follow the same trend. Towards the end of the experiment, as the sulphate reduction rate increased with increasing

sulphate loading rate, the COD consumption as well as the TOC consumption rates increased also, as expected.

According to stoichiometry, 0.67 g of COD is required to reduce one gram of sulphate. However, as shown in Figure 5-4 the actual measured COD:SO_4^{2-} ratio varied from 6 to less than 1 g-COD g^{-1} SO_4^{2-}.

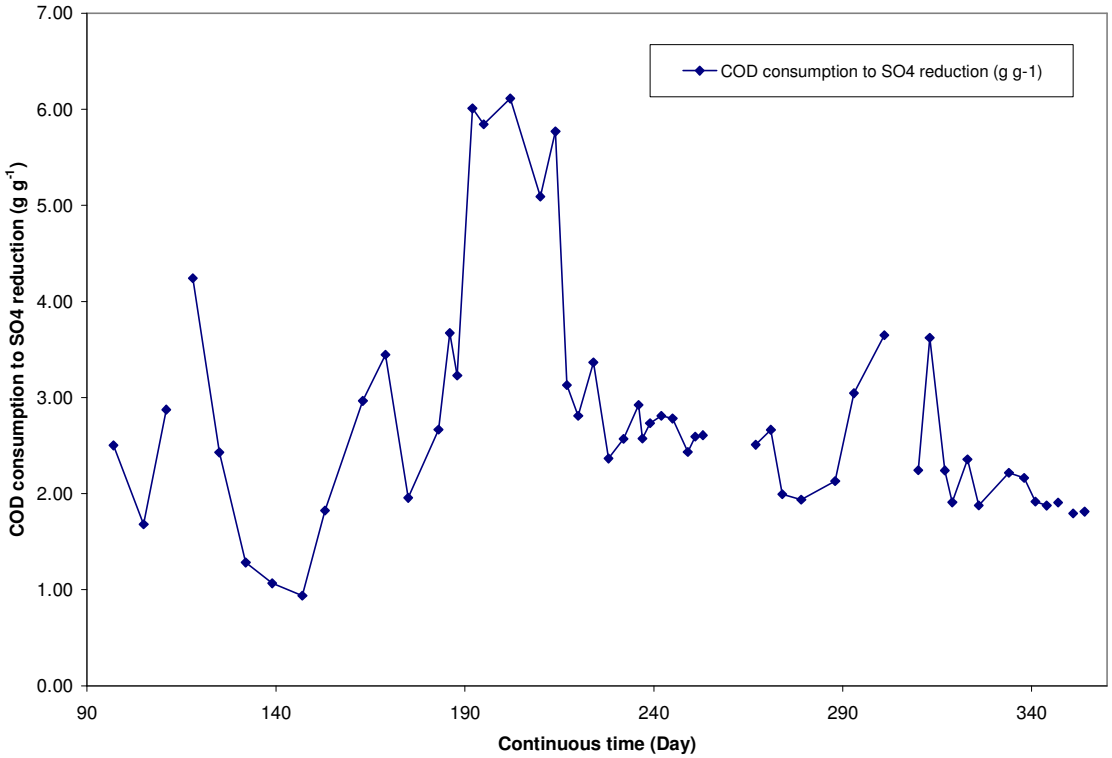

Figure 5-4 Experimental COD consumption to sulphate reduction ratio.

The additional COD consumption, not accounted for by sulphate-reduction, is consumed in other bacterial processes that vary in importance throughout the experiment. The very high COD:SO_4^{2-} ratios coincide with a period when the sulphate influent concentration was very low (~700 mg L^{-1}). Then, from day 214 to the end of the experiment, as the sulphate feed concentration was increased, the COD:SO_4^{-2} ratio decreased from 3 to 1.75 g g^{-1}. Therefore it appears that, at higher sulphate concentrations, more of the carbon in the bioreactor is consumed for sulphate-reduction. In other words, these reactors become more efficient in terms of carbon source usage at higher sulphate loading rates. One explaination for this is that the high sulphate concentrations are more selective for

SRB, which build up in number in the sludge over time. On average, for the whole experiment, the amount of COD consumed was 84%. In the last 50 days of the experiment 38% of this COD went towards sulphate reduction.

To see if carbohydrates are directly consumed in sulphate reduction, I attempted to correlate carbohydrate consumption with sulphate reduction (Figure 5-5). The hypothesis is that if carbonhydrates are directly consumed in sulphate-reduction then as the SRR increased so should have the carbonhydrate consumption rate.

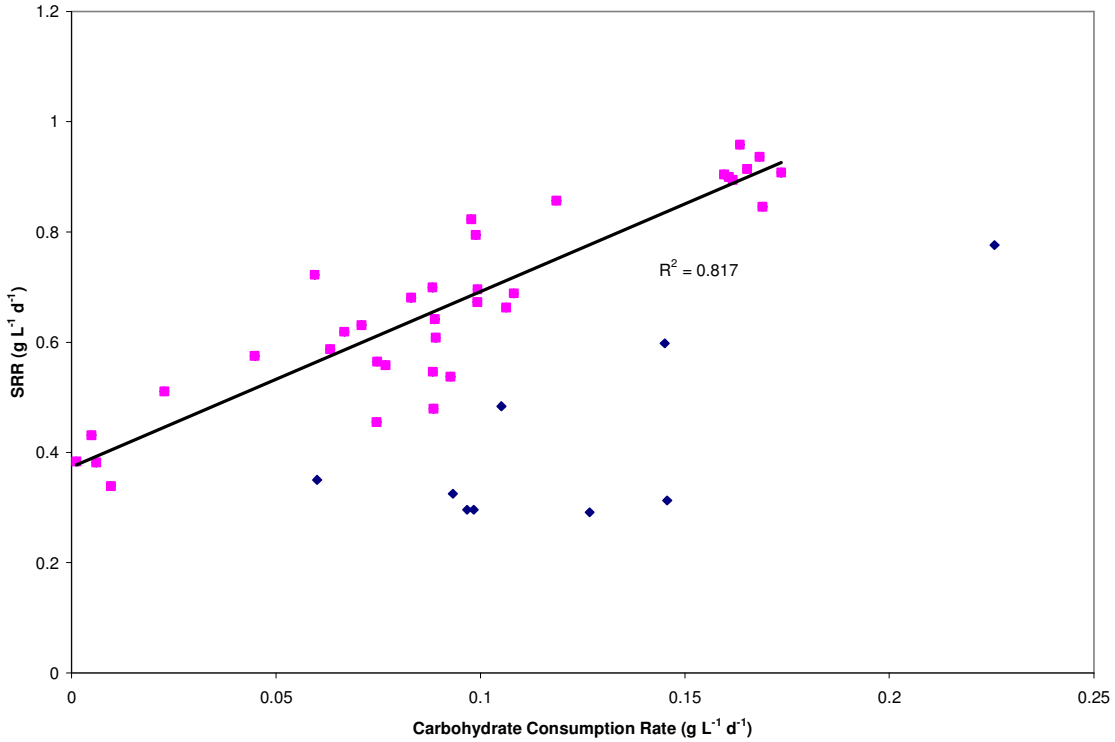

Figure 5-5 Correlation between carbohydrate consumption and sulphate reduction.

Although the trends are difficult to observe due to scatter in the data, there does appear to be a positive correlation. After the outliers were removed from the graph (blue points), a relatively strong ($R^2 = 0.817$) can be seen in Figure 5-5. This suggests that carbohydrate consumption did contribute to the increase in sulphate reduction in the reactor.

5.1.2 The Effect of Sulphide Concentration on Reactor Performance

It is well documented that sulphide can inhibit SRB growth (Maillacheruvu 1993) depending on the pH. In the pH range for optimal SRB growth (6.5 – 8), the bacteria can tolerate sulphide concentrations from 250 to 1,500 mg L^{-1}. In the UBC UASB reactor, the pH was consistently between 7 and 8, where more sulphide is present as HS^-, the less toxic form. Indeed, even though the sulphide concentration in the UBC UASB reactor reached very high concentrations (>1,000 mg L^{-1}) at the end of the experiment, sulphate reduction rates were the highest. However, at this pH most of the sulphide is retained in solution. To remove sulphide from the reactor one could lower the pH of the system, thus shifting the equilibrium of Eqn 2-2 to the left side, leading to more H_2S gas production. This would work well only if less than ~1,000 mg L^{-1} sulphate is reduced, since at low pH the inhibiting sulphide concentration is ~250 mg L^{-1}. For cases where more sulphate needs to be reduced the pH must be increased to prevent inhibition by H_2S. Sulphide may inhibit other bacteria in the system, such as fermenters and acetogens that are useful for producing carbon sources for SRB.

To find out if sulphide inhibition is a factor in the UBC UASB, a stripper column was installed. The design of the stripping column was based on studies by Yamaguchi (1999) and Gangagni Rao (2003). Beginning on Day 236, the suphide stripping column operated for 118 days, during which the design was adjusted in an attempt to improve its performance. Initially the stripping column showed great promise as effluent sulphide concentration decreased by 300 mg L^{-1} in a matter of 3 days. However this could not be maintained. Despite several design changes and experimenting with different nitrogen supply flow rates, the effluent sulphide concentration continuted to increase with the sulphate-reduction rate. In order to find out why the sulphide stripper did not work it was compared with both Yamaguchi (1999) and Gangagni Rao (2003) (Table 5-2).

Table 5-2 Comparison between sulphide stripping columns

System	Flow type	Flow rate ($L\ min^{-1}$)	Packing	Sulphide removal ($mg\ L^{-1}$)
Yamaguchi 1999	Counter-Flow	5-20	No	129
Gangagni Rao 2003	Counter-Flow	1.1	Yes	105 - 189
UBC	Counter-Flow	0.065 – 10	No	~Nil

The UBC stripping column could only be operated under nitrogen flows greater than 0.5 $L\ min^{-1}$ for a few brief periods due to operational problems with gas carry-over into the UASB, which disrupted the sludge bed. When the N_2 flow rate was greater than 0.5 $L\ min^{-1}$ the stripper was effective in reducing the reactor sulphide concentrations to the range 200 – 400 $mg\ L^{-1}$ (Figure 4-7). But, since it was only feasible to maintain a N_2 flow rate of less than 200 $L\ min^{-1}$ no sulphide removal occurred. The other two studies where sulphide removal was achived used N_2 flow rates greater than 1 $L\ min^{-1}$. Therefore if the sulphide-stripping column is to be used it must be designed differently so that gas carryover does not occur.

5.1.3 Biomass Growth in the Bioreactor

For most of the experiment the median size of the granules sampled was ~0.5 mm, although the last two granule samples had smaller median sizes of ~0.12 mm. This indicates that there were always large numbers of the smaller particles in the sludge bed. Figures 5-6 and 5-7 shows the histogram from Day 125 and Day 288, respectively.

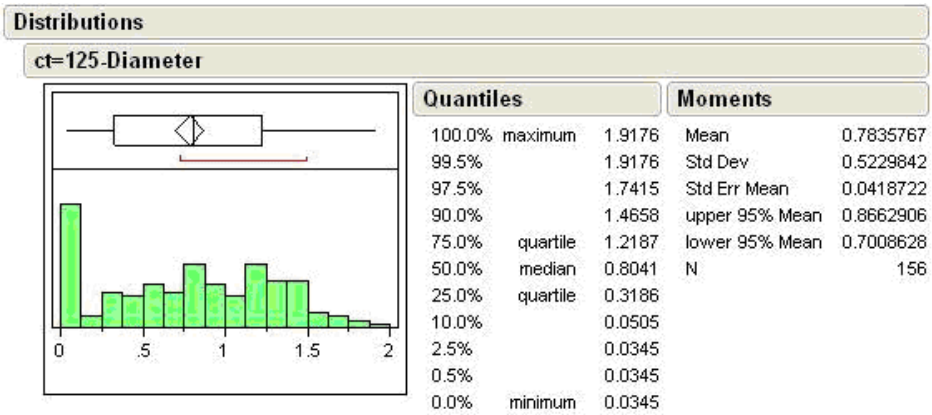

Figure 5-6 Day 125 histogram.

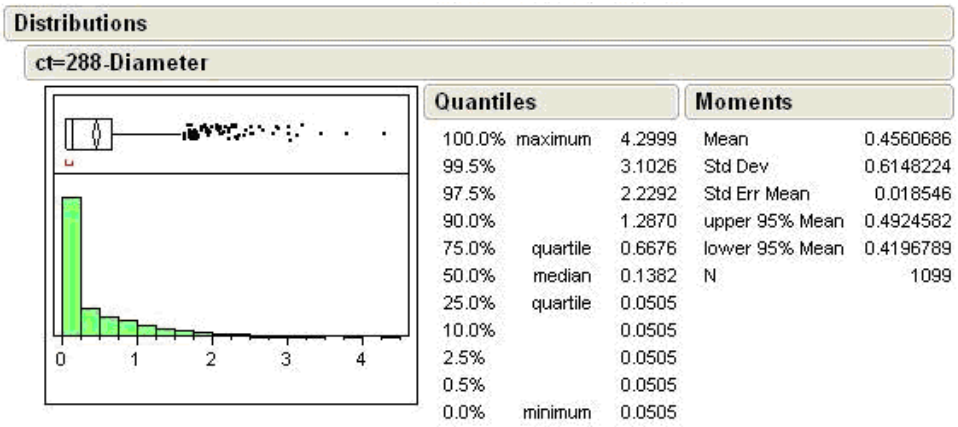

Figure 5-7 Day 288 histogram.

Additional histograms can be found in the appendix. For a better representation of the granule growth during the experiment, a plot of the sizes of each quantile for the respective sample dates was constructed and shown in Figure 5-8.

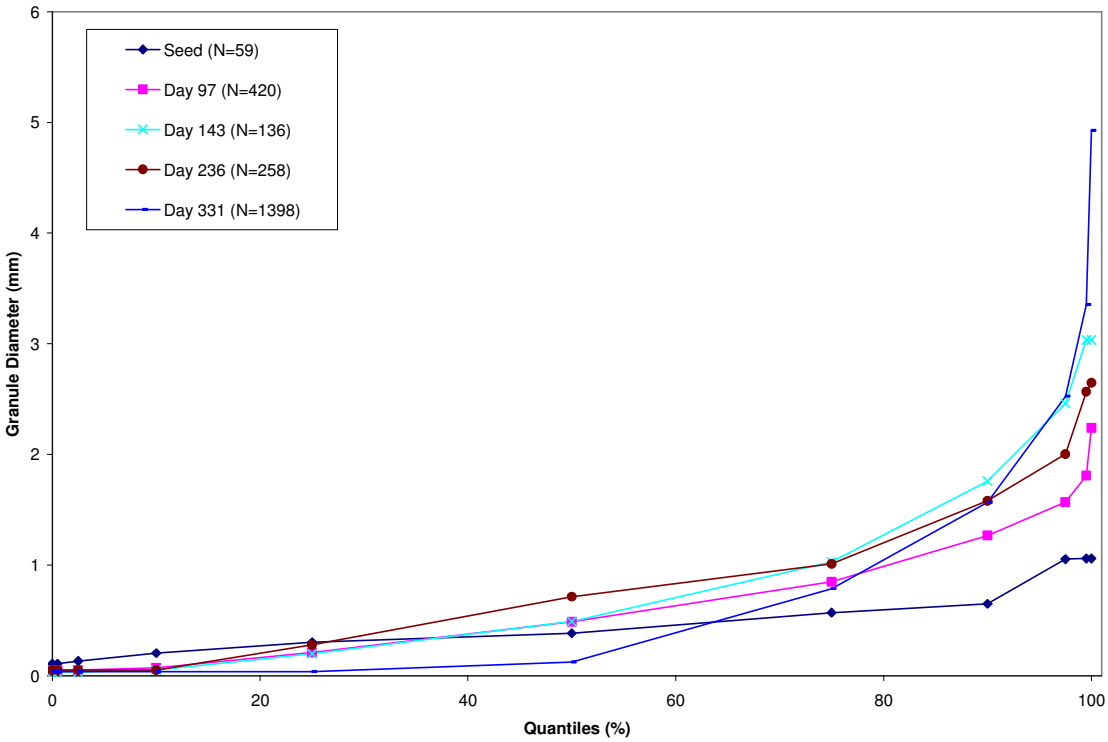

Figure 5-8 Granule size distribution comparison of 5 sample dates.

Each quantile represents a certain granule size, expressed in mm, as shown in the two histograms above. From Figure 5-8 it is clear that the granules have experienced growth during the experiment, at each of the granule sizes (quantiles). One issue that was

encounterd near the latter stages of the experiment was the number of small particles included in the granule samples. For example, Day 288 and Day 331 samples had very small median sizes, at 0.14 mm (Day 288) and 0.12 mm (Day 331). But the Day 125 sample had a median size of 0.80 mm. The difference comes from the number of particles sampled for the three sample dates. Day 125 sample had a sample number of just 156 while Day 288 had a sample number of 1099, and for Day 331, 1398. As such the median sizes of the granules generated from these samples would not be a good representation of the general growth of the granular sludge in the bioreactor. This reactor was originally designed to only have 4 ports (Figure 3-2), with port 4 being used for granule sampling. Once the granules become large and start to compact, the sludge bed became dense and it was difficult to obtain samples. An improvement to the design would be to have 2 or 3 ports at the bottom of the reactor. This would allow for a more even representation of the granule sizes at various location of the reactor.

During the experiment, the sludge bed volume increased by 66% (Figure 4-17). This represents an accumulation of new biomass that is retained in the bioreactor. An increase in concentration of SRB retained in the sludge of the bioreactor also was a contributing factor to the increase in SRR over time.

5.2 Treatment System Comparison

As discussed earlier in Chapter 2, reactor configurations used for sulphate reduction include anaerobic filters, fludized beds, sequencing batch reactors, anaerobic baffles, and gas lift reactors. In addition, both laboratory-scale and full-scale UASB reactors have been used successfully for sulphate reduction since the 1970s. In Table 5-3 the performance of the UBC UASB is compared with all other reactor types, as well as other laboratory and full-scale UASB applications.

The reactor types in Table 5-3 that used defined carbon sources, such as acetate, ethanol or CO_2/H_2, all achieved sulphate reduction rates much higher than that of the UBC UASB. The sulphate reduction rate of the UBC UASB was in the same range as those for the reactors using complex feeds, such as landfill leachate, wastewater or the lactate/molasses mixture. Even though silage leachate contains lactate and ethanol, which are carbon sources used in the defined media reactors; the sulphate reduction rates were much less. This may be due to inhibiting compounds in the silage leachate that were not identified in the organic compound analysis. There may be some design modifications that can be made to the UBC UASB to improve its performance. Below I discuss some of the features that contributed to the high performance of the other reactors, and the applicability of incorporating these features into the UBC UASB.

The gas lift reactor had the highest sulphate reduction rate of 7.5 g L^{-1} d^{-1} using a H_2/CO_2 gas mixture as the only nutrient source for the SRB community. This innovative approach also operated under thermophilic conditions. However, these features would not be suitable when applied to the UBC UASB design, especially during field applications. For one the use of a gas mixture is beyond the reach of farmers, and the cost of heating the entire system would far exceed the financial limitations set out in the scope of this project.

The higher SRR of a fluidized bed reactor, when compared to that of the UASB design, comes from having better mass transfer rates. However, this design required the use of a pure SRB culture, which is not recommended for a farm-based UASB. In addition, the turbulent fluid flow rates and cost of ethanol means that these features would not be suitable for applications in the field design.

The other three reactor designs were eliminated from further considerations in Chapter 2, hence their results are just presented here for comparison. Following is a discussion of the various UASB designs that have seen relative success in sulphate reduction, and their comparison to the UBC UASB design, in terms of the important features that must be considered when designing a UASB bioreactor for sulphate reduction. These important features include sulphate and organic loading rates, the COD/sulphate ratio, type of carbon source, pH, and temperature, retention of the biomass in the reactor as well as toxicity from sulphide or other compounds in the feed or produced in the reactions.

Anaerobic processes are known to have high organic loading rates, typically in the range 12 - 20 gCOD L^{-1} d^{-1} (MetCalf and Eddy 2003), which contributes to development and growth of dense granulated sludge, which is key for high rates of COD consumption. As the loading rate and sludge density increase, higher liquid upflow velocities will flush out other waste solids, while retaining granular sludge, leading to more sludge bed growth (Metcalf and Eddy 2003). Therefore, if the UBC UASB design is scaled up to process higher loading rates, its performance may improve. In addition, higher flow rates in the feed pipes will decrease the likelihood of bacterial activity and clogging in the feed line.

As discussed earlier, stoichiometry states that for every gram of sulphate reduced, 0.67 g of COD is required. Dries et al. (1998) kept the COD/SO_4^{2-} ratio at the stoichiometric value for their entire experiment. By varying the upflow velocity they were able to achieve a maximum SRR of 10 g L^{-1} d^{-1} and up to 98% COD removal efficiency (Dries 1998). In contrast, Vallero et al. (2003) varied their COD/SO_4^{2-} ratio between 10 and 0.5. Their study showed a sulphate removal of over 95% (maximum SRR of 4 g L^{-1} d^{-1}) at the COD/SO_4^{2-} ratio of 10, but the removal efficiency dropped to below 50% after the ratio

was changed to 0.5 (Vallero 2003). For the UBC system, the influent COD concentration remained relatively constant, while the influent sulphate concentration changed as the experiment progressed. Results showed a gradual decrease in COD/sulphate ratio, while the SRR has been on an increasing trend for the entire experiment (Figure 5-9).

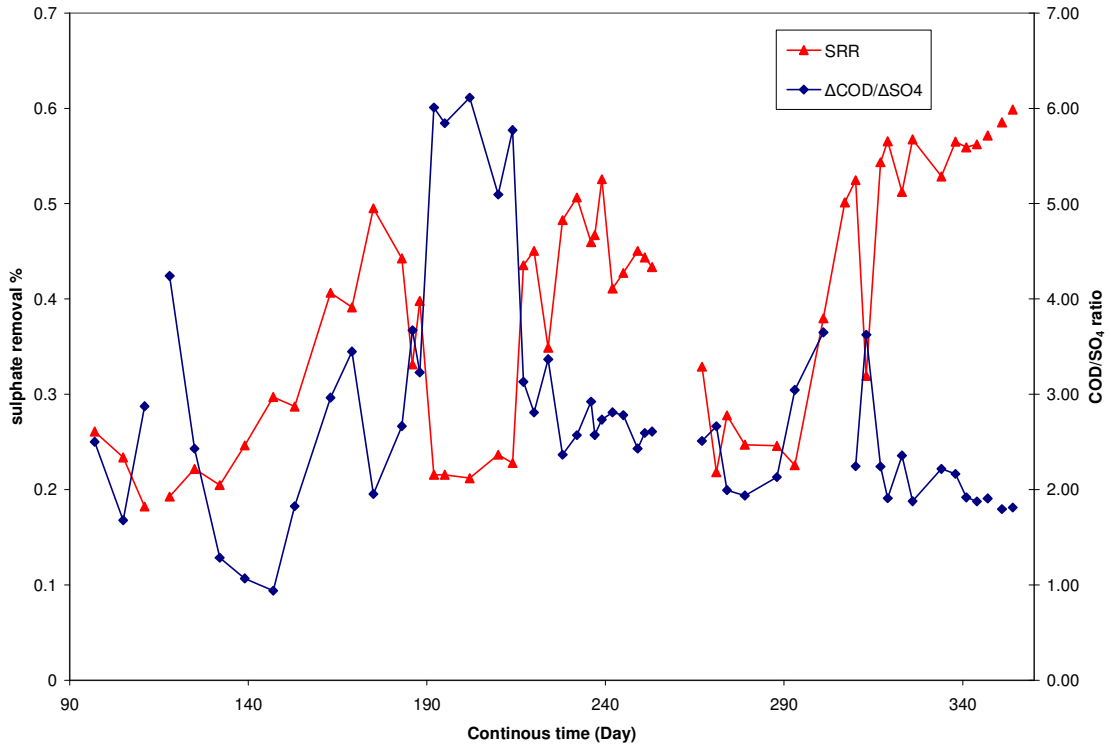

Figure 5-9 Comparison between SRR and COD/SO_4^{2-} ratio for the UBC system.

The UASB reactors listed in Table 5-3 used carbon sources such as ethanol (Scheeren 1991), methanol (Vallero 2003) and acetate (Muthumbi 2001, Dries 1998). Use of a single, defined carbon source is an advantage for SRB as they are able to out-compete other bacteria, especially in the preferred pH range of 7-8. Another study, conducted by Goncalves et al. (2005), used a mixture of lactate with molasses as the carbon/energy source. This study's use of mixed carbon sources is similar to the UBC USAB treatment system with silage. In their 222 days of operation, Goncalves et al. (2005) varied the composition of the carbon sources, producing a maximum SRR of ~0.35 g L^{-1} d^{-1}, while only achieving 34 – 41% COD removal efficiency. The UBC system had a higher SRR (maximum of 0.536 g L^{-1} d^{-1}) and 50 – 95% COD removal efficiency.

Table 5-3 Comparison of the performance of the UBC UASB to other sulphate-reduction bioreactor configurations

Reactor Type	Source	Reactor Volume (L)	Temperature (°C)	pH	Carbon Source	HRT (hr)	Sulphate reduction rate ($g\ L^{-1}\ d^{-1}$)	COD removal (%)
Other								
Anaerobic Filter	Henry *et al.* 2000	3	19-25	7.4-8	Landfill leachate	0.5-2	0.13 - 2.44	50-93
Fluidized bed	Nagpal *et al.* 2000	3	25	~7	Ethanol	5.1	6.33	N/A
Sequencing batch	Krapivina *et al.* 2000	0.7	35	6.5-8	Waste-water	60	1.39	50-70
Anaerobic Baffled	Fox 1996	10	35	7-7.25	Acetate	24	4.5	50
Gas lift	van Houten *et al.* 1997	4.3	55	7	H_2/CO_2	4.5	7.5	
UASB								
	Scheeren 1991	12,000	20-38	6-8	Ethanol	4	6.84	N/A
	Muthumbi *et al.*, 2001	2.3	32	7.5-8.5	Acetate	2-25	1.5 - 14	N/A
	Goncalves *et al.*, 2005	13	35	6.5-8	Lactate/ Molasses		~0.35	34-41
	Dries *et al.*, 1998	2.3	33	7.9	Acetate	1.9-2.5	0.7 - 10	79-98
	Vallero *et al.*, 2003	0.92	55	7	Methanol	7.5	2 - 4	50-100
	UBC	5	20	7.5-8	Silage leachate	83	0.262 - 0.536	50-95

Note: adapted from Table 2-2 in Chapter 2.

5.3 The Suitability of Silage

Silage was chosen for this project since it is an agricultural byproduct formed by fermentation with lactic acid bacteria. Since lactate is a common substrate for SRB, the leachate from silage was deemed a suitable carbon source for the reactor. I found that silage leachate from different sources contained several different organic acids (lactate, acetate), as well as alcohols, phenols and carbohydrates. Table 5-4 summarizes findings from various locations and literature sources.

Table 5-4 Quantification of compounds in silage from various locations and sources.

Compounds	Lanigan Farm	Agassiz Farm*	Krizsan 2007 (g Kg^{-1} DM)
Lactate	Present	0.68 – 2.43 g L^{-1}	49.3
Acetate	Present	0.068 – 0.137 g L^{-1}	28.6
Malate	Present	Not Detected	Not Reported
Fumarate	Present	Not Detected	Not Reported
Formate	Present	Not Detected	2.6
Pyruvate	Present	Not Detected	Not Reported
Propionate	Not tested	Not Tested	1.0
Butyrate	Not tested	Not Tested	6.0
Methanol	Not tested	19 – 46 mg L^{-1}	Not Reported
Ethanol	Not tested	266 mg L^{-1}	6.8
Carbohydrate	Not tested	11.8 – 417.1 mg L^{-1}	33.0
Phenols	Not tested	5.5 mg L^{-1}	Not Reported
Amines	Not tested	Not Tested	4.82

*Note: All Agassiz silage values are from feed port with the exception of carbohydrate, where it is from the influent port.

Using Figure 4-16, Lanigan Farm silage and Agassiz Farm silage were compared. As shown in Table 5-5, it is clear that silage from different locations are not the same in terms of organic acids composition. Some may have a wide variety of fatty acids (like the Lanigan Farm silage and silage used by Krizsan) while others may only contain one or two dominant organic acids (like the Agassiz Farm silage). The Agassiz Farm silage was beneficial for this process since it contained only two types of organics acids: lactate and aceate, both of which are commonly used substrates for SRB. But it is not known what other organic compounds constitute the unknown fraction of the silage leachate TOC, and some of these may be toxic to microbes. Silage leachate proved to be very

successful for supporting sulphate reduction and all of the compounds that we measured were consumed in the reactor. Table 5-5 presents the consumption of each of the organic compounds as a percentage of overall TOC consumption.

Table 5-5 Fraction of organic consumption to overall consumption

Compounds	Quantity (mg L^{-1})	TOC consumption fraction (%)
Lactate	190 – 730	4.93 – 18.2
Acetate	44 – 59	1.10 – 3.25
Methanol	6.4 – 20	0.1 – 0.136
Ethanol	90.4	2.67
Carbohydrate	11.8 – 543	2 – 6
Phenols	2.4	0.027

The consumption of different organic compounds (Table 5-5) strongly supports the presence of many bacterial species in the UASB bioreactor. For example, both lactate-utilizing and acetate-utilizing SRB must be present. Acetate can also be consumed by methanogens, but no gas production was observed in the UASB, therefore it is unlikely that they are present. At the end of the experiment, 2,053 mg L^{-1} of sulphate was reduced in the reactor. This required 1,376 mg L^{-1} of COD, which is only 36% of the total COD that was consumed (3,768 mg L^{-1}). Lactate and acetate consumption accounted for 16.8% (mol/mol) and 1.24% (mol/mol), respectively, of the total organic carbon consumption, and I hypothesize that all of these organic acids were used for sulphate reduction. Other carbon sources most likely used for sulphate reduction include ethanol, which accounted for 2.67% (mol/mol) of the TOC consumed. The remaining 15.3% TOC consumed in sulphate reduction could have come from other alcohols or sugars. The 60% of TOC reduced that was not used directly for sulphate-reduction was consumed for biomass growth, as evidenced by the increasing sludge bed volume, or used for other electron transfer processes. The organic compounds that were measured (in Table 5-5) comprised only a fraction of the total organic carbon. It is not know what the other compounds were. Likely soluble amides, are present also in silage leachate, but these were not measured.

Farmers typically store silage in large silos for 3-4 months before fermentation leads to mold growth, which makes the silage unusable. As a result, several trips were made

during the experiment from UBC to Agassiz to replenish fresh silage. Typically fresh silage will have a sweet smell due to the presence of alcohols and carbohydrates in the tests.

The water-soluble carbohydrate content of silage leachate can vary with each batch of silage. This is due to the type of organic material used (hay or orchard grass), the seasonal conditions during plant growth and the fermentation conditions during silage making. Looking at Figure 4-11, it is clear that the carbohydrate concentration changes with the seasons. The winter of 2008 was especially cold for the Greater Vancouver region. This likely would have slowed the fermentation process, causing a decrease in the organic concentrations available in the silage. This was most likely the reason for the sharp decrease in organic concentration shown in Figures 4-9 to 4-11 between Day 267 and Day 288.

Since silage leachate contains organic substrates that are used by the different bacteria in the consortium, proper storage and handling of silage is needed so as to preserve these. If possible, leachate should be prepared in the same location as where the silage is fermented from hay. If silage is to be transported from the fermentation site, the silage must be bagged and vacuum-sealed to prevent aerobic decomposition of the organic compounds. The silage leachate must be stored at 4ºC if it is not used immediately. In practice, silage leachate can be prepared *in situ* by soaking fresh silage contained in a mesh bag in feed water.

Use of silage leachate in this study was especially beneficial as the leachate provides the necessary nutrients for the bacterial community in the reactor, while at the same time negates the problems associated with using silage in a laboratory scaled setup (eg: line plugging from solids and frequent system failure due to excess solids in the reactor).

All of the particular organic compounds that I measured in the silage were consumed in the UASB bioreactor to some extent (>80%). Organic acids, known to be prefered substrates for SRB, were totally consumed. This indicates the presence of acetate-

utilizing SRB as well as lactate-utilizing SRB. Complete consumption of alcohols usually is indicative of the presence of methanogens in the bioreactor. However, the lack of observed gas formation means that either no methanogens were present or they were growing only very slowly as a result of the high sulphide concentration. Therefore it is likely that the alcohols were consumed by other SRBs. The carbohydrates and phenols were consumed by acidogens and fermentative bacteria (Table 5-6).

Table 5-6 Percents consumed of organic compounds measured.

Test	Amount consumed (%)
TOC	88
Carbohydrate	88
Alcohols	80
Phenols	86
Organic acids	
Lactate	100
Acetate	100

This bioreactor design should be still be applicable for places where the silage organic acid composition differs from that used here, since the process involves a consortium of bacteria containing many species able to grow on a wide range of carbon sources. Using a consortium of bacteria in the bioreactor rather than a pure culture is essential for this very reason; that the composition of complex (and therefore inexpensive) organic sources can vary with different locations and batches.

Chapter 6. Conclusions and Future Work

Conclusions

In Saskatchewan as well as parts of the Okanagan in British Columbia, drinking water sources for livestock are high in sulphate, which is often above the recommended 1,000 mg L^{-1} as set by Canadian Water Quality Guidelines. Therefore, two biological reactors for sulphate-reduction were tested in this thesis. First, a 1,400 L trough-bioreactor, which was in operation for over a year at the Lanigan experimental farm in Saskatchewan, was monitored for sulphate reduction. At 39.6 mg L^{-1} d^{-1}, the SRR for this bioreactor was slow, which means that very large volume troughs (125 m^3 or 2 forty-foot containers for treating 4500 L of water per day) would be required to treat enough water for a typical cattle producer. Therefore, other suitable reactor types were researched in the literature. Based on this review, an upflow anaerobic sludge blanket (UASB) bioreactor configuration was selected for its low footprint, high SRR, and low maintenance. Based on design criteria in Metcalf and Eddy (2003), a 4 L laboratory-scale UASB was constructed and run for over one year with silage leachate as a carbon source and feed sulphate concentrations increasing from 2100 to 3200 mg l^{-1}. Since the seed sludge, containing mature granules, was obtained from an operating UASB bioreactor (Fleishman's Yeast), the acclimation period was only 33 days, which is much shorter than the usual 60 – 90 days required for start-up of these types of bioreactors with laboratory-generated culture. Sulphate reduction for this batch stage was calculated to be 146 and 85.9 mg L^{-1} d^{-1}, while COD consumption during this same period was 4,991 and 4,675 mg L^{-1}. The initial flow rates of sulphate feed water and silage leachate of 0.25 mL min^{-1} and 0.08 mL min^{-1}, respectively, were based on the batch rate data. However, for most of the continuous operation, these flow rates were increased to ~1 mL min^{-1} for sulphate feed and ~0.5 mL min^{-1} for silage leachate feed since the kinetics were much faster in the continuous operation mode.

During continuous operation, the influent COD concentration was maintained fairly constant at 4,000 mg L^{-1}, while the sulphate concentration in the influent was varied from 800-2000 mg L^{-1}. The UASB bioreactor was tested in five phases with different influent sulphate concentrations of 1253, 803.3, 1400, 1787, and 2014 mg L^{-1}. The SRR calculated for each stage was 425, 368, 601, 481, and 845 mg L^{-1} d^{-1}, respectively. We observed that the SRR increased with influent sulphate concentration. The effluent sulphate concentration averaged about 100 mg L^{-1} for the majority of the experiment, with one exception when it reached 1366 mg L^{-1}, due to an unanticipated drop in COD concentration in the system.

Suitability of silage as carbon source for the treatment system was studied with 6 different tests: COD, TOC, carbohydrates, alcohols, phenols, and organic acids. All six test results showed that over 80% of all organics in the silage was consumed by the bacteria during the sulphate reducing process. The sulphate reduction process utilized 20-30% of the COD consumed and 12-24% of the TOC consumed. All of the TOC consumed for sulphate reduction was in the form of lactate consumption. The only positive correlation observed was between the SRR and carbohydrate consumption rate, while both COD and TOC consumption did not show any correlation at all in the sulphate reduction process. In most industrial continuous operation bioreactors, the nutrient feed must be sterile to avoid contamination of the bioreactor or prevent clogging of feed lines due to growth of bacteria biofilms. Bacterial growth in the feed tank and feed lines is especially difficult to prevent when a complex, i.e. nutrient rich, carbon source, such as silage, is used. To minimize this in the laboratory the silage leachate was autoclaved, filtered, and stored at 4℃. The leachate feed tubing had to be kept at 4℃ since we found that bacterial activity in the feed line could degrade the leachate by as much as 40% at room temperature before it enters the bioreactor. Clearly, sterilization and chilling are not feasible for a farm-based process. However, steps that operators could take to minimize biodegradation of the organics before they enter the UASB would be to have a floating lid that prevents oxygen from entering the silage-leachate feed tank and to keep the feed line from the silage feed tank to the influent port on the UASB as short as possible. Also, in larger-scale bioreactors where the feed rates will be faster the

diameter of the feed lines will be larger, which will help to prevent clogging from fine particles and biofilms.

Sulphide concentrations in the UASB ranged from ~600 to ~1,300 mg L^{-1}, increasing as the amount of sulphate reduced in the system increased, which was expected. Based on the fact that 333 mg L^{-1} of sulphide is produced for every 1,000 mg L^{-1} of sulphate reduced, based on stoichiometry, we conclude that no sulphide was lost to the gas phase or as precipitated sulphides in the UASB. Since we suspected that such high sulphide concentrations could be inhibitory to SRB activity, we attempted to remove some of sulphide by stripping with nitrogen. Initially the stripping column was effective at reducing the sulphide concentration by about 300 mg L^{-1}, however, operational problems prevented sustained use of the stripper. Nevertheless, the SRR increased with sulphate loading despite the increasing sulphide concentrations

When the performance of the UBC UASB treatment system was compared to other similar systems in works previously published, the UBC systems' sulphate removal efficiencies and the COD loading rates were either very similar or better. In addition, other system operated either in thermophilic or high mesophilic ranges, whereas the UBC system was operated at ambient temperature for the entire experiment. This is a significant cost saving feature, as heating the system would require considerable amounts of energy.

Future Work

As the development and operation of the laboratory scale UBC UASB treatment system is complete with successful adaption of system to various influent sulphate concentration, as well as good classification of organic compounds in the silage leachate to be used as carbon source for the sulphate reduction process, treatment system scale-up should be the next step to test the ability of treating 5,000 L d^{-1} of groundwater, typical water usage for a cattle farm.

The following recommendations are made for pilot- and full-scale systems:

1. Implementation of the sulphide stripping column early in the continuous operation stage. With a larger operating volume, higher purge gas can be sustained, which could lead to better sulphide reduction.
2. The silage source at different farms in different locations may have different organic compound make-up. It is advisable to perform a complete organic analysis of the silage source at each location.
3. Due to time constraints, a bacterial analysis was not performed. As the UASB granular sludge is known to be a consortium of various bacteria, it is advisable to perform a complete bacterial analysis on the sludge. Combined with the complete organic analysis will provide a better picture of all the biological activities in the UASB reactor.
4. Implementation of the UASB design into the overall sulphate/sulphide treatment system, as well as additions of other treatment units, in order to meet the government livestock water quality guidelines indicated at the beginning of this report.
5. As the system increase in size, consider use of multiple bioreactors in series, which would negate the problem of the height to diameter ratio typically associated with UASB designs.

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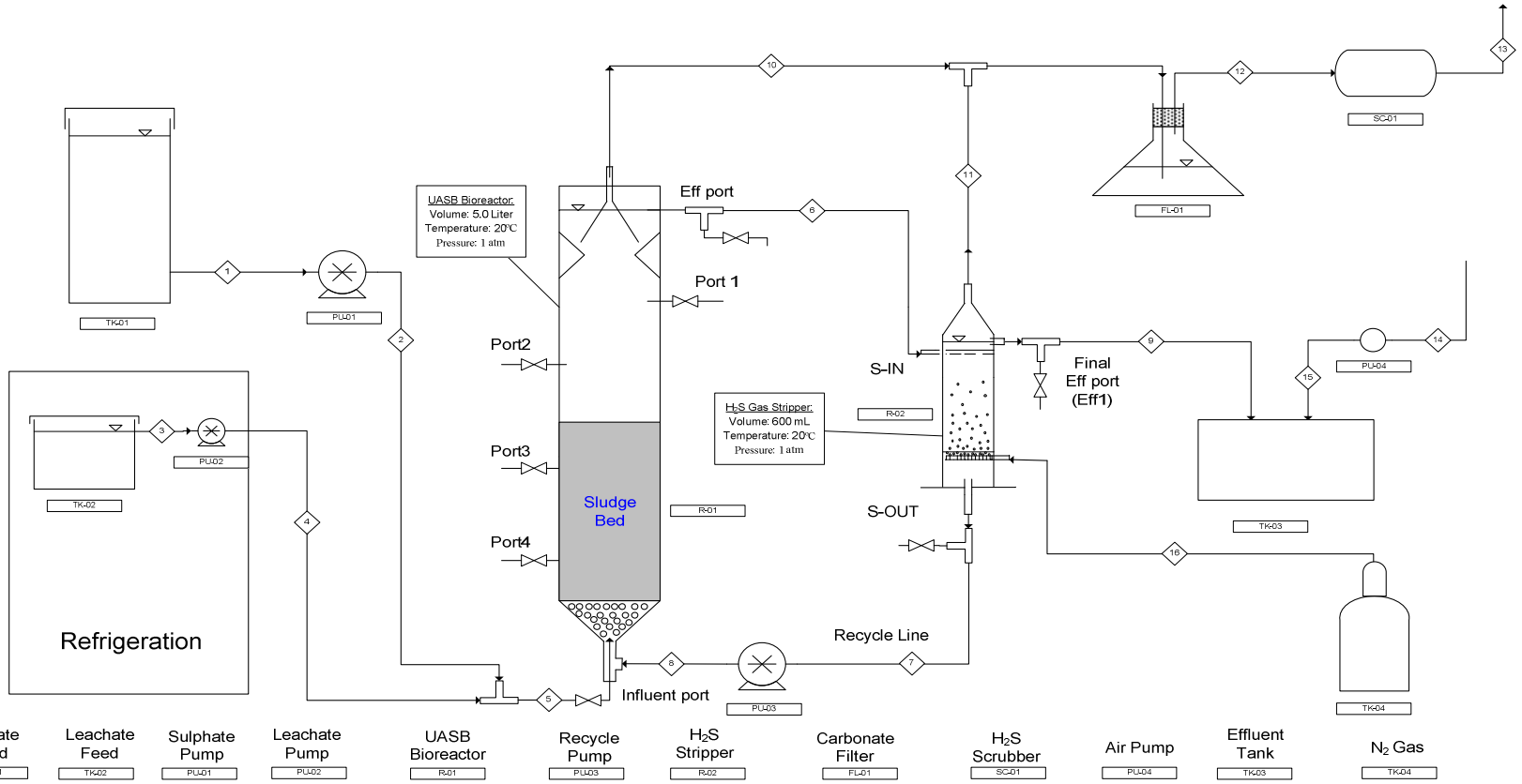
Appendices

Appendix A. UBC UASB Treatment System Drawings.

Figure A-1 UBC UASB treatment system – overall system diagram



Stream Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Phase	L	L	L	L	L	L	L	L	L	G	G	G	G	G	G	G
Flowrate(mL/min)	1	1	0.56	0.56	1.56	86.56	85	85	1.56							
Temperature(°C)	20	20	4	4	10	20	20	20	20	20	20	20	20	20	20	20
Pressure(atm)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Component flowrate (mL/min)																
sulphate(aq)	1	1	0	0	1	trace	trace	trace	trace	0	0	0	0	0	0	0
leachate(aq)	0	0	0.56	0.56	0.56	trace	trace	trace	0	0	0	0	0	0	0	0
sulphide(aq)	0	0	0	0	0	1.56	85	85	1.56	0	0	0	0	0	0	0
methane	0	0	0	0	0	0	0	0	0	trace	trace	trace	trace	0	0	trace
carbon dioxide	0	0	0	0	0	0	0	0	0	trace	trace	0	0	0	0	0
hydrogen sulphide	0	0	0	0	0	0	0	0	0	trace	trace	trace	0	0	0	0
Nitrogen	0	0	0	0	0	0	0	0	0		100	100	100	0	0	100
Air	0	0	0	0	0	0	0	0	0	0	0	0	0	50	50	0

	WBDC Groundwater Treatment System
	UASB Bioreactor Treatment System Diagram
Drawn by: Winton Li	SIZE / FSCM NO / DWG NO / REV: A
August 15, 2008	SCALE / SHEET: 1 OF 1

Figure A-2 UASB bioreactor mechanical diagram

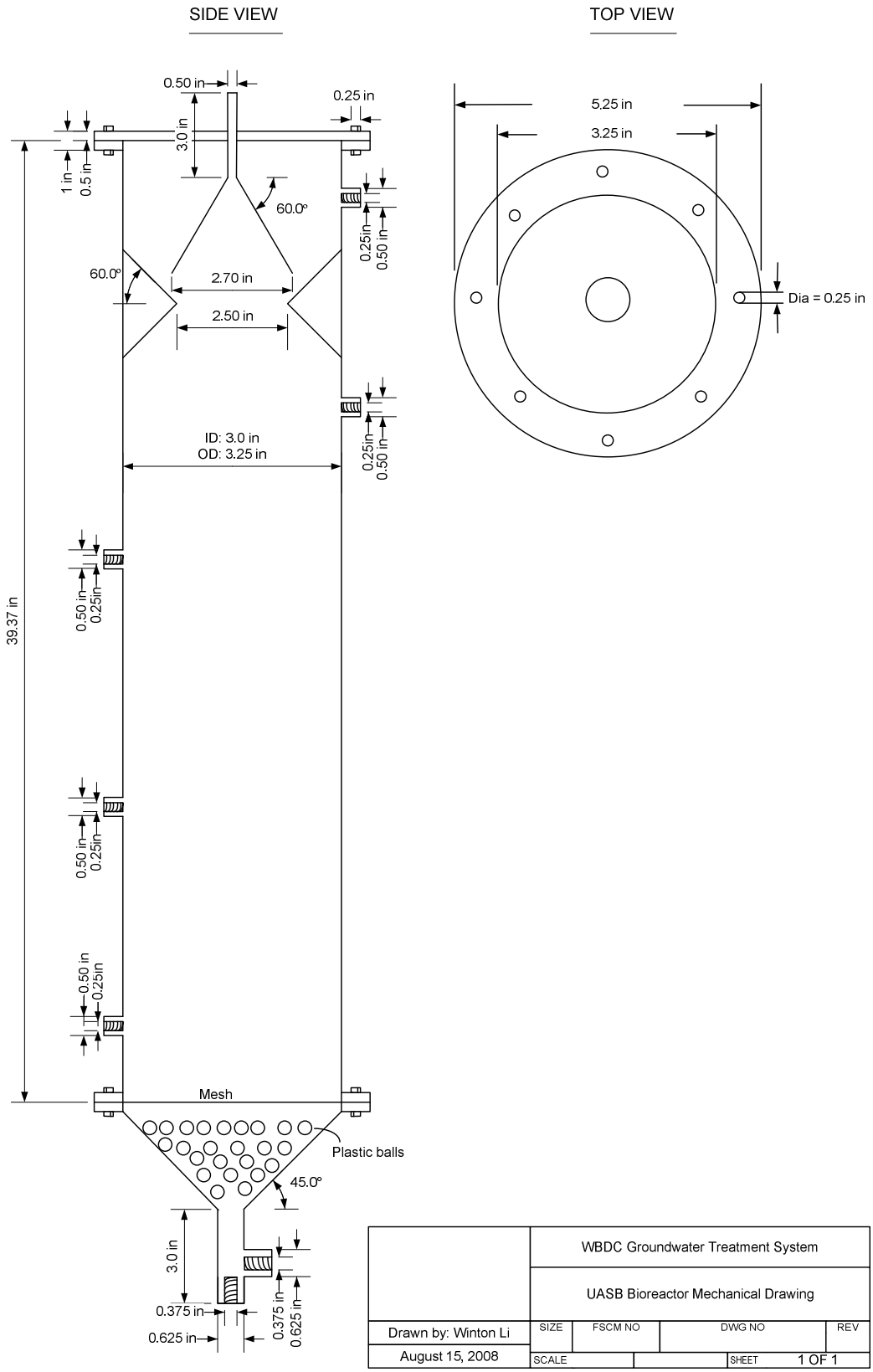
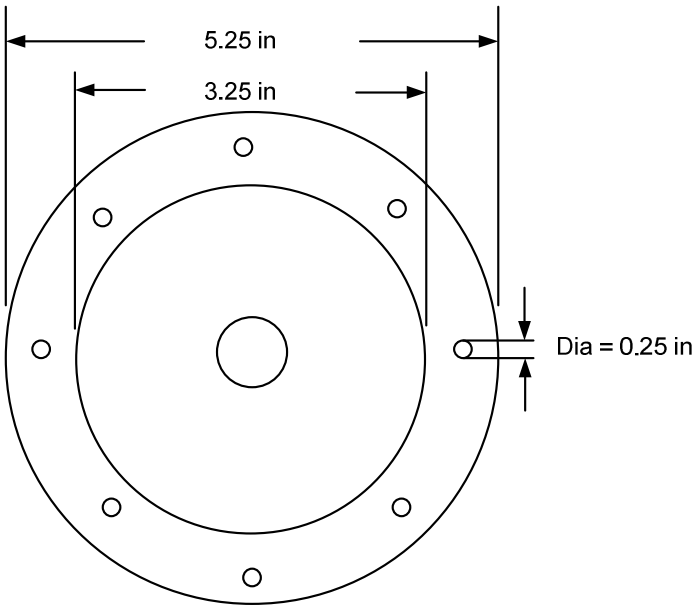


Figure A-3 UASB bioreactor inlet assembly – mechanical diagram

TOP VIEW



SIDE VIEW

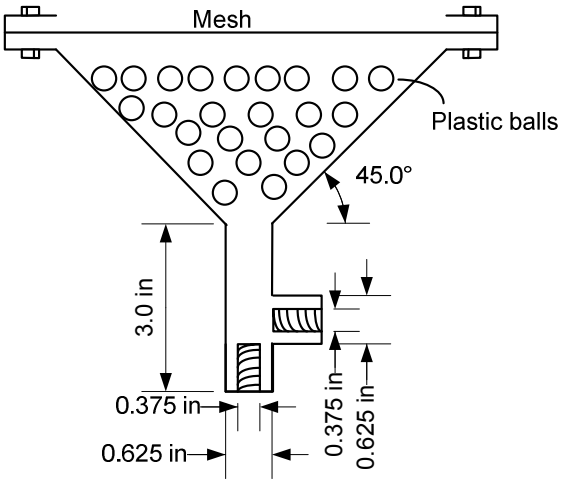


Figure A-4 UASB bioreactor gas solid separator (GSS) assembly – mechanical diagram

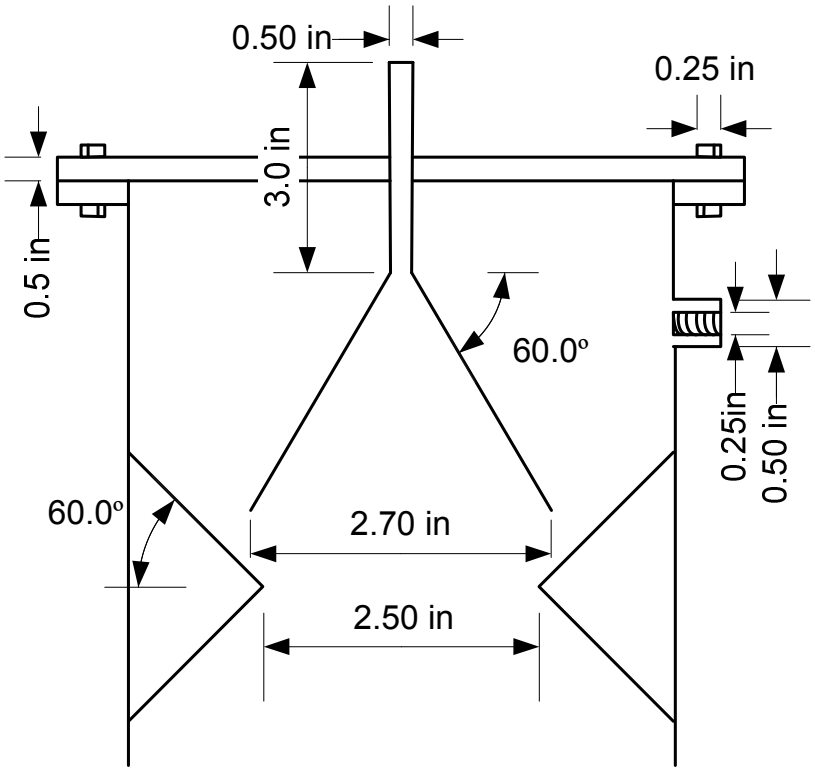


Figure A-5 Sulphide stripper column – diagram

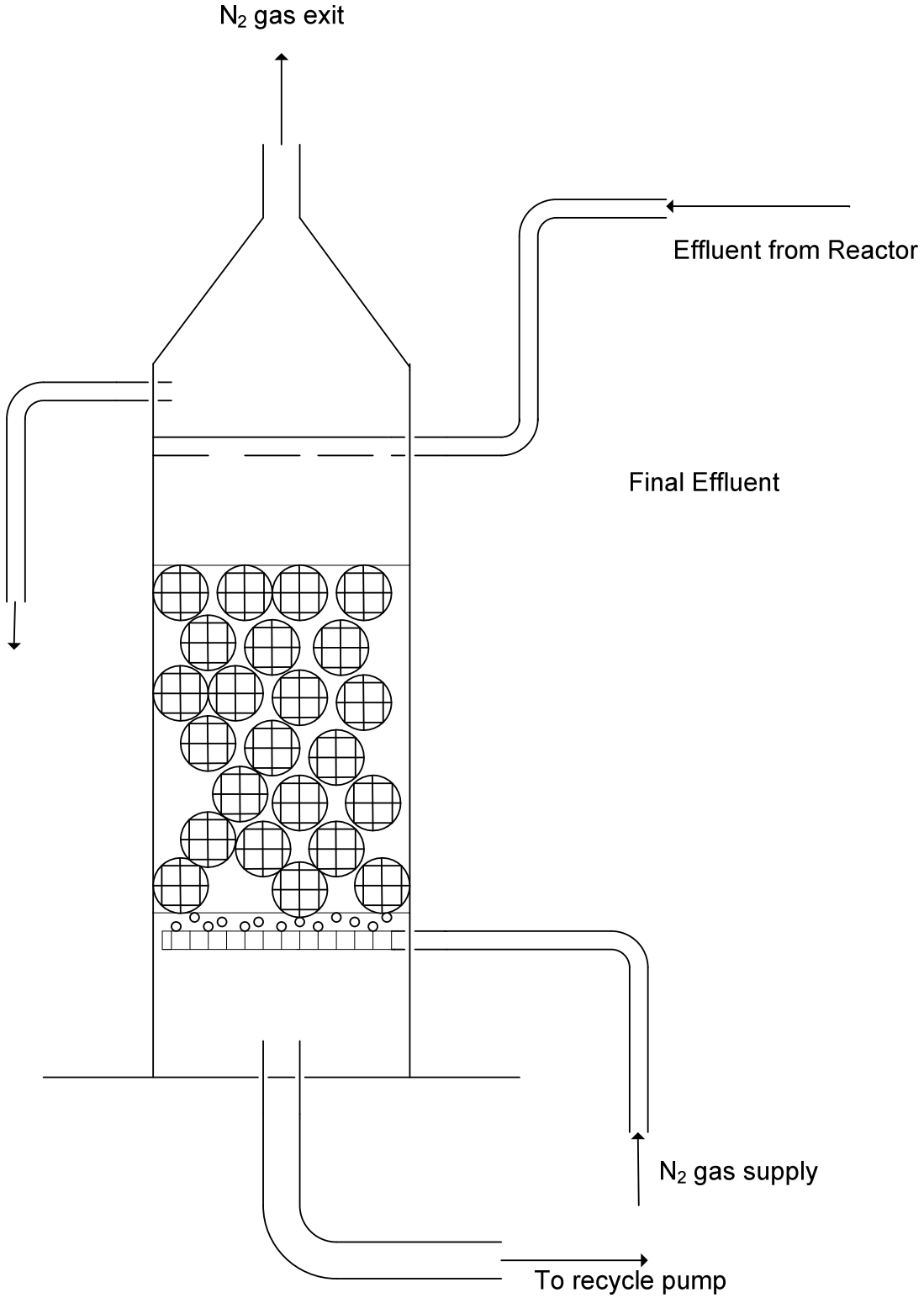
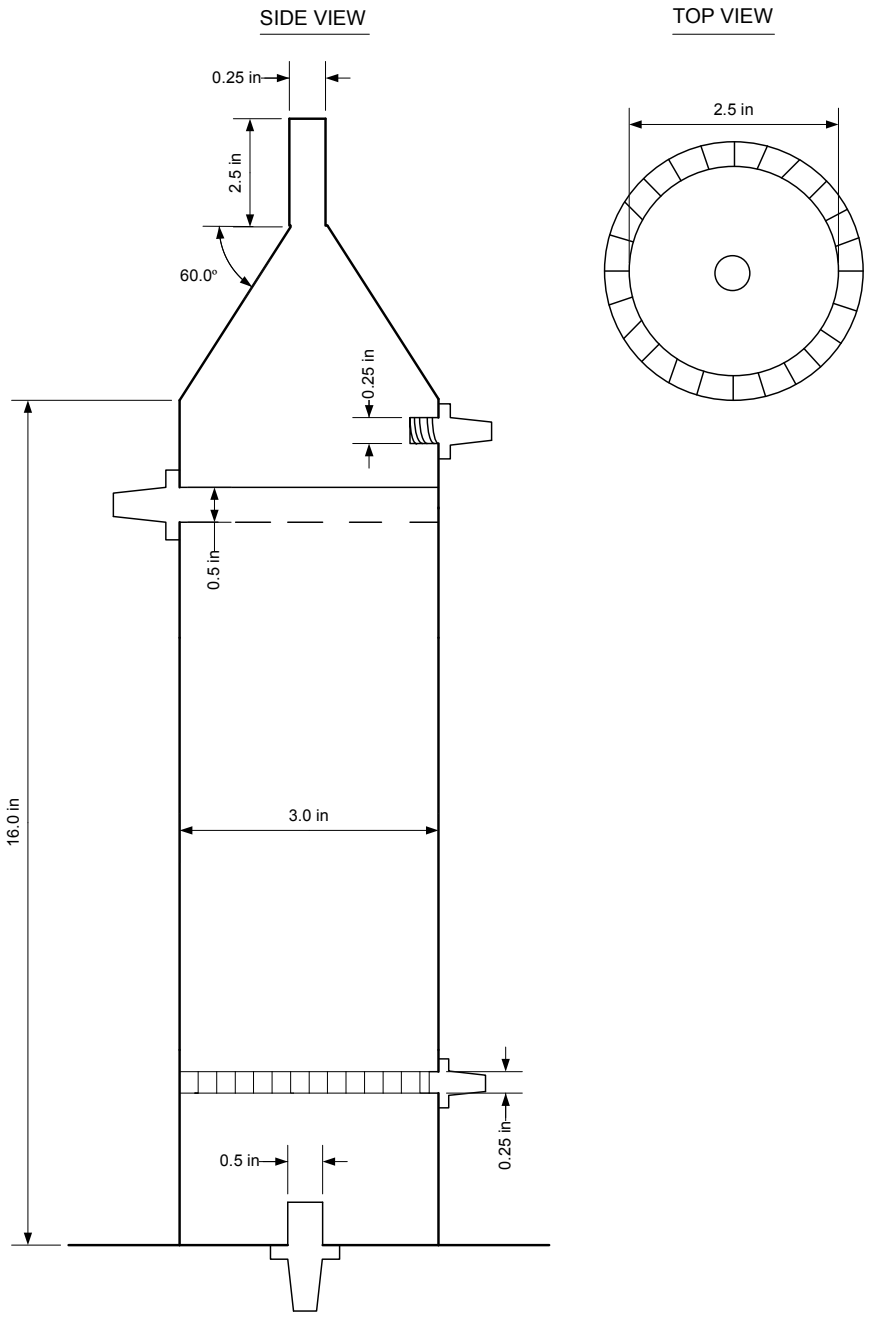


Figure A-6 Sulphide stripper column – mechanical diagram

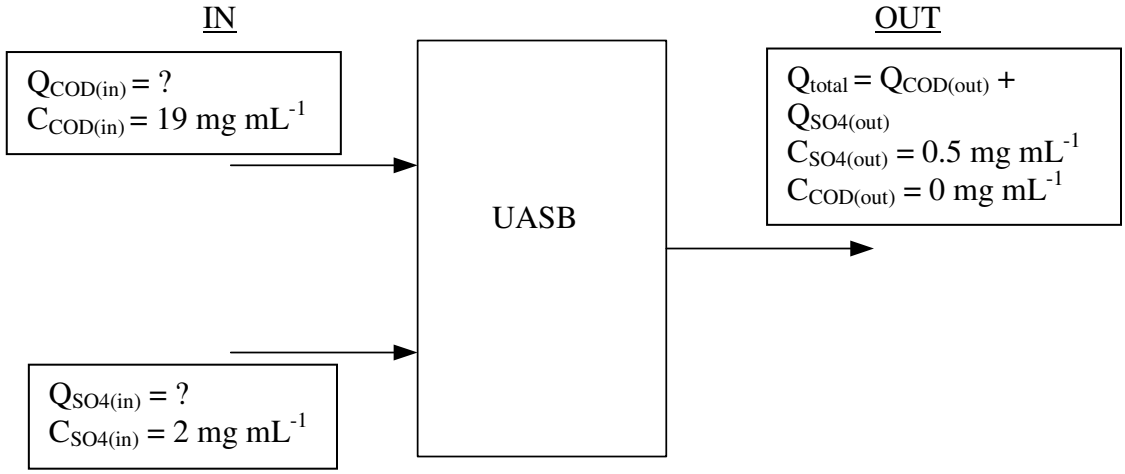


	WBDC Groundwater Treatment System			
	H_2S Stripper Mechanical Diagram			
Drawn by: Winton Li	SIZE	FSCM NO	DWG NO	REV A
August 15, 2008	SCALE		SHEET 1 OF 1	

Appendix B. Sample Calculations

B-1: Influent flowrate Calculations.

To calculate the influent sulphate and COD flowrate, the sulphate and COD balance on the UASB bioreactor is used, base on the following diagram.



Where $Q_{COD(in)}$ = influent COD flowrate (mL min-1)
$C_{COD(in)}$ = influent COD concentration (mg ml-1)
$Q_{SO4(in)}$ = influent sulphate flowrate (mL min-1)
$C_{SO4(in)}$ = influent sulphate concentration (mg mL-1)

Q_{total} = total effluent flowrate (mL min-1)
$Q_{COD(out)}$ = effluent COD flowrate (mL min-1)
$Q_{SO4(out)}$ = effluent sulphate flowrate (mL min-1)
$C_{COD(out)}$ = effluent COD concentration (mg mL-1)
$C_{SO4(out)}$ = effluent sulphate concentration (mg mL-1)

By selecting a COD/sulphate ratio of 4:1, then,

$$\frac{(F_{COD})_{in} - (F_{COD})_{out}}{(F_{SO4})_{in} - (F_{SO4})_{out}} = 4 \qquad \text{Eqn. 1}$$

And assuming first order kinetics,

$$rate = k_1 C_{SO4} \qquad \text{Eqn. 2}$$

Sulphate balance is:

$$F_{in} - F_{out} = (rate)(Volume)$$ Eqn. 3

COD balance is:

$$F_{in} - F_{out} = (rate)(Volume)$$ Eqn. 4

Which becomes:

$$F_{in} = F_{out}$$

As rate is independent of COD.

From Eqn. 1, since $(F_{COD})_{out} = 0$ as $C_{COD(out)} = 0$, then:

$$(F_{COD})_{in} = 4[(F_{SO4})_{in} - (F_{SO4})_{out}]$$ Eqn.5

As $(F_{COD})_{out} = 0$. then $(Q_{COD})_{out} = 0$, hence:

$$(Q_{SO4})_{out} = (Q_{total})_{out} = (Q_{total})_{in} = (Q_{SO4})_{in} + (Q_{COD})_{in}$$ Eqn. 6

Substitute Eqn. 6 into Eqn. 5:

$$[Q_{COD}C_{COD}]_{in} = 4[(Q_{SO4}C_{SO4})_{in} - (Q_{SO4}C_{SO4})_{out}]$$
$$(19\frac{mg}{mL})(Q_{COD})_{in} = 4(2\frac{mg}{mL})(Q_{SO4})_{in} - 4(0.5\frac{mg}{mL})Q_{total}$$
$$19(Q_{COD})_{in} = 8(Q_{SO4})_{in} - 2[(Q_{SO4})_{in} + (Q_{COD})_{in}]$$
$$(Q_{COD})_{in} = \frac{6}{21}(Q_{SO4})_{in}$$ Eqn. 7

Substitute Eqn. 7 into Eqn. 6:

$$(Q_{SO4})_{out} = (Q_{SO4})_{in} + \frac{6}{21}(Q_{SO4})_{in}$$
$$(Q_{SO4})_{out} = \frac{27}{21}(Q_{SO4})_{in}$$ Eqn. 8

From Eqn. 3: $F_{in} - F_{out} = (rate)(Volume)$

$$(Q_{SO4}C_{SO4})_{in} - (Q_{SO4}C_{SO4})_{out} = -k_1 C_{out}(Volume)$$
$$(2\frac{mg}{mL})(Q_{SO4})_{in} - (0.5\frac{mg}{mL})(\frac{27}{21})(Q_{SO4})_{in} = -k_1(0.5\frac{mg}{mL})(5000mL)$$
$$1.357(Q_{SO4})_{in} = -2500k_1$$ Eqn. 9

Then, using kinetics data obtained from the batch mode operation, the influent sulphate and COD flowrates are calculated and shown below.

$k_1(d^{-1})$	$(Q_{SO4})_{in}$ (mL min^{-1})	$(Q_{COD})_{in}$ (mL min^{-1})
-0.0617	0.079	0.023
-0.1256	0.161	0.046
-0.1904	0.244	0.070

From this table, the initial influent flowrates for the start of continuous mode operation are as follows:

$(Q_{SO4})_{in}$ = 0.25 mL min^{-1}
$(Q_{COD})_{in}$ = 0.08 mL min^{-1}

Appendix C. Analytical Methods

C-1 Sulphate

Sulphate concentrations were measured using the barium sulphate turbidimetric method 4500-SO_4^{2-} (APHA 2005). Addition of barium ions, in the form of barium chloride, forms barium sulphate in the presence of sulphate ions in the water. This insoluble precipitate is a suspension of milky crystals of uniform size. These particles affect the amount of light that passes through the solution when it is placed in a spectrophotometer at the wavelength of 450 nm. This direct relationship means that the sulphate concentration can be determined when compared to a standard curve of known sulphate concentrations.

The detection limits for this test ranged from 1 mg L^{-1} to 100 mg L^{-1} with the aid of two buffer solutions. Buffer A has a detection limit between 20 and 100 mg L^{-1}, while Buffer B has a detection limit of 1 to 20 mg L^{-1}. Samples with an expected concentration greater than 100 mg L^{-1} were diluted with dH_2O.

Both color due to dissolved organics and suspended material can affect the accuracy of the optical density reading, therefore samples were filtered through 0.22 µm filter paper prior to analysis. Potential errors caused by the sample color were eliminated by blanking the spectrophotometer with sample water prior to the addition of barium chloride. Steps in the sulphate analysis protocol are listed below (adapted from Brown, 2007):

1. Turn on the spectrophotometer (Biochrom Ultrospec 1000) at least 30 minutes before analyzing samples, to allow time for the lamp to warm up. Set the wavelength to 450 nm.
2. Filter samples using 0.22 µm filter paper. Transfer 10 mL of the filtered samples into a clean 50 mL beaker containing a magnetic stir bar. Dilute the samples with

distilled water, if needed, in order to achieve a sulphate concentration between 1 and 100 mg L^{-1}. Be sure to maintain a total volume of 10 mL in the beaker.

3. Depending on the concentration of the sulphate in the samples, add 2 mL of buffer A or buffer B to the diluted sample.
4. Pour some of the solution into a clean cuvette and use it to zero the spectrophotometer. Pour the liquid back into the beaker.
5. Place the beaker on the magnetic stirrer. Weigh 0.25 g barium chloride and add it to the beaker. Begin timing immediately and let stir for 60 ± 2 seconds at constant speeds.
6. Remove the beaker from the stirrer and pour some of the solution into a clean cuvette. Place the cuvette in the spectrophotometer and take the OD reading after exactly 5 minutes.
7. Tests for each sample were performed in duplicate and sometimes in triplicate. If the replicates are not in acceptable agreement more replicates are performed to improve accuracy.

Reagents:

A. Stock sulphate solution of 100 mg/L to be used as a standard.
B. Buffer A (high concentrations): Dissolve 30g of magnesium chloride ($MgCl_2 \cdot 6H_2O$), 5g of sodium acetate ($CH_3COONa \cdot 3H_2O$), 1.0g of potassium nitrate (KNO_3) and 20mL of acetic acid (CH_3COOH) in 500mL distilled water and make up to 1000mL.
C. Buffer B (low concentrations): Dissolve 30g of magnesium chloride ($MgCl_2 \cdot 6H_2O$), 5g of sodium acetate ($CH_3COONa \cdot 3H_2O$), 1.0g of potassium nitrate (KNO_3), 0.111g sodium sulphate (Na_2SO4) and 20mL of acetic acid (CH_3COOH) in 500mL distilled water and make up to 1000mL.
D. Barium Chloride

C-2 Sulphide

The method used to determine sulphide concentrations was a modified methylene blue method, taken from the standard Methylene Blue Method 4500-S^{2-} in Standard Methods for Water and Wastewater (APHA 2005). The total sulphide concentration measured by this method includes dissolved H_2S, HS^-, and acid-soluble metallic sulphides present as suspended matter in solution. This method is based on the reaction between these sulphides and dimethyl-p-phenylenediamine to produce methylene blue, with the aid of an oxidizing agent, postassium dichromate. This method detects concentrations in the range of 0 to 1.5 mg L^{-1}.

Procedure:

1. Turn on the spectrophotometer and set the wavelength to 625 nm.
2. Dilute samples, if necessary, with the appropriate amount of dH_2O to achieve a sulphide concentration between 0 and 1.5 mg L^{-1}. Transfer 2.5 mL of samples for testing into 4 mL cuvettes.
3. Prepare a blanking solution with 2.5 mL of one sample.
4. To this blanking solution, add 0.167 mL 1+1 H_2SO_4 solution and 1 drop $FeCl_3$ solution at the same time. Cover the cuvette with parafilm and invert once slowly. Wait 12 minutes, then add 0.533 mL $(NH_4)_2HPO_4$ solution. Invert once again then wait 10 minutes before using the solution to blank the spectrophotometer.
5. To the test samples, add 0.167 mL amine-sulphuric acid solution and 1 drop $FeCl_3$ at the same time. Cover the cuvette with parafilm and invert once slowly. Wait 12 minutes then add 0.533 mL $(NH_4)_2HPO_4$. Invert once again then wait 10 minutes. Record the optical density readings from the spectrophotometer.
6. Perform this test in triplicate.

Reagents:

A. Amine-sulphuric acid stock solution: Dissolve 27 g N,N-dimethyl-p-phenylenediamine oxalate in cold mixture of 50 mL concentrated H_2SO_4 and 20 mL distilled water. Cool and dilute to 100 mL with distilled water. The amine

oxalate should be fresh as an old supply may be oxidized and discolored to a degree that results in interfering colors in the test. Store in a dark bottle. When the stock is diluted and used in the procedure with a sulphide-free sample, it must yield a colorless solution.

B. Amine-sulphuric acid reagent: dilute 25 mL amine-sulphuric acid stock solution with 975 mL 1+1 H_2SO_4. Store in a dark glass bottle.
C. Ferric Chloride solution: Dissolve 100 g $FeCl_3$-$6H_2O$ in 40 mL distilled water.
D. Sulphuric acid solution, 1+1 H_2SO_4: Add 1 mL concentrated H_2SO_4 to 1 mL distilled water
E. Diammonium hydrogen phosphate solution: Dissolve 400 g $(NH_4)_2HPO_4$ in 800 mL distilled water.
F. Sodium Sulphide stock solution: Dissolve 5 g crushed Na_2S-$9H_2O$ crystal in 2.5 mL distilled water. At 30ºC, this will yield a saturated Na_2S solution at $9.2434x10^4$ mg L^{-1}.

C-3 Carbon Tests

C-3.1 Soluble Chemical Oxygen Demand

The soluble Chemical Oxygen Demand (sCOD) test that was used is the standard COD method 5220D in Standard Methods for Water and Wastewater (APHA 2005). This test determines the amount of oxygen required to oxidize both organic and inorganic soluble compounds in an acidic dichromate mixture. As the samples taken for analysis were a mixture of soluble and insoluble matter, they were filtered through a 0.22 µm filter paper before testing. Distilled water was used as blank to zero the spectrophotometer. This method detects concentrations in the range of 1 to 1,000 mg L^{-1} O_2.

Procedure:

1. Turn on the digester and preheat to 150ºC.
2. Filter the samples through 0.22 µm filter paper. Dilute samples, if necessary, to achieve the desired sCOD concentration range.
3. In a clean test tube place solutions in the following order:
    1. 2 mL (sample, or blank, or standard)
    2. 1.2 mL digestion solution
    3. 2.8 mL catalyst
4. Cap tightly and invert three times.
5. Place test tube on digester for 2 hours.
6. Remove from digester, cool to room temperature.
7. Turn on spectrophotometer, set wavelength to 600 nm. Allow it to warm-up for 20 minutes.
8. Zero the spectrophotometer with the blank solution.
9. Measure absorbance of sample solutions.
10. Do each sample in duplicates (preferably triplicates).

Reagents:

A. Standard: 0.850 g potassium acid phthalate in 1 L distilled water.

B. Digestion solution (add reagents in the following order):
    1. 167 mL H_2SO_4 in 500 mL distilled water. Mix.
    2. add 17.0 g mercuric sulphate. Mix.
    3. add 10.216 g potassium dichromate. Mix.
    4. make to 1 L with distilled water.
C. Catalyst: 11 g silver sulphate in 2.25 L concentrated H_2SO_4.

C-3.2 Total Organic Carbon

Samples for the Total Organic Carbon (TOC) test were performed on a TOC analyzer in Dr. Madjid Mohseni's lab at the Chemical and Biological Engineering building. This test method uses ultraviolet radiation to convert the organic carbon present in the samples to carbon dioxide. The carbon dioxide produced is measured with an infrared analyzer. The calibration curve used for this testing procedure has a detection range of 0 – 100 mg L^{-1} reported as Non-Purgeable Organic Carbon (NPOC).

Procedure

1. Start the TOC analyzer program by double clicking *TOC-Control V* on Windows Desktop.
2. Double click *Sample Table Editor* in *TOC-Control V* window. A new window will open up.
3. In *File* menu, click *New*, then select *Sample Run*, press *OK*. Then press *OK* again to accept the *General Information* window.
4. On top menu bar, click on the *autogenerate* icon. The *Sample Group Wizard* window will pop up.
5. Select *Calibration Curve*, then click on *Browse* icon, and select appropriate calibration file to be used. Click *Next* to continue.
6. Enter number of samples, and Start vial position.
7. Click *Next* twice, then click *Finish* to close the window.
8. The *Sample Loader* window will pop up. Place samples onto loader tray according to positions highlighted in blue.

9. Click *OK* to accept settings. This window will close automatically and the sample table window is populated with sample names and IDs.
10. Select first cell of *Sample Name* column, change the sample names accordingly.
11. Select *Connect* icon on top menu bar.
12. Once connection is finished, and all settings are ok, the traffic light/start icon will light up.
13. Select the *traffic light/start* icon.
14. Save the sample table as txt file in appropriate location.
15. A standby window will pop up, select *keep running*, then click *standby* tab.
16. *Sample loader* window will pop up again, double check sample names with those on sample loader tray, then select *OK*.
17. Analyzer will now start analyzing whole sample set.

Equipment:

Analyzer: Shimadzu TOC-V cpH with ASI-V sample loader

Software: Shimadzu TOC-Control V

C-3.3 Carbohydrate

Tests for total carbohydrates were performed using a variation of the classic Anthrone Method (Ludwig 1956). Carbohydrates in solution readily react with concentrated sulfuric acid and, in the presence of anthrone, form a blue-green complex that absorbs light at a wavelength of 620 nm (Mohan 2003). This test has a detection range of 0 – 1 mg L^{-1} expressed as glucose equivalents.

Procedure:

1. Turn on spectrophotometer and set the wavelength to 620 nm. Allow it to warm-up for 20 minutes.
2. Remove samples from freezer and place in 20ºC water bath.
3. Filter thawed samples through 0.22 µm filter paper. Dilute samples if necessary.
4. Place water bath on hotplate and bring to boil.

5. Add 1 mL sample to clean glass test tube. Also prepare 1 mL blank sample with distilled water.
6. Add 4 mL Anthrone reagent to test tubes containing sample and blank.
7. Cap tightly and invert three times.
8. Loosen cap and place samples along with blank in boiling water bath for 10 minutes.
9. Remove samples and blank from water bath and allow to cool.
10. Zero spectrophotometer with blank solution.
11. Record absorbance of sample solutions.
12. Test each sample in triplicates.

Reagents:

A. Standard: 1 mg L^{-1} glucose solution
B. Reagent: Anthrone Reagent – 0.2%$_{w/v}$ in conc. Sulfuric Acid

C-3.4 Organic Acids – High Performance Liquid Chromotography

Through the use of external standards, this test determined the concentrations of several individual organic acids present in the silage leachate and unconsumed organic acids in the effluent samples.

Procedure:

1. Turn on HPLC pump and Absorbance Detector. It will take approximately 30 minutes to warm up.
2. Check that the correct column is installed. Also check eluent levels.
3. Filter sample with 0.22 µm filter paper. Dilute if necessary.
4. In Windows NT desktop, double click the Breeze® icon to open the program.
5. Click the equilibrate tab once, and change flow to 100% organic acids to purge the column.
6. Inject approximately 75 µL sample (3 syringe injections) into the sample port.

7. Click the Inject tab once, enter the name of the sample, sample volume, run time, select organic acids as eluent in new window. Select Run to start the injection.
8. When a second window pops up, count 10 seconds then turn black knob around injection port clockwise, then count 10 more seconds and return knob to original position.
9. Sample will now be injected and results will show up in new window at the end of the run.

Reagent:

Eluent: 13 mM H_2SO_4 solution in nano-pure water, filtered through glass Buchner funnel with Waters 0.2 µM Super-200 membrane filter paper under vacuum suction and de-gassed for one hour.

Equipment:

Hardware: Waters 1525 Binary HPLC Pump with manual injection port
Waters 2487 Dual λ Absorbance Detector
Waters IC-Pak Ion Exclusion 50Á 7 µm 7.8x300 mm HPLC Column

Software: Waters® Breeze™ (Version 3.20)

C-3.5 Dissolved Oxygen (DO)

The method used for measuring dissolved oxygen levels within the UASB bioreactor was the Rhodazine D Method, which is based on test method A of the ASTM-D-5543 Standard Test Methods for Low-Level Dissolved Oxygen in Water (ASTM 1999). Chemetrics Inc. provides this test in a simple test kit called Oxygen CHEMets (Cat. No. C-7501). In this test, Rhodazine D reacts with dissolved oxygen present in the water sample to produce a pale pink color that is proportional to the dissolved oxygen concentration in the water sample (ASTM 1999). The particular kit used in this work was for low dissolved oxygen concentration with the range of 0 – 10 mg L^{-1} O_2.

Procedure:

1. Flush sample port with sample water.

2. Fill the sample tube with sample water.
3. Insert the CHEMet ampoule with the tapered tip at the bottom of the sample tube.
4. Gently press the ampoule toward the wall of the sample tube to break the tip. Allow the ampoule to fill with sample water.
5. Invert the ampoule several times to mix the sample water with the reagents in the ampoule
6. Compare the color of the solution in the ampoule with the color chart within 30 seconds of mixing.
7. Record the concentration with the best match in color.

C-3.6 Alcohols and Phenols

Quantification of both alcohols and phenols present in the silage leachate can provide a more complete picture of the chemical make-up of the organic material in the silage leachate. However, the best methods for determining alcohols and phenols were through the use of a GC/MS analyzer. In order to efficiently utilize the time on the experiments, samples for these tests were send out to Bodycote Testing Group in Calgary, Alberta to determine the quantity of alcohols and phenols in the silage leachate. For these tests, Bodycote used the US EPA method number 8000 for testing alcohols in water, and the APHA direct photometric method, 5530D, for testing phenols in water.

C-4 Particle Size Distribution of the Granules

This procedure was conducted in order to determine the growth profile of the granules within the reactor over the operation period of the experiment. Photographs of sludge granule samples were taken during various times and analyzed for particle size distribution.

These samples were taken from Port 4 of the reactor and using a spatula, a random mass of granules (roughly the size of a pencil eraser head) was placed into a clean Petri dish. These granules were washed with 3 aliquots of 20 mL distilled water to remove as much of the floating fiber materials as possible. A final aliquot of 20 mL distilled water was added and the granules were spread out on the Petri dish. A digital picture was taken with a Canon A610 digital camera and analyzed for PSD using Image J.

ImageJ procedure:

1. Open ImageJ software by clicking on its icon.
2. Load the image to be processed by clicking on *Open* in the *File* menu, then select the correct file.
3. Click on line draw icon on menu bar. 
4. Draw line on picture with length equal to 1cm on graph paper backing.
5. On top menu bar, click on *Analyze*, and then click on *Set Scale*, change units and length corresponding to graph paper. Click *Apply* to close the window.
6. Click on *Image* on top menu bar, move cursor to *Type*, then click on *8-bit* to convert image to grayscale.
7. Click on *Image* on top menu bar, move cursor to *Adjust*, then select *Threshold*. Adjust the redness bar until background noise is minimized. Click *Apply* to close the window.
8. Click on *Process* on top menu bar, move cursor to *Binary*, select *Make Binary* to digitize image. This image can now be saved.
9. Click on *Analyze* on top menu bar, select *Analyze Particles*. ImageJ will calculate number of particles and area of each particle. A *Results* window and *Area Distribution* window will open up.

10. The results table can now be saved and analyzed with JmpIN.

JmpIN Procedure:

1. Open JmpIN software by clicking on its icon.
2. In the *Jmp Starter* window, click on *Open Data Table*.
3. In the appropriate file folder, select and open the text file that the results table is saved to.
4. Insert new column by double clicking on the first cell of an empty column.
5. Change column heading to *Diameter* by double clicking column heading.
6. Right click on column heading; select *Formula* to open *Formula Editor*.
7. Enter formula for *Diameter* calculation using *Area column*. Select *Apply*.
8. Click on *Analyze* on top menu bar, then click on *Distribution* to open Distribution window.
9. Select and drag the *Diameter* column heading to the *Y, Columns*, then click on *OK*. A distribution table is created, displaying all necessary information.
10. This distribution table can now be copied to Word and saved for future comparisons.

Appendix D. Sample Analysis Reports

Figure D-1: Analytical report of Sept.18/2007 sample by Bodycote

Bodycote TESTING GROUP

Analytical Report

Bill To:	University of British Columbia	Project:		Lot ID:	**575438**
Report To:	University of British Columbia	ID:	UASB	Control Number:	A008262
	#218 2360 East Mall	Name:		Date Received:	Sep 24, 2007
	Vancouver, BC, Canada	Location:		Date Reported:	Oct 16, 2007
	V6T 1Z3	LSD:		Report Number:	1051064
Attn:	Amber Lee	P.O.:	Visa-A-JDQF56818		
Sampled By:	Winton Li	Acct code:			
Company:	UBC-CHBE				

	Reference Number	575438-1	575438-3	575438-5	
	Sample Date	Sep 18, 2007	Sep 18, 2007	Sep 18, 2007	
	Sample Location				
	Sample Description	Reactor Effluent / Sample #1	Silage Leachate (Batch #2) / Sample #3	Silage Leachate (Batch #3) / Sample #5	
	Matrix	Water	Water	Water	
Analyte	**Units**	**Results**	**Results**	**Results**	**Detection Limit**
Alcohol Screen - Water					
Methanol	mg/L	8	35	46	5
Ethanol	mg/L	<5	<5	<5	5
2-Propanol	mg/L	<5	<5	<5	5
1-Propanol	mg/L	<5	<5	<5	5
1-Butanol	mg/L	<5	<5	<5	5
1-Pentanol	mg/L	<5	<5	<5	5

Bodycote TESTING GROUP

Analytical Report

Bill To:	University of British Columbia	Project:		Lot ID:	**575438**
Report To:	University of British Columbia	ID:	UASB	Control Number:	A008262
	#218 2360 East Mall	Name:		Date Received:	Sep 24, 2007
	Vancouver, BC, Canada	Location:		Date Reported:	Oct 16, 2007
	V6T 1Z3	LSD:		Report Number:	1051064
Attn:	Amber Lee	P.O.:	Visa-A-JDQF56818		
Sampled By:	Winton Li	Acct code:			
Company:	UBC-CHBE				

	Reference Number	575438-2	575438-4	575438-6	
	Sample Date	Sep 18, 2007	Sep 18, 2007	Sep 18, 2007	
	Sample Location				
	Sample Description	Reactor Effluent / Sample #2	Silage Leachate (Batch #2) / Sample #4	Silage Leachate (Batch #3) / Sample #6	
	Matrix	Water	Water	Water	
Analyte	**Units**	**Results**	**Results**	**Results**	**Detection Limit**
Aggregate Organic Constituents					
Phenol	mg/L	0.69	5.0	5.5	0.001

Approved by:

Randy Neumann, BSc
Vice President, Environmental

Bodycote TESTING GROUP

Methodology and Notes

Bill To:	University of British Columbia	Project:		Lot ID:	**575438**
Report To:	University of British Columbia	ID:	UASB	Control Number:	A008262
	#218 2360 East Mall	Name:		Date Received:	Sep 24, 2007
	Vancouver, BC, Canada	Location:		Date Reported:	Oct 16, 2007
	V6T 1Z3	LSD:		Report Number:	1051064
Attn:	Amber Lee	P.O.:	Visa-A-JDQF56818		
Sampled By:	Winton Li	Acct code:			
Company:	UBC-CHBE				

Method of Analysis

Method Name	Reference	Method	Date Analysis Started	Location
Alcohols - Water	US EPA	* US EPA method, 8000	15-Oct-07	BTG Calgary
Phenol in water	APHA	* Direct Photometric Method, 5530 D	16-Oct-07	BTG Edmonton

**Bodycote method(s) based on reference method*

References

APHA	Standard Methods for the Examination of Water and Wastewater
US EPA	US Environmental Protection Agency Test Methods

Comments:

Please direct any inquiries regarding this report to our Client Services group.
Results relate only to samples as submitted.

Figure D-2. Analytical report of 2008 samples by Bodycote

Bodycote TESTING GROUP

Analytical Report

Bill To:	University of British Columbia	Project:	
Report To:	University of British Columbia	ID:	UASB
	2360 East Mall	Name:	
	Vancouver, BC, Canada	Location:	
	V6T 1Z3	LSD:	
Attn:	Winton Li	P.O.:	58930
Sampled By:		Acct code:	
Company:			

Lot ID:	**631924**
Control Number:	A047174
Date Received:	Jul 22, 2008
Date Reported:	Aug 6, 2008
Report Number:	1136268

	Reference Number	631924-1	631924-2	631924-3	
	Sample Date				
	Sample Location				
	Sample Description	Silage Leachate (June/2008)	Reactor effluent (Mar/2008)	Reactor effluent (May/2008)	
	Matrix	Water	Water	Water	
Analyte	**Units**	**Results**	**Results**	**Results**	Nominal Detection Limit
Alcohol Screen - Water					
Methanol	mg/L	19	<5	<5	5
Ethanol	mg/L	266	<5	<5	5
2-Propanol	mg/L	<5	<5	<5	5
1-Propanol	mg/L	20	<5	<5	5
1-Butanol	mg/L	20	<5	<5	5
1-Pentanol	mg/L	<5	<5	<5	5

Approved by:

Michael Yohemas, BSc
General Manager

Bodycote TESTING GROUP

Methodology and Notes

Bill To:	University of British Columbia	Project:	
Report To:	University of British Columbia	ID:	UASB
	2360 East Mall	Name:	
	Vancouver, BC, Canada	Location:	
	V6T 1Z3	LSD:	
Attn:	Winton Li	P.O.:	58930
Sampled By:		Acct code:	
Company:			

Lot ID:	**631924**
Control Number:	A047174
Date Received:	Jul 22, 2008
Date Reported:	Aug 6, 2008
Report Number:	1136268

Method of Analysis

Method Name	Reference	Method	Date Analysis Started	Location
Alcohols - Water	US EPA	* US EPA method, 8000	24-Jul-08	BTG Calgary

**Bodycote method(s) based on reference method*

References

US EPA — US Environmental Protection Agency Test Methods

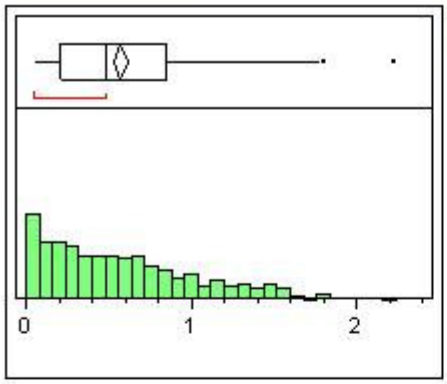
Comments:

Please direct any inquiries regarding this report to our Client Services group.
Results relate only to samples as submitted.

Appendix E. Granules Analysis Results

Distributions

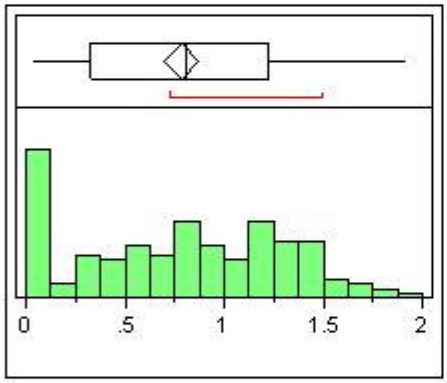
ct=97-Diameter



Quantiles			Moments	
100.0%	maximum	2.2398	Mean	0.5750074
99.5%		1.8070	Std Dev	0.4419763
97.5%		1.5676	Std Err Mean	0.0215662
90.0%		1.2678	upper 95% Mean	0.6173988
75.0%	quartile	0.8485	lower 95% Mean	0.5326159
50.0%	median	0.4879	N	420
25.0%	quartile	0.2096		
10.0%		0.0714		
2.5%		0.0505		
0.5%		0.0505		
0.0%	minimum	0.0505		

Distributions

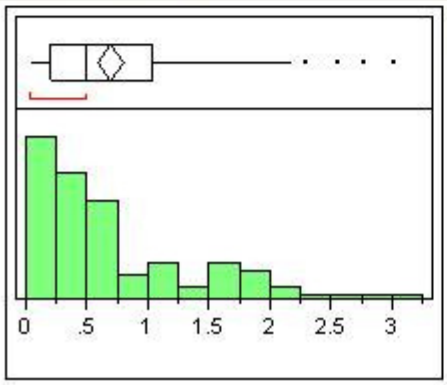
ct=125-Diameter



Quantiles			Moments	
100.0%	maximum	1.9176	Mean	0.7835767
99.5%		1.9176	Std Dev	0.5229842
97.5%		1.7415	Std Err Mean	0.0418722
90.0%		1.4658	upper 95% Mean	0.8662906
75.0%	quartile	1.2187	lower 95% Mean	0.7008628
50.0%	median	0.8041	N	156
25.0%	quartile	0.3186		
10.0%		0.0505		
2.5%		0.0345		
0.5%		0.0345		
0.0%	minimum	0.0345		

Distributions

ct=143-Diameter



Quantiles			Moments	
100.0%	maximum	3.0341	Mean	0.695849
99.5%		3.0341	Std Dev	0.6656901
97.5%		2.4611	Std Err Mean	0.0570825
90.0%		1.7584	upper 95% Mean	0.8087405
75.0%	quartile	1.0274	lower 95% Mean	0.5829574
50.0%	median	0.4912	N	136
25.0%	quartile	0.1994		
10.0%		0.0505		
2.5%		0.0333		
0.5%		0.0333		
0.0%	minimum	0.0333		

Figure E-1 Granule sample size distributions for Day 97, 125, and 143.

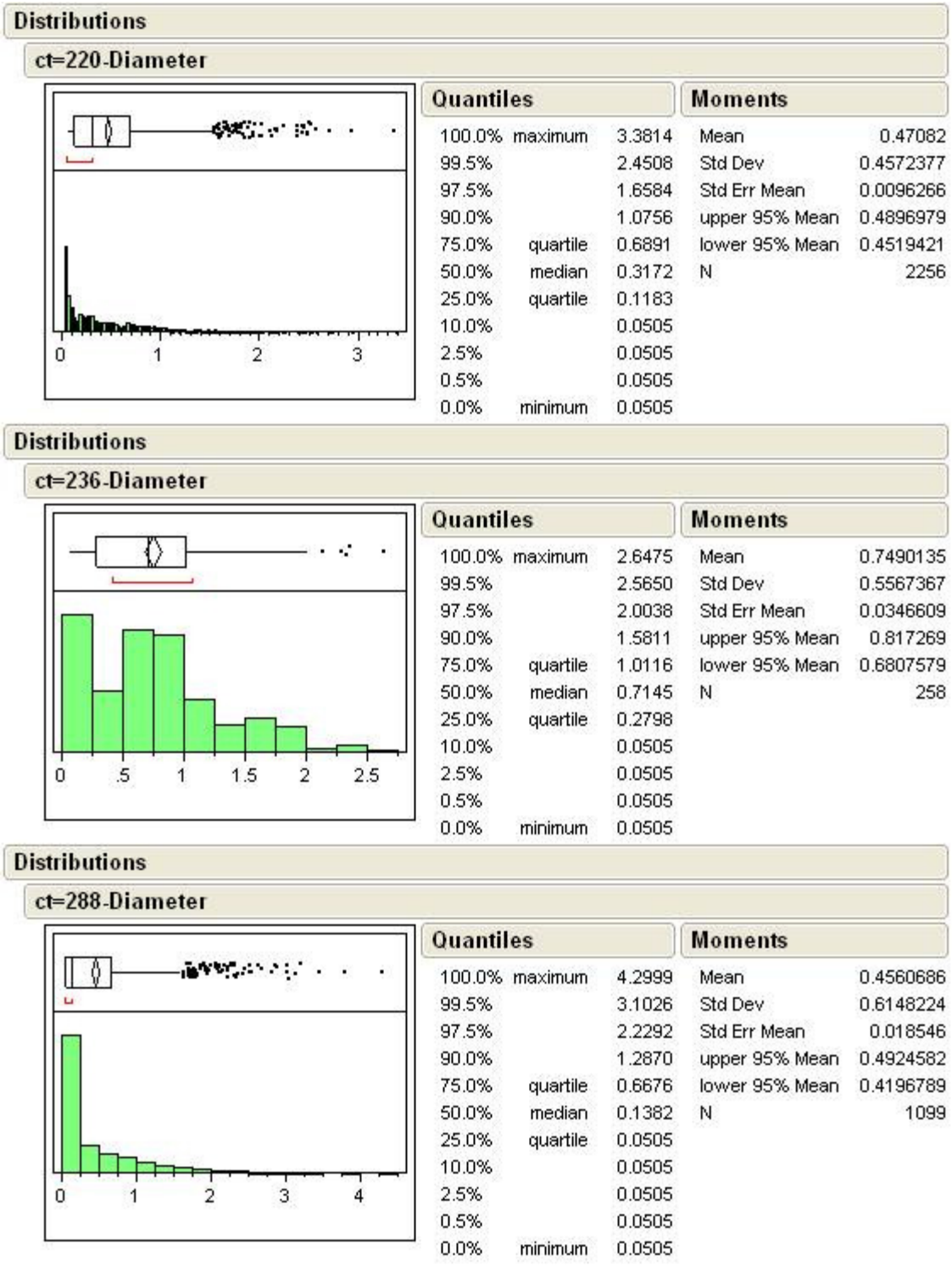


Figure E-2 Granule sample size distributions for Day 220, 236, and 288.

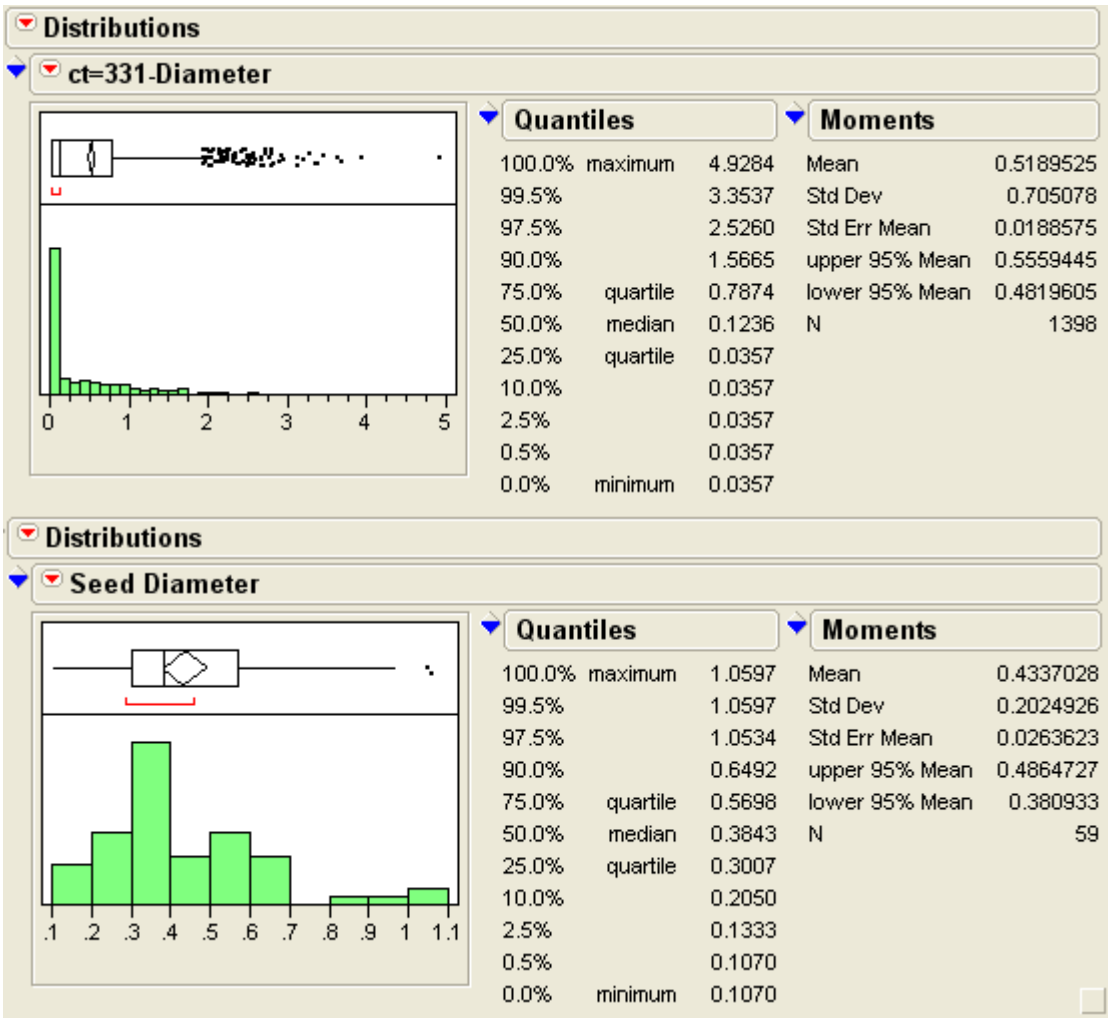


Figure E-3 Granule sample size distributions for Day 0 and 331.

Appendix F. Correlations between SRR, influent sulphate concentration and organic consumptions.

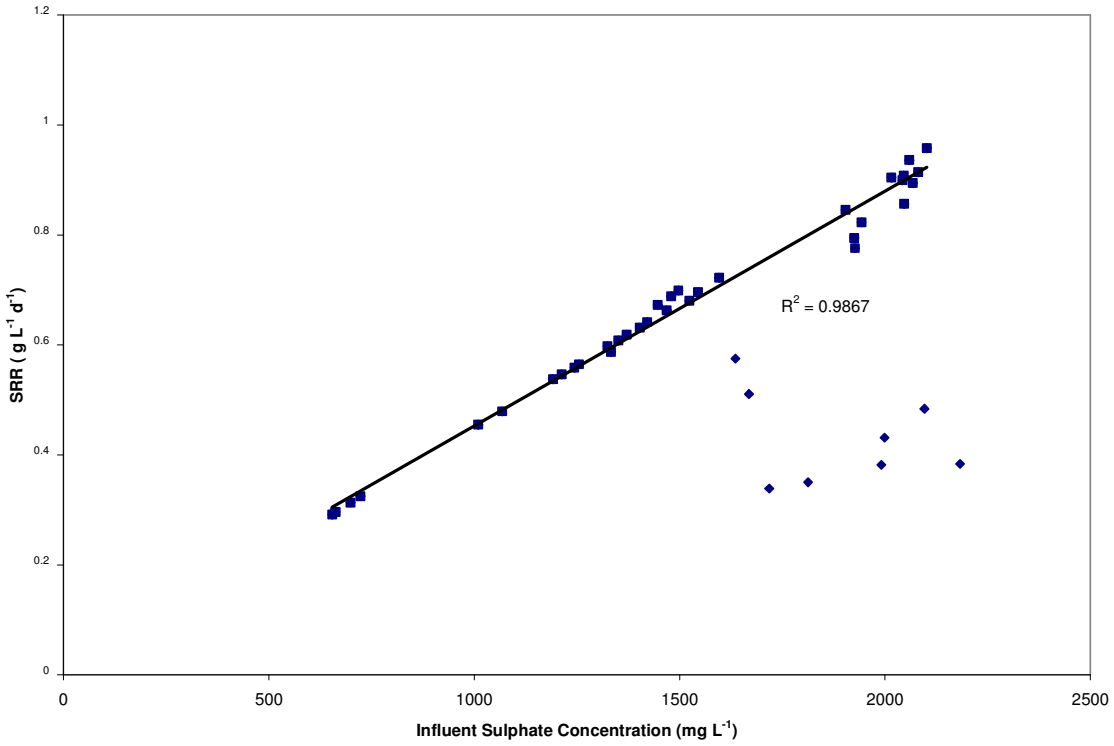


Figure F-1 Correlation between SRR and influent sulphate concentration

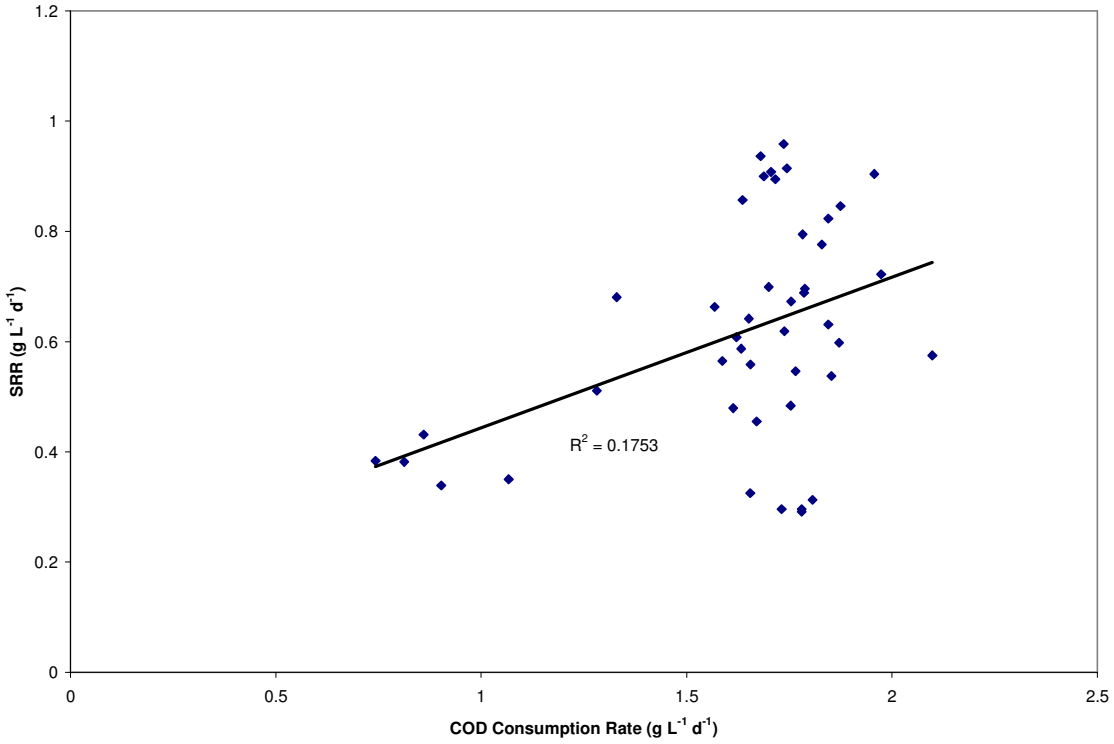


Figure F-2 Correlation between SRR and COD consumption rate.

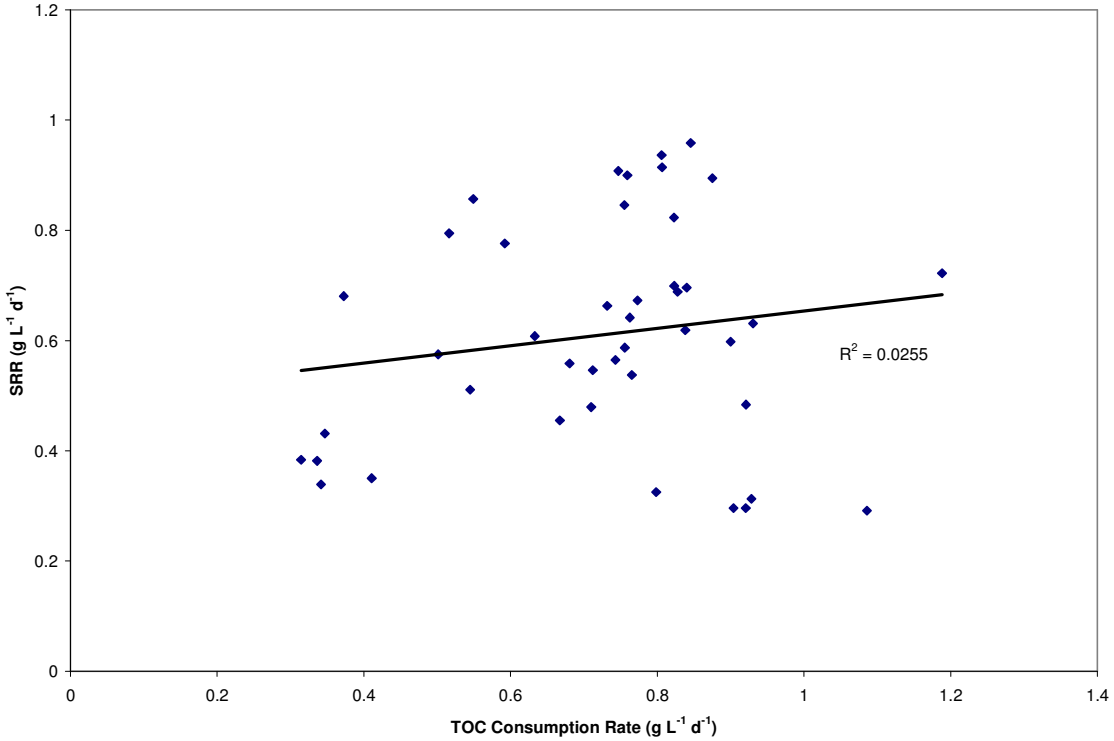


Figure F-3 Correlation between SRR and TOC consumption rate.

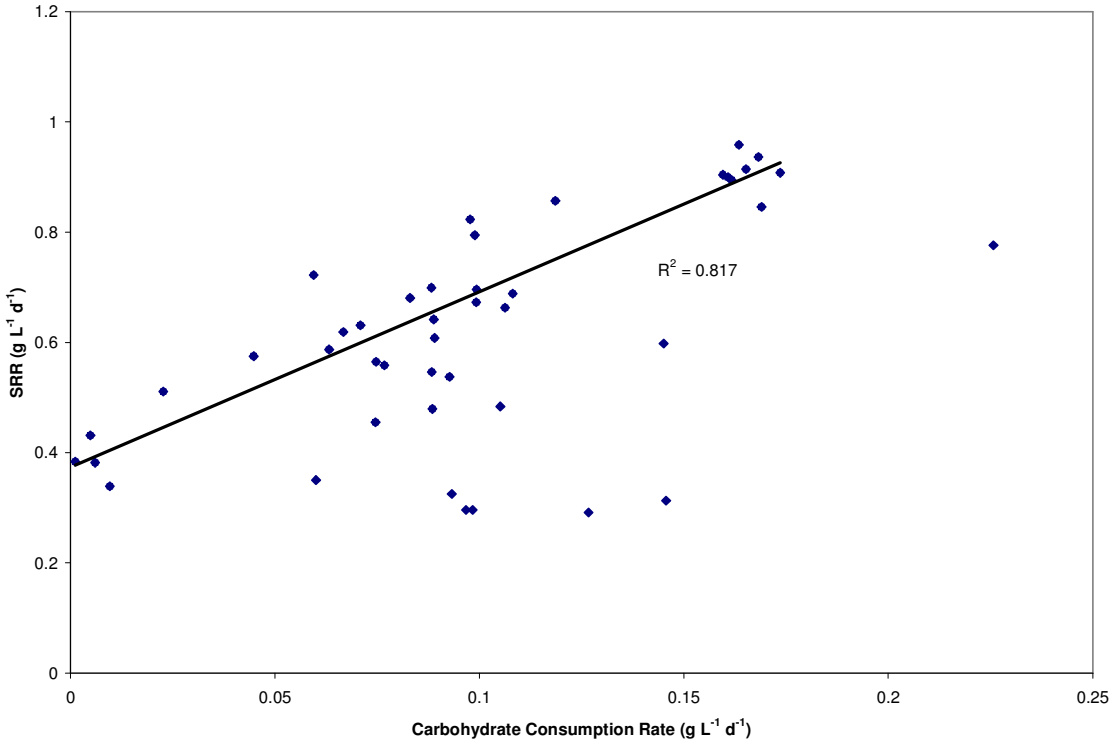


Figure F-4 Correlation between SRR and carbohydrate consumption rate.

Appendix G: UBC UASB Experiment Results

Table G-1 Batch mode operation results

Date	Day	pH	Sulphate	Std Dev	Sulphide	Std Dev	COD	Std Dev	TOC	Std Dev
11-Jun-07	0	7.75	2747	33.11	41.23	3.165	7110	654.2	2658	26.16
15-Jun-07	4	7.75	2493	79.18	51.62	1.152	2814	725.8	987.0	22.63
17-Jun-07	6	7.75	2536	40.03	49.28	2.477	2199	148.5	514.3	11.74
20-Jun-07	9	7.75	1433	30.07	44.79	1.210	2220	101.0	571.3	3.111
22-Jun-07	11	7.75	1392	10.76	45.71	0.845	2251	199.6	450.1	8.980
25-Jun-07	14	7.5	1357	20.48	90.11	5.296	6358	31.43	3348	22.63
28-Jun-07	17	7	2468	23.97	108.9	0.648	6054	48.01	907.4	21.28
30-Jun-07	19	7.5	2262	36.75	122.9	2.872	5844	48.01	1771	50.91
2-Jul-07	21	7.5	2158	11.17	128.1	3.281	5457	48.01	1256	147.1
4-Jul-07	23	7.75	1974	79.52	129.2	3.433	4671	72.58	1910	334.5
6-Jul-07	25	7.75	1890	25.71	191.8	1.256	3927	65.42	1489	263.8
11-Jul-07	30	8	1261	22.08	337.9	1.044	2157	155.0	990.7	447.4
13-Jul-07	32	8	1179	2.598	344.3	1.304	1863	31.43	776.3	327.7

Table G-2 Continuous mode sulphate concentrations and hydraulic retention times.

													Sulphate									HRT	
																			(mL/min)	(g/L/D)			
Date	Day	pH	eff	Std Dev	port1	Std Dev	port2	Std Dev	port3	Std Dev	inf	Std Dev	feed	Std Dev	ΔSO4	sulphate removal (%)	Std Dev	Q(SO4)	SRR		total Q	HRT
14-Jul-07	0	8	1117	14.29														0.25				
15-Jul-07	1	8	1617	6.49																		
16-Jul-07	2	8	2185	1.30																		
17-Jul-07	3	8	2535	11.69																		
18-Jul-07	4	8	3280	10.39																		
19-Jul-07	5	8	2475	3.90			2430	5.20														
20-Jul-07	6	8	3694	9.09			3698	3.90										0				
21-Jul-07	7	8	3781	11.69			3839	11.69														
22-Jul-07	8	8	3625	5.20			3315	7.79														
25-Jul-07	11	8	2884	3.90			2588	2.60										0.2				
26-Jul-07	12	8	2652	5.20			2321	5.20														
27-Jul-07	13	8	2335	9.09			2202	2.60														
28-Jul-07	14	8	2152	7.79			1946	3.90														
29-Jul-07	15	8	1931	6.49			1825	5.20					2143	5.20								
1-Aug-07	18	8	1601	10.39			1379	7.79					2138	5.20								
2-Aug-07	19	8	1258	2.60			1050	7.79					2131	3.90								
3-Aug-07	20	8	942	5.20			833	3.90					2132	2.60								
5-Aug-07	22	8	598	5.20			513	6.49					2139	6.49								
7-Aug-07	24	8	428	5.20			366	6.49					2146	6.49								
9-Aug-07	26	8	350	3.90	349	2.60			350	3.90			2125	5.20								
11-Aug-07	28	8	72	0.78	74	1.04			85	1.04												
12-Aug-07	29	8	73	0.78	76	0.78			80	1.30			2149	5.20				0.69				
14-Aug-07	31	8	126	0.78	137	1.56			144	1.30			2148	11.69								
16-Aug-07	33	8	162	1.56	81	2.08			85	1.82			2171	7.79								
18-Aug-07	35	8	70	0.52	71	0.52			70	0.78			2140	7.79								
20-Aug-07	37	8	70	0.26	72	0.26			71	0.52			2118	11.69								
22-Aug-07	39	8	35	0.26	76	0.52			73	1.04			2120	19.48								
24-Aug-07	41	8	70	0.52	77	1.30			74	0.26			2156	5.20								

26-Aug-07	43	8	76	0.52	71	0.26			73	0.78			2152	10.39									
28-Aug-07	45	8	74	1.04	73	0.26			77	0.52			2137	11.69									
30-Aug-07	47	8	69	0.26	70	0.52			71	0.26			2165	7.79									
7-Sep-07	55	8	102	0.52	70	0.52			71	0.78			2174	5.20									
13-Sep-07	61	8	70	0.52	71	0.26			76	0.78			2198	12.99									
20-Sep-07	68	8	47	0.52	23	0.52			23	0.32			2196	9.09									
27-Sep-07	75	8	84	0.39	26	0.52			24	0.19			2204	10.39				0.7			0.7	4.960317	
6-Oct-07	84	8	98	0.13	20	0.52			20	0.26			2194	9.09				0.7			0.7	4.960317	
13-Oct-07	91	8	151	0.71	19	0.13			20	0.26			2092	23.38				0.7			0.7	4.960317	
19-Oct-07	97	8	37	0.58			28.8	0.19			1331	7.79			1294	97.22	8.38	0.7	0.261		0.7	4.960317	
27-Oct-07	105	8	135	0.71	128	0.26			126	0.32	1295	7.79			1160	89.58	8.51	0.7	0.234		0.7	4.960317	
2-Nov-07	111	8	29	0.00			23.58	0.00			932.8	0.00			903.8	96.89	0.00	0.7	0.182		0.7	4.960317	
7-Nov-07	116												1758	0.00			0.00	0.7			0.7	4.960317	
9-Nov-07	118	8	125	0.14			118	0.34			1081	4.11			956	88.44	4.25	0.7	0.193		0.7	4.960317	
16-Nov-07	125	8	67	0.34			62	0.21			1167	5.48			1100	94.26	5.82	0.7	0.222		0.7	4.960317	
23-Nov-07	132	8	141	0.41			139	0.34			1157	8.22			1016	87.81	8.63	0.7	0.205		0.7	4.960317	
30-Nov-07	139	8	15	0.34			10	0.21			1237	8.22			1222	98.79	8.56	0.7	0.246		0.7	4.960317	
8-Dec-07	147	8	17	0.27			16	0.21			1491	4.11			1474	98.86	4.39	0.7	0.297		0.7	4.960317	
14-Dec-07	153	8	99	0.75			71	0.27			1523	10.96			1424	93.50	11.71	0.7	0.287		0.7	4.960317	
24-Dec-07	163	8	17	0.21			9	0.21			1244	2.74			1227	98.63	2.95	1.15	0.406		1.15	3.019324	
30-Dec-07	169	8	11	0.14			12	0.27			1192	2.74			1181	99.08	2.88	1.15	0.391		1.15	3.019324	
5-Jan-08	175	8	29	0.27			18	0.34			1524	6.85			1495	98.10	7.13	1.15	0.495		1.15	3.019324	
13-Jan-08	183	8	15	0.41			14	0.27			1351	8.22			1336	98.89	8.63	1.15	0.442		1.15	3.019324	
16-Jan-08	186	8	10	0.41			10	0.34			1010	8.22	1709	10.96	1000	99.01	8.63	1.15	0.331		1.15	3.019324	
18-Jan-08	188	8	12	0.34			11	0.34			1213	10.96			1201	99.01	11.30	1.15	0.398		1.15	3.019324	
22-Jan-08	192	8	11	0.62			8	0.41			662	12.33			651	98.34	12.95	1.15	0.216		1.15	3.019324	
25-Jan-08	195	8	11	0.69			9	0.41			662	6.85			651	98.34	7.54	1.15	0.216		1.15	3.019324	
2-Feb-08	202	8	14	0.41			9	0.55			654	6.85			640	97.86	7.26	1.15	0.212		1.15	3.019324	
10-Feb-08	210	8	9	0.34			8	0.21			723	8.22			714	98.76	8.56	1.15	0.236		1.15	3.019324	
14-Feb-08	214	8	11	0.41			10	0.41			699	9.59	2600		688	98.43	10.00	1.15	0.228		1.15	3.019324	
17-Feb-08	217	8	10	0.21			9	0.34			1324	8.22			1314	99.24	8.43	1.15	0.435		1.15	3.019324	
20-Feb-08	220	8	11	0.48			10	0.48			1371	6.85			1360	99.20	7.33	1.15	0.450		1.15	3.019324	

24-Feb-08	224	8	15	0.27			14	0.34			1068	8.22			1053	98.60	8.50	1.15	0.349		1.15	3.019324
28-Feb-08	228	8	12	0.21			10	0.27			1469	16.44			1457	99.18	16.65	1.15	0.483		1.15	3.019324
3-Mar-08	232	8	16	0.34			11	0.21			1545	10.96			1529	98.96	11.30	1.15	0.506		1.15	3.019324
7-Mar-08	236	8	16	0.21			9	0.27			1403	5.48			1387	98.86	5.69	1.15	0.459		1.15	3.019324
8-Mar-08	237	8	11	0.21			10	0.27			1421	8.22			1410	99.23	8.43	1.15	0.467		1.15	3.019324
10-Mar-08	239	8	9	0.21			10	0.14			1596	9.59			1587	99.44	9.80	1.15	0.526		1.15	3.019324
13-Mar-08	242	8	14	0.27							1255	6.85			1241	98.88	7.13	1.15	0.411		1.15	3.019324
16-Mar-08	245	8	43	0.27			47	0.14			1333	5.48			1290	96.77	5.76	1.15	0.427		1.15	3.019324
20-Mar-08	249	8	8	0.21			10	0.34			1497	4.11	2671	5.48	1489	99.47	4.32	1.05	0.450		1.05	3.306878
22-Mar-08	251	8	13	0.27			12	0.41			1480	9.59			1467	99.12	9.87	1.05	0.444		1.05	3.306878
24-Mar-08	253	8	14	0.27			13	0.41			1447	9.59	2755	13.70	1433	99.03	9.87	1.05	0.433		1.05	3.306878
28-Mar-08	257	8	286	1.64	*	*												1.05			1.05	3.306878
4-Apr-08	264	8	99	0.41	*	*												1.05			1.05	3.306878
7-Apr-08	267	8	581	2.74			582	4.11			1669	8.22			1088	65.19	10.96	1.05	0.329		1.05	3.306878
11-Apr-08	271	8	996	4.80			1019	3.43			1718	16.44			722	42.03	21.24	1.05	0.218		1.05	3.306878
14-Apr-08	274	8	1080	10.96			1137	9.59			1999	13.70			919	45.97	24.66	1.05	0.278		1.05	3.306878
19-Apr-08	279	8	1366	5.48	*		1174	6.85			2183	21.93	3282	27.22	817	37.43	27.41	1.05	0.247		1.05	3.306878
28-Apr-08	288	8	1178	5.48	*		1156	4.11			1991	13.70			813	40.83	19.18	1.05	0.246		1.05	3.306878
3-May-08	293	8	1067	9.59	*		1008	5.48			1813	13.70			746	41.15	23.29	1.05	0.226		1.05	3.306878
11-May-08	301	8	331	4.11			312	8.22			1636	10.96			1305	79.77	15.07	1.01	0.380		1.01	3.437844
17-May-08	307	8	216	0.69			220	0.82			1939	16.44			1723	88.86	17.13	1.01			1.01	
20-May-08	310	8	122	1.37			98	1.64			1925	19.19			1803	93.66	20.56	1.01	0.524		1.01	3.437844
23-May-08	313	8	998	5.48			1020	8.22			2096	19.19			1098	52.39	24.67	1.01	0.319		1.01	3.437844
27-May-08	317	8	75	0.82			87	0.82			1943	16.44			1868	96.14	17.26	1.01	0.543		1.01	3.437844
29-May-08	319	8	103	0.55			90	0.82			2047	10.96			1944	94.97	11.51	1.01	0.565		1.01	3.437844
2-Jun-08	323	8	166	9.31			106	21.41			1927	25.42			1761	91.39	34.73	1.01	0.512		1.01	3.437844
5-Jun-08	326	8	76	2.33			83	1.75			2046	18.32			1970	96.29	20.65	1	0.567		1	3.472222
13-Jun-08	334	8	69	1.61			78	1.75			1904	17.76			1835	96.38	19.37	1	0.528		1	3.472222
17-Jun-08	338	8	54	0.44			45	0.39			2016	15.50			1962	97.32	15.94	1	0.565		1	3.472222
20-Jun-08	341	8	127	0.70			134	0.87			2068	11.84			1941	93.86	12.54	1	0.559		1	3.472222
23-Jun-08	344	8	91	0.49			92	0.78			2043	13.98			1952	95.55	14.47	1	0.562		1	3.472222
24-Jun-08	345	*																1			1	

26-Jun-08	347	8	97	0.59			88	0.68			2081	11.84			1984	95.34	12.43	1	0.571		1	3.472222
	347	*																1			1	
30-Jun-08	351	8	27	0.78			24	0.34			2059	17.76			2032	98.69	18.54	1	0.585		1	3.472222
2-Jul-08	353	*																1			1	
3-Jul-08	354	8	23	0.40			19	0.44			2102	15.50			2079	98.91	15.90	1	0.599		1	3.472222
	354	*																				
4-Jul-08	355																					

Table G-3 Continuous mode sulphide concentrations

		Sulphide													
Date	Day	eff	Std Dev	port1	Std Dev	port2	Std Dev	port3	Std Dev	S-IN	Std Dev	S-OUT	Std Dev	inf	Std Dev
14-Jul-07	0	385.5	1.25												
15-Jul-07	1	127.2	0.94												
16-Jul-07	2	129.6	1.25												
17-Jul-07	3	150.7	0.94												
18-Jul-07	4	316.2	0.94												
19-Jul-07	5	62.8	0.31			166.6	0.00								
20-Jul-07	6	121.7	0.08			166.6	0.00								
21-Jul-07	7	96.0	1.25			365.2	0.63								
22-Jul-07	8	138.3	1.57			437.1	1.57								
25-Jul-07	11	161.3	1.57			544.7	0.94								
26-Jul-07	12	152.7	1.25			540.0	0.63								
27-Jul-07	13	184.8	0.94			658.5	1.57								
28-Jul-07	14	125.2	1.25			606.7	1.57								
29-Jul-07	15	178.6	1.57			586.3	0.94								
1-Aug-07	18	261.3	0.94			725.1	1.88								
2-Aug-07	19	41.8	1.57			604.2	1.25								
3-Aug-07	20	225.9	0.94			728.0	1.57								
5-Aug-07	22	142.7	1.57			542.3	1.88								
7-Aug-07	24	205.6	1.57			554.2	1.25								
9-Aug-07	26	271.3	1.25	635.2	1.88			647.8	1.57						
11-Aug-07	28	24.3	1.88	563.5	1.25			470.3	1.57						
12-Aug-07	29	27.6	1.57	647.8	1.57			719.6	1.57						
14-Aug-07	31	28.3	1.88	654.3	1.88			620.2	1.88						
16-Aug-07	33	129.2	2.50	656.7	1.57			680.8	1.88						
18-Aug-07	35	10.8	0.31	647.2	8.14			683.9	14.40						
20-Aug-07	37	4.6	0.41	587.4	6.89			553.3	3.13						
22-Aug-07	39	8.3	0.58	580.1	35.38			542.7	4.38						
24-Aug-07	41	3.8	0.17	523.0	4.07			525.2	2.82						

26-Aug-07	43	51.6	0.72	494.9	8.14			504.6	13.15								
28-Aug-07	45	7.1	0.06	604.0	10.33			572.8	19.41								
30-Aug-07	47	3.9	0.27	604.7	20.66			683.5	8.14								
7-Sep-07	55	78.5	1.41	547.8	17.84			526.5	26.61								
13-Sep-07	61	5.7	0.81	731.1	20.97			555.3	20.35								
20-Sep-07	68	21.7	0.11	555.5	6.89			509.9	6.26								
27-Sep-07	75	7.4	0.09	510.3	5.64			478.0	6.26								
6-Oct-07	84	21.4	0.13	568.3	3.76			521.4	2.50								
13-Oct-07	91	3.5	0.06	466.9	4.38			473.6	3.76								
19-Oct-07	97																
27-Oct-07	105	358.0	4.38			501.4	5.64								0.483	0.031	
2-Nov-07	111	478.0	3.76	624.5	5.64			564.3	3.13						0.361	0.047	
7-Nov-07	116	474.4	1.25			614.8	3.13								0.726	0.063	
9-Nov-07	118	521.8	1.88			670.1	2.50								0.194	0.009	
16-Nov-07	125	631.6	1.88			795.0	3.76								0.205	0.006	
23-Nov-07	132	342.5	2.50			706.4	3.76								0.221	0.003	
30-Nov-07	139	515.2	3.76			737.9	1.88								0.199	0.016	
8-Dec-07	147	650.2	4.38			787.0	5.01								0.156	0.005	
14-Dec-07	153	617.4	1.88			452.7	4.38								0.205	0.006	
24-Dec-07	163	428.0	3.13			729.0	3.13								0.302	0.008	
30-Dec-07	169	540.8	5.01			755.1	2.50								0.105	0.005	
5-Jan-08	175	460.3	13.77			675.0	10.64								0.164	0.002	
13-Jan-08	183	399.6	4.38			710.9	10.02								0.246	0.011	
16-Jan-08	186	478.4	13.15			677.2	15.03								0.152	0.038	
18-Jan-08	188	421.8	18.16			577.2	13.77								0.221	0.016	
22-Jan-08	192	422.2	10.02			599.7	3.13								0.137	0.022	
25-Jan-08	195	360.2	7.51			602.4	5.64								0.197	0.013	
2-Feb-08	202	354.9	3.76			475.8	6.89								0.259	0.013	
10-Feb-08	210	451.0	6.89			574.9	6.89								0.257	0.014	
14-Feb-08	214	404.5	5.01			495.2	6.89								0.153	0.020	
17-Feb-08	217	558.6	5.01			742.3	8.14								0.239	0.009	
20-Feb-08	220	622.8	11.90			807.4	32.56								0.155	0.003	

24-Feb-08	224	655.5	30.68			829.1	72.00							0.152	0.000		
28-Feb-08	228	672.8	0.00			799.8	73.26							0.135	0.019		
3-Mar-08	232	538.6	34.44			745.4	13.77							0.098	0.002		
7-Mar-08	236	558.6	1.25			831.7	1.88							0.131	0.002		
8-Mar-08	237	325.2	1.88			431.9	2.50							0.117	0.003		
10-Mar-08	239	260.2	1.25			333.2	3.13							0.129	0.005		
13-Mar-08	242	278.8	2.50											0.116	0.005		
16-Mar-08	245	456.3	3.13			486.8	1.25							0.208	0.003		
20-Mar-08	249	523.1	2.50			534.6	2.50							0.132	0.003		
22-Mar-08	251	513.0	4.38			526.7	5.01							0.205	0.013		
24-Mar-08	253	364.6	3.76			422.6	1.88							0.138	0.014		
28-Mar-08	257	420.4	5.01														
4-Apr-08	264	816.2	3.76														
7-Apr-08	267	787.4	4.38											0.354	0.009		
11-Apr-08	271	643.1	3.13											0.270	0.003		
14-Apr-08	274	644.4	3.76											0.201	0.013		
19-Apr-08	279	582.9	1.88											0.242	0.005		
28-Apr-08	288	612.1	10.64			659.1	29.43							0.170	0.006		
3-May-08	293	589.1	0.63			620.1	3.13							0.211	0.005		
11-May-08	301	662.2	1.25			703.3	21.91							0.319	0.008		
17-May-08	307	805.2	5.64			808.7	0.63										
20-May-08	310	445.2	57.60			595.3	10.64										
23-May-08	313	151.7	8.14			197.3	1.25										
27-May-08	317	421.8	133.36			1050.4	456.44										
29-May-08	319	638.7	115.83			367.7	193.47										
2-Jun-08	323	280.8	63.00			928.1	360.27										
5-Jun-08	326	836.6	23.58			879.4	14.61										
13-Jun-08	334	714.7	139.95			687.2	246.32										
17-Jun-08	338	926.6	94.09			1273.1	473.54										
20-Jun-08	341	731.2	11.30			718.2	12.02										
23-Jun-08	344	838.7	5.90			839.8	5.90			374.1	7.53	382.6	12.09				
24-Jun-08	345	878.2	26.40			908.9	11.52			410.4	2.56	404.8	8.60				

26-Jun-08	347	929.3	50.29			969.7	44.99			390.9	19.96	408.3	3.99			
	347	1008.7	33.66			1041.7	31.00			417.5	12.41	394.5	1.35			
30-Jun-08	351															
2-Jul-08	353															
3-Jul-08	354															
	354					355.0				411		395				
4-Jul-08	355					358.0										

Table G-4 Continuous mode COD concentrations

										COD									
								expt	COD	expt	expt	theo	COD consump	COD	Q(leachate)	COD loading			
Date	Day	eff	port1	port2	port3	inf	feed	ΔCOD	Consumption rate	ΔgCOD/ΔgSO4	inf(COD)/inf(SO4)	ΔCOD	for SO4 red.	Removal(%)	mL/min	g/L/D	COD:S		
14-Jul-07	0	1454													0.08				
15-Jul-07	1	1501													0.1				
16-Jul-07	2	1470																	
17-Jul-07	3	1360																	
18-Jul-07	4	1163																	
19-Jul-07	5	1137		1449															
20-Jul-07	6	1702		1732															
21-Jul-07	7	951		967											0.3				
22-Jul-07	8	1360		1706															
25-Jul-07	11	2287		2381															
26-Jul-07	12	2303		2539															
27-Jul-07	13	2036		2397			9673												
28-Jul-07	14	2444		2601															
29-Jul-07	15	1847		1879			5351												
1-Aug-07	18	1706		1879			10380												
2-Aug-07	19	1816		2146			10364												
3-Aug-07	20	1879		1596			10349												
5-Aug-07	22	2161		2413			10679												
7-Aug-07	24	1941		2146			10302												
9-Aug-07	26	1879	1973		2083		10270												
11-Aug-07	28	1643	1580		1549														
12-Aug-07	29	1281	1250		1266		10364								0.17				
14-Aug-07	31	731	920		826		10333												
16-Aug-07	33	951	1014		1109		10694												
18-Aug-07	35	731	1109		1061		10349												
20-Aug-07	37	763	1030		1030		10176												
22-Aug-07	39	700	889		873		10302												
24-Aug-07	41	480	1030		904		10270												

26-Aug-07	43	590	936		904		10317												
28-Aug-07	45	433	810		904		10097												
30-Aug-07	47	574	779		841		10254												
7-Sep-07	55	606	999		1093		10396												
13-Sep-07	61	653	1156		1014		10443												
20-Sep-07	68	763	1297		1486		10474												
27-Sep-07	75	1219	1156	1109			10710									0.333			
6-Oct-07	84	669	826		983		10600									0.333			
13-Oct-07	91	920	1250				10396									0.333			
19-Oct-07	97	873	936			4110		3237	0.96	2.50	3.09	866.98	0.27	78.76	0.333	0.31	9.26		
27-Oct-07	105	889	841			2837	10726	1948	0.58	1.68	2.19	777.2	0.40	68.66	0.333	0.19	6.57		
2-Nov-07	111	166				2764		2598	0.77	2.87	2.96	605.546	0.23	93.99	0.333	0.25	8.89		
7-Nov-07	116														0.333				
9-Nov-07	118	417		606		4471	9516	4054	1.21	4.24	4.14	640.52	0.16	90.67	0.333	0.39	12.41		
16-Nov-07	125	637		700		3309	9877	2672	0.79	2.43	2.84	737	0.28	80.75	0.333	0.26	8.51		
23-Nov-07	132	1297		1423		2601	9972	1304	0.39	1.28	2.25	680.72	0.52	50.13	0.333	0.13	6.74		
30-Nov-07	139	763		716		2067	10066	1304	0.39	1.07	1.67	818.74	0.63	63.09	0.333	0.13	5.01		
8-Dec-07	147	684		731		2067	10254	1383	0.41	0.94	1.39	987.58	0.71	66.91	0.333	0.13	4.16		
14-Dec-07	153	142		167		2739		2597	0.77	1.82	1.80	954.08	0.37	94.82	0.333	0.25	5.40		
24-Dec-07	163	392		350		4030	10424	3638	1.66	2.96	3.24	822.09	0.23	90.27	0.43	0.45	9.72		
30-Dec-07	169	533		733		4604		4071	1.85	3.45	3.86	791.27	0.19	88.42	0.43	0.50	11.59		
5-Jan-08	175	167		225		3089	10357	2922	1.33	1.95	2.03	1001.65	0.34	94.59	0.43	0.36	6.08		
13-Jan-08	183	500		767		4063		3563	1.62	2.67	3.01	895.12	0.25	87.69	0.43	0.44	9.02		
16-Jan-08	186	700		833		4371	8726	3671	1.67	3.67	4.33	670	0.18	83.99	0.43	0.45	12.98		
18-Jan-08	188	708		808		4587		3879	1.77	3.23	3.78	804.67	0.21	84.57	0.43	0.48	11.34		
22-Jan-08	192	575		667		4487		3912	1.78	6.01	6.78	436.17	0.11	87.19	0.43	0.48	20.33		
25-Jan-08	195	667		692		4471		3804	1.73	5.84	6.75	436.17	0.11	85.08	0.43	0.47	20.26		
2-Feb-08	202	725		808		4637		3912	1.78	6.11	7.09	428.8	0.11	84.36	0.43	0.48	21.27		
10-Feb-08	210	567		733		4204	9774	3637	1.65	5.09	5.81	478.38	0.13	86.51	0.43	0.45	17.44		
14-Feb-08	214	700		650		4670		3970	1.81	5.77	6.68	460.96	0.12	85.01	0.43	0.49	20.04		
17-Feb-08	217	583		683		4695	9475	4112	1.87	3.13	3.55	880.38	0.21	87.58	0.43	0.51	10.64		
20-Feb-08	220	567		667		4387		3820	1.74	2.81	3.20	911.2	0.24	87.08	0.43	0.47	9.60		

24-Feb-08	224	542		667		4088		3546	1.61	3.37	3.83	705.51	0.20	86.74	0.43	0.44	11.48	
28-Feb-08	228	725		792		4171	9974	3446	1.57	2.37	2.84	976.19	0.28	82.62	0.43	0.43	8.52	
3-Mar-08	232	575		783		4504		3929	1.79	2.57	2.92	1024.43	0.26	87.23	0.43	0.49	8.75	
7-Mar-08	236	608		667		4662	9558	4054	1.84	2.92	3.32	929.29	0.23	86.96	0.43	0.50	9.97	
8-Mar-08	237	442		517		4071		3629	1.65	2.57	2.86	944.7	0.26	89.14	0.43	0.45	8.59	
10-Mar-08	239	475		517		4812	9774	4337	1.97	2.73	3.02	1063.29	0.25	90.13	0.43	0.54	9.05	
13-Mar-08	242	484				3971		3487	1.59	2.81	3.16	831.47	0.24	87.81	0.43	0.43	9.49	
16-Mar-08	245	533		492		4121	9491	3588	1.63	2.78	3.09	864.3	0.24	87.07	0.43	0.44	9.27	
20-Mar-08	249	525		500		4146		3621	1.70	2.43	2.77	997.63	0.28	87.34	0.58	0.60	8.31	
22-Mar-08	251	484		500		4288	9525	3804	1.79	2.59	2.90	982.89	0.26	88.71	0.58	0.64	8.69	
24-Mar-08	253	434		484		4171	9458	3737	1.75	2.61	2.88	960.11	0.26	89.59	0.58	0.62	8.65	
28-Mar-08	257														0.58			
4-Apr-08	264														0.58			
7-Apr-08	267	550		608		3280	9391	2730	1.28	2.51	1.97	728.96	0.27	83.23	0.58	0.46	5.90	
11-Apr-08	271	625		625		2548		1923	0.90	2.66	1.48	483.74	0.25	75.47	0.58	0.32	4.45	
14-Apr-08	274	583		558		2415		1832	0.86	1.99	1.21	615.73	0.34	75.86	0.58	0.31	3.62	
19-Apr-08	279	608		567		2190	9774	1582	0.74	1.94	1.00	547.39	0.35	72.24	0.58	0.26	3.01	
28-Apr-08	288	592		600		2323	9591	1731	0.81	2.13	1.17	544.71	0.31	74.52	0.58	0.29	3.50	
3-May-08	293	517		533		2789	9774	2272	1.07	3.05	1.54	499.82	0.22	81.46	0.58	0.38	4.62	
11-May-08	301	658		717		5420	9408	4762	2.10	3.65	3.31	874.35	0.18	87.86	0.52	0.71	9.94	
17-May-08	307																	
20-May-08	310	309		417		4354	8509	4045	1.78	2.24	2.26	1208.01	0.30	92.90	0.52	0.61	6.79	
23-May-08	313	542		517		4521	8559	3979	1.75	3.62	2.16	735.66	0.18	88.01	0.52	0.60	6.47	
27-May-08	317	533				4720	10024	4187	1.84	2.24	2.43	1251.56	0.30	88.71	0.52	0.63	7.29	
29-May-08	319	583				4296	9957	3713	1.64	1.91	2.10	1302.48	0.35	86.43	0.52	0.56	6.30	
2-Jun-08	323	395				4546	9458	4151	1.83	2.36	2.36	1179.87	0.28	91.31	0.52	0.62	7.08	
5-Jun-08	326	900				4601	9280	3701	1.71	1.88	2.25	1319.9	0.36	80.44	0.6	0.64	6.75	
13-Jun-08	334	500				4568	9169	4068	1.87	2.22	2.40	1229.45	0.30	89.05	0.6	0.70	7.20	
17-Jun-08	338	311				4557	9014	4246	1.96	2.16	2.26	1314.54	0.31	93.18	0.6	0.73	6.78	
20-Jun-08	341	844				4568	9058	3724	1.72	1.92	2.21	1300.47	0.35	81.52	0.6	0.64	6.63	
23-Jun-08	344	916				4579	9303	3663	1.69	1.88	2.24	1307.84	0.36	80.00	0.6	0.63	6.72	
24-Jun-08	345																	

26-Jun-08	347	872				4657	9525	3785	1.74	1.91	2.24	1329.28	0.35	81.28	0.6	0.65	6.71	
	347																	
30-Jun-08	351	1066				4712	9236	3646	1.68	1.79	2.29	1361.44	0.37	77.38	0.6	0.63	6.87	
2-Jul-08	353																	
3-Jul-08	354	933				4701	9203	3768	1.74	1.81	2.24	1392.93	0.37	80.15	0.6	0.65	6.71	
	354																	
4-Jul-08	355																	

Table G-5 Continuous mode TOC and carbohydrate concentrations

		TOC									Carbohydrate							
Date	Day	eff	port1	port2	port3	inf	feed	ΔTOC	TOC Consumption rate	TOC removal	eff	Std Dev	inf	Std Dev	Δcarbohydrate	Carb Consumption rate	carb removal	Δcarb/ΔTOC
14-Jul-07	0	884																
15-Jul-07	1	772																
16-Jul-07	2	387																
17-Jul-07	3	466																
18-Jul-07	4	355																
19-Jul-07	5	354		337														
20-Jul-07	6	313		304														
21-Jul-07	7	296		292														
22-Jul-07	8	418		603														
25-Jul-07	11	985		909														
26-Jul-07	12	925		908														
27-Jul-07	13	879		884														
28-Jul-07	14	1067		1061														
29-Jul-07	15	837		807			2267											
1-Aug-07	18	729		830			6936											
2-Aug-07	19	810		813			7737											
3-Aug-07	20	854		853			7244											
5-Aug-07	22	862		848			6769											
7-Aug-07	24	871		910			6292											
9-Aug-07	26	838	819		796		7132											
11-Aug-07	28	631	525		530													
12-Aug-07	29	367	309		305	3383	7796	3016										
14-Aug-07	31	227	164		165	3222	7425	2995										
16-Aug-07	33	189	190		198	3570	8225	3381										
18-Aug-07	35	221	284		280	3536	8147	3315										
20-Aug-07	37	391	266		278	3817	8796	3426										
22-Aug-07	39	257	210		219	3301	7606	3044										
24-Aug-07	41	207	260		269	3278	7552	3071										

26-Aug-07	43	267	262		256	3649	8408	3382												
28-Aug-07	45	311	311		301	3683	8486	3372												
30-Aug-07	47	341	324			3659	8431	3318												
7-Sep-07	55	388	445			3847	8864	3459												
13-Sep-07	61	399	452			4492	10350	4093												
20-Sep-07	68	252	519		519	5117	11790	4865												
27-Sep-07	75	534	445	445		3218	7414	2684												
6-Oct-07	84	245	303		305	3830	8826	3585												
13-Oct-07	91	152	334		310	4626	10660	4474				16.4	0.5	382.3	57	365.9	0.11	95.71		
19-Oct-07	97	1191		1166		2090		899	0.27	43.01		23.8	7.3	69.5	13.1	45.7	0.01	65.76	0.87	
27-Oct-07	105	123	131		114	1337		1214	0.36	90.80		14.1	2.5	94.5	12.9	80.4	0.02	85.08	2.41	
2-Nov-07	111	112		104		1829		1717	0.51	93.88		10.1	0.7	106.7	15.9	96.6	0.03	90.53	2.11	
7-Nov-07	116	99		92		2426	8836	2327	0.69	95.92							0.00			
9-Nov-07	118	99		92		2426		2327	0.69	95.92		15.3	4	194.3	12.4	179	0.05	92.13	2.95	
16-Nov-07	125	174		161		1787		1613	0.48	90.26		25	1.7	150.1	3.6	125.1	0.04	83.34	2.80	*
23-Nov-07	132	139		128		1365		1226	0.36	89.82		25.7	0.5	185	3.1	159.3	0.05	86.11	4.67	*
30-Nov-07	139	166		159		1063	7345	897	0.27	84.38		26.9	1.9							*
8-Dec-07	147	147		135		1044	9393	897	0.27	85.92		24.4	2.8	385.7	9.8	361.3	0.11	93.67	13.84	*
14-Dec-07	153	76		76		1555	9400	1479	0.44	95.11		22.3	2.7	259.4	5.3	237.1	0.07	91.40	6.10	*
24-Dec-07	163	180				1675	11320	1495	0.68	89.25		15.8	2.4	184.6	3.1	168.8	0.08	91.44	4.03	*
30-Dec-07	169	215				1897		1682	0.77	88.67		15.6	0.3	219.4	5.3	203.8	0.09	92.89	4.30	*
5-Jan-08	175	149				968	2153	819	0.37	84.61		15.8	1	198.4	64.2	182.6	0.08	92.04	7.55	*
13-Jan-08	183	143				1534		1391	0.63	90.68		12.9	1.8	208.6	40.5	195.7	0.09	93.82	5.10	
16-Jan-08	186	168				1634		1466	0.67	89.72		11	0.6	175	8.4	164	0.07	93.71	4.01	
18-Jan-08	188	190				1755		1565	0.71	89.17		17.9	2.1	212.1	8.6	194.2	0.09	91.56	4.43	
22-Jan-08	192	237				2223		1986	0.90	89.34		17.6	0.7	230.2	5.3	212.6	0.10	92.35	3.83	
25-Jan-08	195	266				2289		2023	0.92	88.38		11.8	1.8	228	7.1	216.2	0.10	94.82	3.78	
2-Feb-08	202	315				2701		2386	1.09	88.34		10.9	0.3	289.3	16.7	278.4	0.13	96.23	4.12	
10-Feb-08	210	221				1976		1755	0.80	88.82		13.4	1.1	218.4	8.9	205	0.09	93.86	4.15	
14-Feb-08	214	270				2310		2040	0.93	88.31		11.8	3.1	331.9	8	320.1	0.15	96.44	5.54	
17-Feb-08	217	221				2199		1978	0.90	89.95		20.4	0.7	339.2	6.7	318.8	0.15	93.99	5.80	
20-Feb-08	220	226				2068	6194	1842	0.84	89.07		28.4	4.5	175.1		146.7	0.07	83.78	2.84	

24-Feb-08	224	204				1764		1560	0.71	88.44		23	4	217.5	4.3	194.5	0.09	89.43	4.41	
28-Feb-08	228	198				1806		1608	0.73	89.04		13.4	1.9	247	11.1	233.6	0.11	94.57	5.17	
3-Mar-08	232	164				2010		1846	0.84	91.84		12.6	1.3	230.8	8.2	218.2	0.10	94.54	4.34	
7-Mar-08	236	171				2216		2045	0.93	92.28		25.3	0.6	181.2	0.7	155.9	0.07	86.04	2.81	
8-Mar-08	237	171				1847		1676	0.76	90.74		10.1	1	205.4	8.1	195.3	0.09	95.08	4.23	
10-Mar-08	239	196				2807		2611	1.19	93.02		12.2	1.3	142.9	3.6	130.7	0.06	91.46	1.86	
13-Mar-08	242	204				1837		1633	0.74	88.89		9.9	0.6	174.2	7.3	164.3	0.07	94.32	3.58	
16-Mar-08	245	194				1855		1661	0.76	89.54		10.7	3.1	149.8	1.5	139.1	0.06	92.86	3.00	
20-Mar-08	249	180				1933		1753	0.82	90.69		17.4	1	205.4	23.4	188	0.09	91.53	3.89	
22-Mar-08	251	183				1946		1763	0.83	90.60		10.9	1.9	241.3	10	230.4	0.11	95.48	4.74	
24-Mar-08	253	168				1815	5954	1647	0.77	90.74		19.1	0.6	230.5	5	211.4	0.10	91.71	4.66	
28-Mar-08	257																			
4-Apr-08	264																			
7-Apr-08	267	167				1328		1161	0.55	87.42		6	2.3	54.4	44	48.4	0.02	88.97	1.46	
11-Apr-08	271	141				869		728	0.34	83.77		3.6		24.2	17.9	20.6	0.01	85.12	0.95	
14-Apr-08	274	132				871		739	0.35	84.85		4.5	1.6	14.9	10.2	10.4	0.00	69.80	0.48	
19-Apr-08	279	122				792		670	0.31	84.60		9.3	5.1	11.8	7.2	2.5	0.00	21.19	0.13	
28-Apr-08	288	119				835		716	0.34	85.75		4.2	1.1	17.1	1.9	12.9	0.01	75.44	0.62	
3-May-08	293	131				1006		875	0.41	86.98		5.5	1.9	133.4	10.2	127.9	0.06	95.88	5.09	
11-May-08	301	131				1269	6559	1138	0.50	89.68		28.2	1.7	129.9	12	101.7	0.04	78.29	3.21	
17-May-08	307																			
20-May-08	310	212				1384		1172	0.52	84.68		17.4	0.7	241.9	7.4	224.5	0.10	92.81	6.49	
23-May-08	313	211				2301		2090	0.92	90.83		18.6	1	257.2	19.5	238.6	0.11	92.77	4.15	
27-May-08	317	214				2081		1867	0.82	89.72		45.3	1.7	267.2	20.9	221.9	0.10	83.05	4.27	
29-May-08	319	263				1509		1246	0.55	82.57		38.6	6.4	307.7	11.5	269.1	0.12	87.46	7.13	
2-Jun-08	323	227				1571		1344	0.59	85.55		30.3	6.7	542.5	87.3	512.2	0.23	94.41	13.04	
5-Jun-08	326	279				1900		1621	0.75	85.32		40.5	4.4	417.1	2.4	376.6	0.17	90.29	7.93	
13-Jun-08	334	236				1875		1639	0.76	87.41		40.8	2	407.6	3.7	366.8	0.17	89.99	7.83	
17-Jun-08	338											46.5	3.3	392.8	3.7	346.3	0.16	88.16		
20-Jun-08	341	299				2198		1899	0.88	86.40		44.8	3.7	395.7	7.7	350.9	0.16	88.68	6.39	
23-Jun-08	344	321				1969		1648	0.76	83.70		67	5.4	415.9	4.7	348.9	0.16	83.89	7.09	
24-Jun-08	345																			

26-Jun-08	347	310				2060		1750	0.81	84.95		59.8	4.7	418.3	5.4	358.5	0.17	85.70	6.96	
	347																			
30-Jun-08	351	335				2084		1749	0.81	83.93		50.5	4.4	415.7	8.4	365.2	0.17	87.85	7.01	
2-Jul-08	353																			
3-Jul-08	354	361				2196		1835	0.85	83.56		54.8	1.6	409.5	7.1	354.7	0.16	86.62	6.46	
	354																			
4-Jul-08	355																			

Table G-6 Continuous mode lactate and acetate concentrations

		Lactate				Acetate			
Date	Day	effluent	influent	feed	Lactate/TOC	effluent	influent	feed	Acetate/TOC
14-Jul-07	0	0		450					
15-Jul-07	1								
16-Jul-07	2								
17-Jul-07	3								
18-Jul-07	4								
19-Jul-07	5								
20-Jul-07	6								
21-Jul-07	7								
22-Jul-07	8								
25-Jul-07	11								
26-Jul-07	12								
27-Jul-07	13			90					
28-Jul-07	14								
29-Jul-07	15								
1-Aug-07	18			580					
2-Aug-07	19	0				95			
3-Aug-07	20								
5-Aug-07	22								
7-Aug-07	24								
9-Aug-07	26								
11-Aug-07	28								
12-Aug-07	29								
14-Aug-07	31								
16-Aug-07	33								
18-Aug-07	35								
20-Aug-07	37								
22-Aug-07	39								
24-Aug-07	41								

26-Aug-07	43										
28-Aug-07	45										
30-Aug-07	47										
7-Sep-07	55										
13-Sep-07	61										
20-Sep-07	68										
27-Sep-07	75										
6-Oct-07	84										
13-Oct-07	91			2430				125			
19-Oct-07	97	0	430		19.35	43					
27-Oct-07	105	0	160		5.33		42		1.41		
2-Nov-07	111										
7-Nov-07	116										
9-Nov-07	118										
16-Nov-07	125	0	250	820	6.27		52	68	1.31		20ml samples stored in two 10ml falcon tubes.
23-Nov-07	132										Noticeble color differences in solution,
30-Nov-07	139										indicating difference in concentration
8-Dec-07	147										
14-Dec-07	153	0	250	1380	6.84		44	94	1.21		20ml samples thoroughly mixed
24-Dec-07	163			1060				137			
30-Dec-07	169			1240				107			
5-Jan-08	175	0	200	680	9.88		45	84	2.24		
13-Jan-08	183										
16-Jan-08	186										
18-Jan-08	188										
22-Jan-08	192										
25-Jan-08	195										
2-Feb-08	202										
10-Feb-08	210										
14-Feb-08	214										
17-Feb-08	217										
20-Feb-08	220										

24-Feb-08	224	0	190		4.93		64		1.67		
28-Feb-08	228										
3-Mar-08	232										
7-Mar-08	236										
8-Mar-08	237										
10-Mar-08	239										
13-Mar-08	242										
16-Mar-08	245										Sulphate feed = 2600mg/L
20-Mar-08	249										
22-Mar-08	251										
24-Mar-08	253	0	250	1150	6.14		67		1.65		
28-Mar-08	257										
4-Apr-08	264										All eff samples taken from eff1 from today.
7-Apr-08	267										Sulphate feed = 2755mg/L, COD feed = 8992mg/L
11-Apr-08	271										
14-Apr-08	274	0	100		5.47		59		3.25		Sulphate feed = 3282mg/L, COD feed = 9774mg/L
19-Apr-08	279										eff1: taken from top of sulfide extraction column
28-Apr-08	288										values marked with (*)
3-May-08	293										
11-May-08	301	0									
17-May-08	307										*only samples for sulfate/sulfide test taken today
20-May-08	310										
23-May-08	313										
27-May-08	317										
29-May-08	319										
2-Jun-08	323										
5-Jun-08	326	0	730		18.22		44		1.10		
13-Jun-08	334										
17-Jun-08	338	0	700	1930	16.18		61	121	1.42		
20-Jun-08	341										sulfide test results w/o ZnAc
23-Jun-08	344										
24-Jun-08	345										only samples for sulfide test taken today

3-Jul-08	354	0	760	2160	16.75		56	112	1.24		
	354										sulfide test results w/o ZnAc
4-Jul-08	355										only S test with 2mL ZnAc performed today