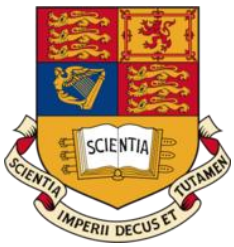

Imperial College London

Submerged Anaerobic Membrane Bioreactors: Fouling, Phage Removal and Flowsheet Models

A thesis submitted for the degree of Doctor of Philosophy

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It is declared that the work presented in this thesis is the candidates own, all else has been appropriately referenced.

Abstract

This thesis focuses on the Submerged Anaerobic Membrane Bioreactor (SAMBR). The aim of this work was threefold; firstly, to investigate the effect of certain system parameters on membrane fouling in the SAMBR; secondly, to monitor phage removal in the SAMBR; and, finally to assess the viability of anaerobic wastewater treatment processes (including the SAMBR) to treat domestic sewage (rather than sludge) for full scale operations in the UK. Using a Kubota flat sheet membrane with 0.4µm pores, the critical flux was found to be 11.8 $lm^{-2}h^{-1}$ (litres per meter squared per hour or LMH), similar to those found by other researchers. The existence of a critical gassing rate was investigated ('there exists a critical gassing rate which when reached causes a steep rise in transmembrane pressure (TMP)'), and was determined to be 4 litres per minute (LPM) or 2.4 $m^3m^{-2}h^{-1}$; more interestingly, this appeared to happen at the changeover between a slug flow regime and bubble flow. The viscosity of the biomass in the SAMBR was found to be 2.5 times greater than water with the colloid fraction having the largest impact on the overall viscosity. The build-up of foulants on the membrane was thought to be the cause of a 10 fold increase in molecular weight cut off that was observed after operation beyond the critical flux and gassing rate. In addition, after extensive fouling some removal of volatile fatty acids (VFAs) was observed from 3.35% acetate removal to 5.9% removal of isovalerate, and this was not likely to be due to degradation across the membrane, but was thought to be due to electrostatic repulsion by the biofilm.

The removal of bacteriophages by the SAMBR was used as a model for the removal of pathogenic viruses. Before critical operation (and the resulting jump in TMP), the smallest phage (MS-2) showed removals of between 1.8 - 2.1 log removal value (LRV), while the larger T4 phage showed removals from 5.1 - 5.3 log. Once critical operation had occurred, and the TMP increased, the T4 phage had a log removal greater than 7. The MS-2 phage, after operation beyond the critical parameters, showed a log removal dependence on the gas scouring rate. The LRV varied from 3.0 at a low gassing rate up to 5.5 at the highest gas scour, and this was thought to be due to concentration polarisation effects. The effect of activated carbon on phage removal was also investigated; while PAC had little effect, the addition of GAC to the SAMBR actually caused an increase in phage throughput. Finally, a range of potential flowsheets for anaerobic wastewater treatment were modelled. It can be concluded from this work that anaerobic treatment is a practical and promising alternative to conventional activated sludge plants. In addition, the SAMBR was found to be the most favourable anaerobic unit. However, it was noted that there is still a lack of full scale data for this unit, thus further emphasising the importance of research into this technology.

Acknowledgements

First of all I would like to thank my supervisor Professor David Stuckey for taking me on as a PhD student. I am grateful to David for his motivating enthusiasm for the subject of anaerobic wastewater treatment and also for our many and varied discussions both related to the subject and on non-academic matters.

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It is important for me to acknowledge the help and support of my family during my PhD. Their comments, ranging from “Eeww you’re working with sewage” to “S*urely*, you will be finished soon”, were invaluable to my self-esteem throughout this project.

Many thanks also to the various housemates, labmates and othermates who supplied the copious quantities of tea, cake and sympathetic ears required to complete this work. Finally to all the postgrads who advised me not to take on a PhD: Thanks for not saying “I told you so” during the difficult parts.

Notation

ABR	anaerobic baffle reactor
BMP	biochemical methane potential
CAPEX	capital expenditure
COD	chemical oxygen demand
CSTR	continuous stirred tank reactor
ECP	extracellular polymeric substances
EPS	extracellular polymeric substances
GAC	granular activated carbon
HRT	hydraulic retention time
LMH	litres per meter squared per hour
LPM	litres per minute
LRV	log removal value
MBR	membrane bioreactor
MWCO	molecular weight cut off
OPEX	operational expenditure
PAC	powdered activated carbon
PCTE	track-etched polycarbonate
PEFE	track-etched polyester
PTFE	polytetraflouroethylene
PFD	process flow diagram
RPM	rotations per minute
SAMBR	submerged anaerobic membrane bioreactor
SEC	size exclusion chromatography
SMP	soluble microbial products
SRT	solids retention time
STW	sewage treatment works

TMP	trans-membrane pressure
TSS	total suspended solids
UASB	upflow anaerobic sludge blanket
VFA	volatile fatty acids
VSS	volatile suspended solids
WWTP	wastewater treatment plant
μ_{max}	maximum growth rate (T^{-1})

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Chapter 1. Introduction

Wastewater treatment is important to maintain the environment in a condition such that it can be enjoyed by the general populace, and to meet emissions legislation. In recent times, stricter laws have been put in place demanding increasingly cleaner effluents. While wastewater treatment plants strive for cleaner effluents, it is also desirable to maximise process efficiency, and to reduce the cost and environmental impact of the plant. In addition, concerns about global warming have forced people to address the issues of carbon footprint (greenhouse gas emissions), energy use, and solids disposal in wastewater treatment plants (WWTP). In order to meet increasingly strict effluent legislation while reducing carbon emissions there is an increased pressure on developing new wastewater treatment technologies.

In general waste streams are treated biologically as this method is cheaper than physical or chemical treatments. Biological treatments can be split into two further types: aerobic and anaerobic. Aerobic treatment uses aerobic bacteria along with dissolved oxygen to digest the waste organics which are broken down to carbon dioxide, water and more biomass.

In anaerobic treatment no oxygen is involved and the waste is broken down into methane, water, and a small amount of biomass. Anaerobic treatment has, in the past, often been regarded as a slow and ineffective method due to the slower growth rate of anaerobic bacteria, especially for low strength wastewater (<500mg/l COD). However, if it were possible to retain all the biomass, whilst still removing the clean effluent at an acceptable rate, then the challenge of slow growth rate can be overcome. The slow growth rate of anaerobic biomass also has advantages over the faster growing aerobic biomass. The excess biomass produced in an aerobic reactor has to be disposed of which adds considerably to the cost of running a plant. An anaerobic reactor produces much less excess sludge volume (>90%) compared to aerobic digestion (Arros-Alileche *et al.*, 2008). In addition, anaerobic treatment does not require aeration energy and produces a source of energy (methane) in the off gas, thereby reducing the energy cost of operation.

There are a large number of technologies and reactor designs involving anaerobic waste treatment, from the established sludge digesters used in a conventional wastewater treatment plant (WWTP), to the more recently developed submerged anaerobic membrane bioreactors (SAMBR), (Hu and Stuckey 2006). Each reactor type has its own advantages and drawbacks; for example the anaerobic baffled reactor (ABR), which is a very simple anaerobic design, has the

advantage of potentially taking on the role of a primary settler as well as a secondary digester (Stuckey 2010). The drawback of this reactor is that, due to the flow dynamics of the design, the ABR requires a very large footprint. Similarly, there are many advantages and disadvantages for the upflow anaerobic sludge blanket (UASB), SAMBR and many other types of anaerobic treatment. In order to assess the suitability of all these different technologies, some sort of decision making model is required.

Membrane reactors are a relatively recent technology that allows the hydraulic retention time (HRT) to be controlled completely independently of the solids retention time (SRT). Cells are too large to pass through the membrane, so the SRT can theoretically be infinite. In the year 2000 there were over 500 aerobic membrane bioreactors (MBRs) used for wastewater treatment, with many more intended to be built (Stephenson *et al.*, 2000). MBRs are still a relatively new form of wastewater treatment; however, they have several advantages over conventional treatment. Since 2000 the global market for MBRs has grown at between 11.6 and 12.7% per annum (Santos *et al.*, 2011).

Figure 1-1 shows a conventional sewage treatment process, but in our proposed flowsheet the entire aerobic secondary treatment section would be replaced by a SAMBR unit. With this substitution the need for a sludge digester, drying bed and disposal would be greatly reduced because of the minimal excess sludge produced in anaerobic treatment.

The effluent from an MBR has passed through a membrane filter and hence contains no solids or bacteria, and few if any bacterial pathogens. Therefore, the effluent requires less post processing than a conventional treatment plant effluent. The footprint of a MBR treatment plant is also a lot smaller than that of a conventional plant, because the membrane unit can perform the same tasks as the aeration tank and settler. Most of the membrane units currently in industrial use are aerobic units, and hence have some of the same difficulties as conventional aerobic treatment; a large waste sludge production and large energy usage. The extra sludge produced by aerobic biomass must undergo further treatment and disposal (usually anaerobically), generating more waste.

Anaerobic treatment produces less excess sludge, and therefore the problem of sludge disposal is minimised. The separation of solids retention time (SRT) and hydraulic retention time (HRT) achieved by the membrane reactor means that an anaerobic membrane reactor can be used to reap the benefits of both membrane and anaerobic treatment technologies; creating a wastewater treatment method that produces minimal surplus waste, is energy

efficient and covers a small geographical footprint. This work will focus on submerged anaerobic membrane bioreactors (SAMBR).

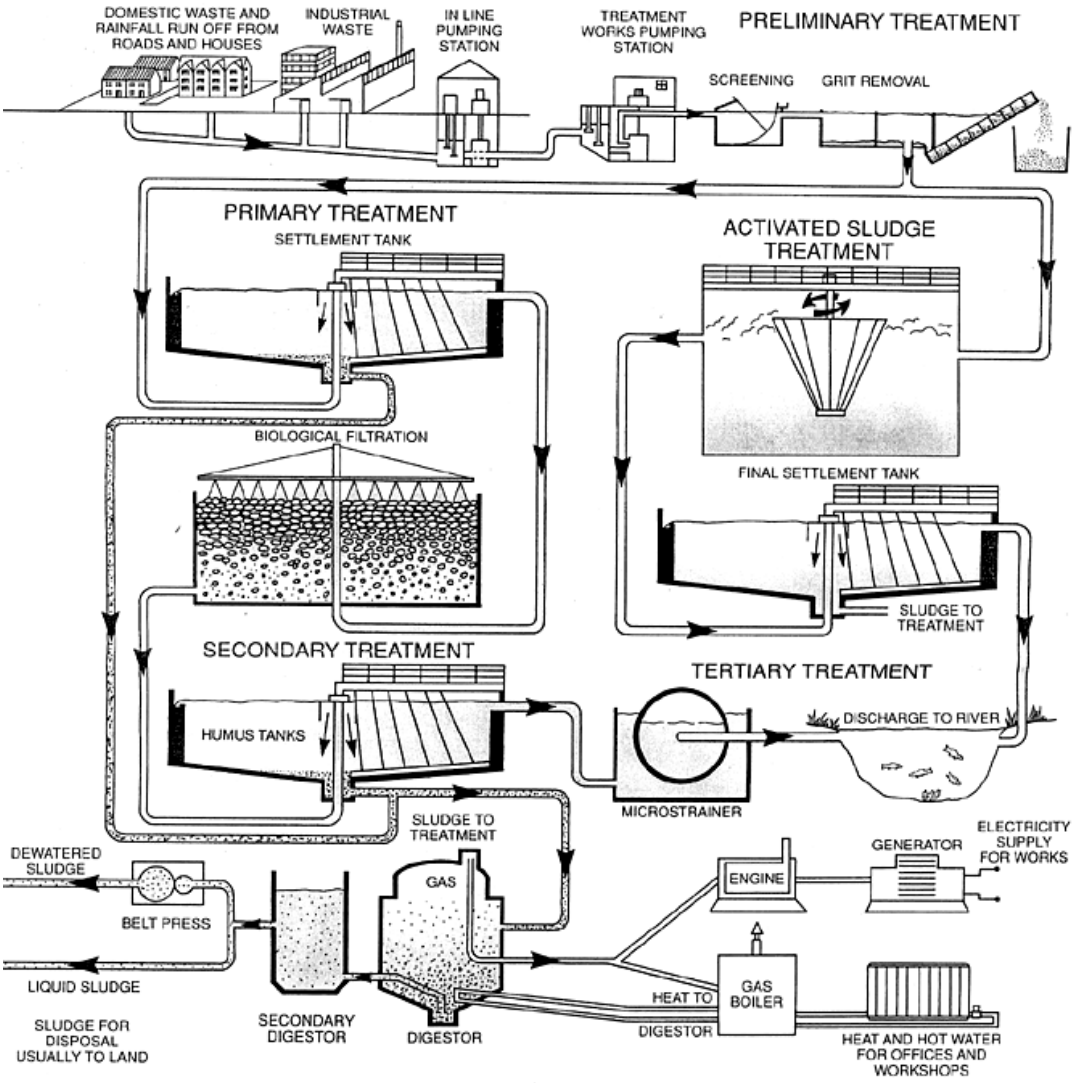


Figure 1-1 Conventional aerobic sewage treatment plant (Water UK, 2012)

One of the main concerns for MBRs in general is the problem of membrane fouling (Le-Clech *et al.*, 2006). As a biofilm builds up on the membrane surface, more energy (in the form of pumping power) has to be put in to achieve the same output (effluent flux). As can be seen in Figure 1-2, 'fouling' is the most common research keyword associated with MBRs, and this gives some indication of the importance of understanding and mitigating the problem of fouling in this field. In this work the main fouling mitigation method considered is gas scouring, where gas bubbles are forced across the surface of the membrane.

While membrane fouling is commonly considered to be a detrimental aspect of MBR technology, it must be pointed out that the biofilm layer can also be beneficial. The fouling layer through pore blocking and restriction can achieve a cleaner effluent due to screening out further fine particulates Vyrides and Stuckey (2011).So while it is important to control the extent of fouling on the membrane, it not always desirable to remove fouling completely.

Figure 1-2 Word cloud representing research keywords associated with MBR papers (Santos *et al.*, 2011)

The epidemiological aspect of waste water treatment should also be considered alongside the environmental and pollution control. As well as discharging an effluent with a low organic content, it is important to ensure the effluent is as pathogen free as possible. One big advantage of the membrane reactors is that the pore size of the membrane is such that the membrane completely retains all bacteria, and therefore the SAMBRs will completely retain any bacterial pathogen. With viral pathogens, however, more careful study is required. The membrane pore sizes used in MBR technology are usually in the region of 200-400nm, while viruses vary in size from 20-200nm, and therefore there is considerable potential for a viral pathogen to enter the effluent stream.

It has been shown that most units in a wastewater treatment plant show a degree of virus removal (Leong, 1983). The standard of virus removal in conventional WWTP units, however, is not usually rigorous enough, and further tertiary treatment is required for an effluent to meet epidemiological standards. There are a variety of options available for tertiary pathogen removal, and most of these involve dosing the effluent with chemicals such as chlorine. While chemical dosing is an effective method of pathogen removal, in the process of inactivating viruses they can produce disinfection by-products (DBPs) which have been the cause for some health concerns.

Frequently bacteriophages are used as a model for virus behaviour due to the hazards involved with using pathogenic viruses. There have been a number of promising studies demonstrating phage removal in aerobic MBRs (Chiemchaisri *et al.*, 1992; Lv *et al.*, 2006; Shang *et al.*, 2005; Ueda and Horan, 2000). However, there is very little information available on the removal performance of viruses in submerged anaerobic membrane bioreactors, and none regarding the treatment of domestic wastewater using these reactors.

The aim of this project was threefold. Firstly, focussing on the SAMBR reactors; the role of fouling on the membrane was investigated. Particular attention was paid to the critical parameters that cause fouling build-up such as flux, gas scour and viscosity. The effect that fouling had on small particle screening was also considered.

Secondly, the role of the SAMBR in virus removal was investigated. Large and small bacteriophages were used as viral indicators for this project. Since the gas scour rate is often the simplest method used to control fouling on the membrane surface, the effect of gas scour rate on the removal of phages was also investigated. The effect of extended operation especially beyond the critical values determined in the previous section was also analysed.

Finally, the role of anaerobic technology in domestic wastewater treatment was considered. The aim of this section was to come up with a recommended treatment flowsheet, involving anaerobic unit(s), and to compare and contrast this new flowsheet with a conventional wastewater treatment process using aerobic activated sludge.

Chapter 2. Literature Review

This chapter presents an overview of the field, and reviews the current knowledge available in the literature. It begins with the fundamentals of anaerobic digestion and membrane bioreactors upon which this work is based. The rest of the review focuses on aspects of membrane fouling with respect to the operation of submerged anaerobic membrane bioreactors (SAMBR), and the mechanisms of viral and phage removal in wastewater treatment plants in general, with a focus on MBRs.

2.1 Anaerobic digestion

Anaerobic digestion involves the conversion of organic molecules into carbon dioxide and methane without the presence of oxygen. The process of anaerobic digestion, carried out by bacteria and archaea (methanogens), is multifaceted and complex. Figure 2-1 shows a simplified version of how large molecules are broken down to methane in a process called 'series metabolism' whereby the slowest step controls the rate of the process (Speece, 1996). The first step involves the hydrolysis of complex compounds into simpler organic compounds such as sugars, amino acids and peptides. The process of breaking down volatile acids and other molecules continues until acetate, carbon dioxide and hydrogen are produced. Once the compounds have been broken down into these intermediates, the acetate, carbon dioxide and hydrogen can be converted into methane via the process of methanogenesis. Approximately 2/3 of the methane produced in anaerobic digestion is from converted acetate, while the rest is from the conversion of CO_2 and H_2 (Speece, 1996). The main bacterial groups involved in series metabolism are discussed below. It is important to understand the microbiology of anaerobic digestion, because this enables us to be able to increase process efficiency.

2.1.1 Hydrolytic Bacteria

Hydrolysis is the first step involved in the breakdown of organic matter. Before bacteria can begin the oxidation reactions the particles or large polymers must be hydrolysed to smaller molecules that can be transported across the cell membrane (Rittmann and McCarty, 2001). During this step large macromolecules such as proteins, carbohydrates, and lipids are broken down into smaller monomers such as amino acids, alcohols, fatty acids and sugars. Hydrolysis is usually carried out by enzymes like cellulase, lipase and protease produced by the biomass. The rate of hydrolysis depends on many factors, the most important are pH, cell age (determined by the SRT), and the organic content of the wastewater. At low temperatures the conversion rate of some molecules is significantly reduced. Some examples of hydrolytic

bacteria include: *Bacillus, Clostridium, Micrococcus* and *Staphylococcus*- these bacteria are commonly facultative, meaning that they can hydrolyse compounds in the presence or absence of oxygen (Ljungdahl *et al.*, 2003; Zehnder, 1988).

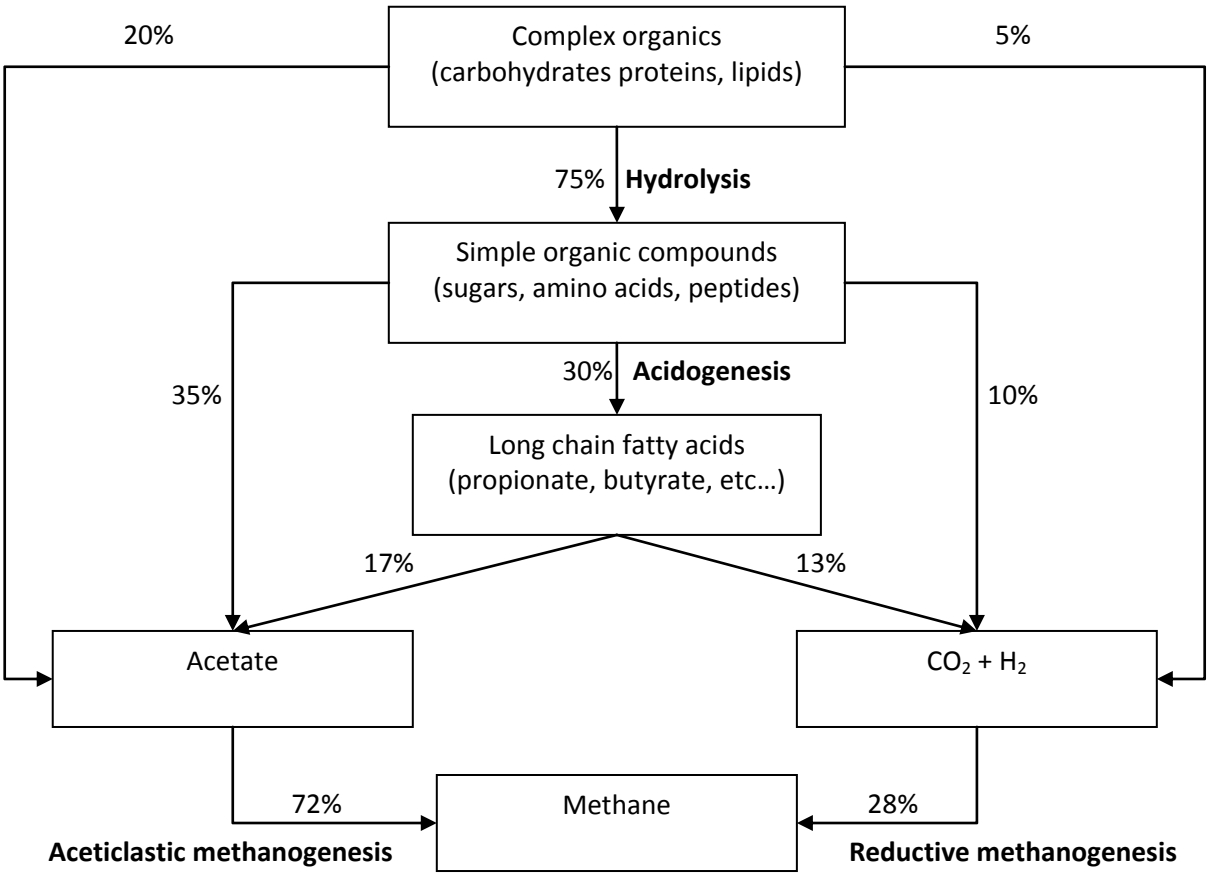


Figure 2-1 Simplified summary of anaerobic digestion (Freese and Stuckey, 2004; McCarty and Smith, 1986)

2.1.2 Acidogenic bacteria

Acidogenic or fermentative bacteria further hydrolyse the amino acids, fatty acids and sugars from the hydrolysis step into intermediary products, acetate and hydrogen. These bacteria have a minimum doubling time of around 30 minutes, and hence are fast growing. As an example glucose is utilised by the acidogenic bacteria as follows (Mosey, 1983);

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COO^- + 2CO_2 + 2H^+ + 4H_2 \qquad \Delta G^0 = +9.6 \text{ kJ mol}^{-1} \qquad \mathbf{1}$$

$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COO^- + 2H_2O + 2H^+$ ΔG^0=+30.6 kJ mol^{-1} **2**

The low positive Gibbs free energy of reaction 1 means that it is the preferred reaction pathway despite being positive; this reaction produces acetate and hydrogen which are the main substrates for methanogens, the partial pressure of the hydrogen needs to be less than 10^{-4} atm for reaction 1 to work.

Since both reactions 1 and 2 have a positive Gibbs free energy they appear to be unfavourable, and the only way the reactions can happen is due to the syntrophic nature of anaerobic digestion. The acidogenic reactions are coupled with methanogenesis (see reaction 4) which rapidly consumes the hydrogen produced in reaction 1, giving a negative overall Gibbs energy. Because the overall Gibbs free energy is negative the reactions can happen.

2.1.3 Acetogenic bacteria

Acetogenic bacteria are a subset of the acidogenic bacteria, and these oxidise volatile fatty acids (VFAs) and alcohols to acetate, CO_2 and H_2. Interestingly, while acetogens have been termed as obligates or strict anaerobes, Karnholz *et al.* (2002) demonstrated their ability to tolerate and consume small amounts of O_2. Some examples of acetogenic bacteria include *Syntrophobacter wolinii* and *Syntrophonomonas wolfei* (Janssen *et al.*, 2009). These bacteria can be further divided into two groups; hydrogen producing bacteria, and hydrogen consuming bacteria (Zehnder, 1988).

Hydrogen producing bacteria

The reaction mechanism of the hydrogen producing bacteria has a large positive Gibbs free energy of reaction and is thermodynamically unfavourable under standard conditions; see the example below from Nollet *et al.* (1996). The hydrogen producing reactions will stop if the concentration of dissolved hydrogen becomes too high. Under ideal conditions the growth rate of these bacteria is still relatively slow with a doubling time in the region of 1.5 - 4 days (Mosey and Hughes, 1975).

$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + 2H_2 + H^+$ ΔG^0=+48.1 kJ mol^{-1} **3**

Hydrogen consuming bacteria

Hydrogen consuming acetogenic bacteria reduce the hydrogen concentration and hence support the hydrogen producing bacteria. They convert dissolved carbonates to acetates with

the addition of hydrogen. These bacteria have a comparatively faster growth rate with a minimum doubling time of around 10 hours.

$$4H_2 + 2HCO_3^- + H^+ \longrightarrow CH_3COO^- + 4H_2O \qquad \Delta G^{0'} = -104.6 \text{ kJ mol}^{-1} \qquad \mathbf{4}$$

2.1.4 Methanogens

Methanogens are strictly Archaea not bacteria, and are involved in the final step of anaerobic digestion which is the conversion of acetate and hydrogen into methane and carbon dioxide. These microorganisms can also be split into two groups; the first group, lithotrophic methanogens, convert CO_2 (in its dissolved bicarbonate form) and H_2 to CH_4 in reductive methanogenesis. These compete with the hydrogen consuming acetogens for HCO_3^- and H^+. The second group, acetotrophic methanogens, convert acetate to methane and bicarbonate in acetoclastic methanogenesis. Examples of lithotrophic and acetotrophic methanogens are *Methanobacterium* and *Methanospirillum,* respectively. About 70% of the methane produced in anaerobic digestion occurs through acetoclastic methanogenesis; since these bacteria have a minimum doubling time of 2-3 days, methanogenesis is usually the rate limiting step in anaerobic digestion (Speece, 1996). Therefore, it is important to ensure that conditions are favourable for methanogenesis; methanogens work best at neutral pH conditions, between 6.5 and 8.2 (Speece, 1996). It is also important to ensure that nutrients are in excess for the biomass so that the degradation rate is not limited by this factor (Owen *et al.*, 1979).

2.1.5 Role of Hydrogen

Equations 1 to 4 also demonstrate the importance of hydrogen the anaerobic digestion process. If the pH of the biomass drops (due to an increase in H+ concentration), the equilibria of the reactions will shift, for example in the acidogenic step an increase in H^+ will lead to a tendency for propionate production over acetate production (see equations 1 and 2). While this reduces the capacity for methane production, it does allow the potential for H_2 production; however since the H^+ to H_2 reaction requires a large amount of electron transfer it is energetically unfavourable.

2.1.6 Monod kinetics

It is desirable to model the growth of bacteria in the anaerobic process so that the effect of different variables can be predicted. Due to the complex nature of anaerobic digestion, the derivation of an exact model is difficult. The simplest model which most others are based on is

the Monod equation (Monod, 1949). The Monod equations shown in 2-1 and 2-2 relate the growth rate of the bacteria to the substrate concentration; and while the equations are empirically derived, they are applicable to a wide range of microbial systems.

$$\mu = \frac{\mu_m \cdot S}{(K_s + S)} \qquad \textbf{2-1}$$

$$\frac{dX}{dt} = \frac{\mu_m \cdot S}{(K_s + S)} \cdot X \qquad \textbf{2-2}$$

Where: μ = specific biomass growth rate (g VSS produced g VSS present^{-1} d^{-1})

μ_m = max specific biomass growth rate (g VSS produced g VSS present^{-1} d^{-1})

S = substrate concentration (g l^{-1})

K_s = half saturation concentration (g l^{-1})

X = biomass concentration (g l^{-1})

An extra term 'b' is usually included in the equation to account for cell decay so the adjusted Monod equation becomes as 2-3.

$$\frac{dX}{dt} = \frac{\mu_m \cdot S}{(K_s + S)} \cdot X - b \qquad \textbf{2-3}$$

Another important factor to model in anaerobic treatment is the biomass yield, and this can be expressed as in 2-4.

$$Y_{x/s} = \frac{\mathrm{dX/dt}}{\mathrm{dS/dt}} \qquad \textbf{2-4}$$

Table 2-1: Typical Monod kinetic constants for the digestion of some common substrates (Pavlostathis and Giraldo-Gomez, 1991)

Substrate	Process	K (gCOD $gVSS^{-1}$ d^{-1})	K_s (mg COD l^{-1})	μ_{max} (d^{-1})	Yield (gVSS $gCOD^{-1}$)	b (d^{-1})
Carbohydrates	Acidogenesis	1.33-70.6	22.5-630	7.2-30	0.14-0.17	6.1
Long-chain fatty acids	Anaerobic oxidation	0.77-6.77	105-3180	0.085-0.55	0.04-0.11	0.01-0.015
Short-chain fatty acids*	Anaerobic oxidation	6.2-17.1	12-500	0.13-1.20	0.025-0.047	0.01-0.027
Acetate	Aceticlastic Methanogenesis	2.6-11.6	11-421	0.08-0.7	0.01-0.054	0.04-0.037
H_2/CO_2	Methanogenesis	1.92-90	$4.8x10^{-5}$-0.6	0.05-4.07	0.017-0.045	0.088

*: except acetate

The yield gives an indication of the volume of excess sludge which needs to be disposed of, and is one of the most significant costs involved in anaerobic treatment. Also a low yield means that most of the carbon entering the reactor is being turned into methane and carbon dioxide. The values of each parameter vary widely depending on the conditions such as temperature, pH, feed type etc. Some typical values that might be expected for mesophilic operation are shown in Table 2-1.

2.2 Anaerobic reactor types

The use of anaerobic technology for the wastewater industry has given rise to a number of different reactor types each with their own benefits and draw backs. Recent developments in research on the upflow anaerobic sludge blanket (UASB), anaerobic baffled reactor, (ABR), and submerged anaerobic membrane bioreactor (SAMBR) are presented in this section.

2.2.1 Anaerobic Baffled Reactors (ABR)

The anaerobic baffled reactor (ABR) was developed by McCarty and co-workers as a simpler alternative to the anaerobic rotating biological contactor (McCarty, 1981). The ABR has been shown to offer significant advantages over more conventional units as summarised in Table 2-2. The ABR reactor is designed to direct the wastewater to flow over and under a series of baffles, through several compartments, as it passes from the inlet to the outlet. A sludge blanket settles in the bottom of each compartment which degrades the organics as they pass through, as shown in Figure 2-2 (McCarty and Bachman, 1992). The methane produced is collected in the headspace of each compartment. In recent years there have been over 700 small scale installations of ABRs in SE asia, in addition there are also working examples of large scale ABRs for example there are four ABRs in Thailand 7000m^3d^{-1} treating starch waste water and pig slurry (Stuckey, 2010).

Perhaps the most significant study of those summarised is that by Orozco (1997); this study demonstrates the successful operation of an ABR treating domestic sewage at 15°C. A brief summary of the design data for this plant is shown in Table 2-3. Although a detailed economic study was not presented, construction costs for the baffled reactor were said to be 20% less than those for UASB reactors in Columbia running at ambient temperature, and five times less than a conventional activated sludge plant for a small town.

Table 2-2 Advantages and disadvantages associated with the anaerobic baffled reactor (Barber and Stuckey, 1999)

Advantages		
Construction	**Biomass**	**Operation**
1 Simple design	1 No requirement for biomass with unusual settling properties	1 Low HRT
2 No moving parts	2 Low sludge generation	2 Intermittent operation possible
3 No mechanical mixing	3 High solids retention times	3 Extremely stable to hydraulic shock loads
4 High void volume	4 Retention of biomass without fixed media or a solid-settling chamber	4 Protection from toxic materials in influent
5 Reduced clogging	5 No special gas or sludge separation required	5 Long operation times without sludge wasting
6 Reduced sludge bed expansion		6 High stability to organic shocks
8 Low operating costs		
Disadvantages		
1 High capital costs of a shallow tank		1 Hard to maintain good distribution of influent
		2 Hard to maintain high liquid up-flow velocity
		3 Low number of units installed worldwide

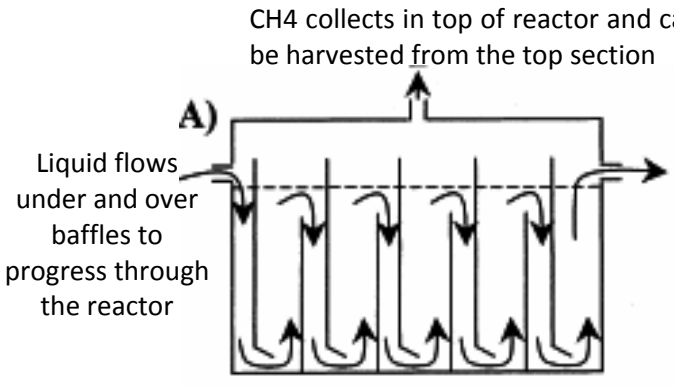


Figure 2-2 Standard ABR design (Barber and Stuckey, 1999)

Table 2-3 Design data for ABR designed in Tenjo, Colombia (Orozco, 1997).

1) Temperature	15°C
2) Number of reactors	2
3) Reactor Volume m^3	394 m^3 (197 m^3 each)
4) Reactor dimensions (height:length:width) (m)	2.7: 17: 4.3
5) Number of compartments	8
6) Liquid upflow velocity	3.00 m/h
7) Packing material	Plastic boxes
8) HRT	10.3

Table 2-4 Summary of ABR studies on low strength wastewater (COD < 1000 mg/l), adapted from (Barber and Stuckey, 1999)

Substrate	Volume (l)	Chambers	Inlet COD (mg/l)	COD removal (%)	HRT (h)	Temp (°C)	Reference
Synthetic greywater	8	6	480	63-58	48-84	25-33	(Witthauer and Stuckey, 1982)
Sucrose	75	11	344-500	85-93	6-12	13-16	(Orozco, 1988)
Municipal wastewater	350	3	264-906	90	4.8-15	18-28	(Garuti *et al.*, 1992)
Slaughterhouse wastewater	5.16	4	450-550	75-90	2.5-26	25-30	(Polprasert *et al.*, 1992)
Domestic sewage/ industrial waste	394000	8	315	70	10.3	15	(Orozco, 1997)
Soluble and colloidal wastewater	10	8	500	80	6	35	(Langenhoff and Stuckey, 2000)
Dilute milk	10	8	500	60	10	10	(Langenhoff *et al.*, 2000)

A novel feature of the ABR is that the first compartment in the reactor can be used as a primary settler, to collect settleable solids as well as anaerobically digesting the organics. In a collaborative project between WS Atkins and Imperial College, supported by 6 water companies, a working ABR that also functioned as a primary settler was developed. The ABR in this case was retrofitted into an existing primary tank at the wastewater treatment works in Ellesmere Port. One of the expected benefits of this project was a reduced sludge production, and the authors reported a 40% decrease

in sludge production. Specific gas production tests were also carried out on the sludge from each compartment; other than for the first compartment, each sludge sample showed a specific gas production between 0.76-0.79 m^3CH_4/kg VSS $_{destroyed}$ which was similar to digested sludge (0.77 m^3CH_4/kg VSS $_{destroyed}$). The initial compartment had a slightly higher specific gas production of 0.94 m^3CH_4/kg VSS $_{destroyed}$ (Clark *et al.*, 2000). Table 2-4 shows a summary of reported studies on ABRs treating low strength wastewater. It can be seen that the COD removals range from 60 to 90%, while the operating temperatures show feasibility has been demonstrated at temperatures as low as 10°C.

2.2.2 Upflow anaerobic sludge blanket (UASB) reactor

The upflow anaerobic sludge blanket (UASB) reactor is the most common type of commercial anaerobic reactor, in 2000 there were over 500 installations worldwide (Tchobanoglous *et al.*, 2003). The UASB reactor was developed in the late 1970s by Lettinga *et al.* (2001), and the principle of the reactor is to pass the waste stream in an upflow mode through a settling sludge blanket. While many of the current reactors have been installed in tropical climates, there is evidence showing that UASB reactors are and can provide satisfactory COD removals at lower temperatures (Lester *et al.*, 2009; Lettinga *et al.*, 1983; Uemura and Harada, 2000).

Uemura and Harada (2000) successfully operated a UASB reactor treating domestic sewage at 13°C. The authors consistently achieved soluble COD removals above 80%, for wastewater with an influent COD between 300-500 mg/l. Lettinga *et al.* (1983) also investigated the operation of a UASB reactor on raw domestic waste (COD 330-1200 mg/l) at temperatures between 12°C and 26°C. The authors achieved COD removals that varied from 70-78% with temperature, and the suspended solids removal also varied with temperature from 60-80%. A further summary of the literature on low temperature treatment of sewage by UASB reactors is shown in Table 2-5.

Table 2-5 Sewage treatment by UASB reactors at low temperatures (adapted from Lester et al, 2009)

Influent	COD (g/l)	OLR ($kg_{COD}/m^3.d$)	Temp (°C)	HRT (h)	COD_{rem} (%)	Reference
Crude	0.35-0.60	1.37-2.34	6-32	6.0	60-87	(Singh and Viraraghavan, 2003)
Crude	0.30	1.03	7-30	7.0	76	(Agrawal *et al.*, 1997)
Crude	0.20-1.30	5.00	10-28	3.0-24.0	48-82	(Lew *et al.*, 2004)
Crude	0.30	0.60	8-20	12.0	67	(Lettinga *et al.*, 1980)
Synthetic	0.30	0.96	10	12.5	83	(Gomec *et al.*, 2004)
Crude and pre-settled	0.34-0.46		13	8.0	59-65	(Elmitwalli *et al.*, 1999)
Crude	0.15-0.60	0.80-3.10	13-25	4.7	64-70	(Uemura and Harada, 2000)
Crude	0.27-0.85	0.92-2.91	15-25	7.0	66-88	(Lester *et al.*, 2009)

2.2.3 Submerged Anaerobic Membrane Bioreactors (SAMBRs)

The idea of using a membrane with the conventional aerobic wastewater treatment system has been around for many years, and these would become known as membrane bioreactors (MBRs) (Judd, 2006). The membrane works as an alternative to sedimentation, acting as a physical barrier to separate bio-solids from the clean effluent. In recent years this technology has been extended to anaerobic use as well, and these have become known as submerged anaerobic membrane bioreactor, termed SAMBRs (Hu and Stuckey 2006). The technology can also be referred to the anaerobic immersed membrane bioreactor (AniMBR) or anaerobic membrane bioreactor (AnMBR), however, in this work the SAMBR acronym will be used.

This work focuses on the submerged membrane configuration of anaerobic bioreactors, where the membrane is submerged inside the body of the reactor. The differences between submerged and sidestream (where the membrane is situated in a separate unit) reactors are discussed in section 2.3.1. In a submerged MBR, the membrane sits in a biomass tank, a transmembrane pressure is created; either by the hydrostatic head alone, or by a combination of this with suction applied on the permeate side, to draw the effluent through while the biomass is retained with the reactor. Gas is bubbled across the membrane surface to prevent the build-up of foulants and solid matter.

Liao *et. al.* (2006) produced a comprehensive review on the application of anaerobic membrane reactors. Since this paper there have been several pertinent papers published on the successful operation of SAMBR reactors to treat various types of wastewater, and these are summarised in Table 2-6. It can be seen from this table that many of the preconceptions about anaerobic technology are misconceived.

It is often claimed that anaerobic treatment cannot cope with low retention times; however, the study by Hu and Stuckey (2006) showed that high COD removals (above 90%) were achievable in a SAMBR with a three hour hydraulic retention time (HRT). Further to this there are several studies that demonstrate COD removals above 90% with retention times below 6 hours (Aquino *et al.*, 2006; Christian *et al.*, 2011; Herrera-Robledo *et al.*, 2011). In the past it has also been assumed that anaerobic treatment is ineffective for the degradation of low strength (typically COD<500mg/l) wastewaters. Table 2-6, however, shows several examples of studies where low strength wastewater has been treated with a 90% or greater COD removal (Aquino *et al.*, 2006; Herrera-Robledo *et al.*, 2011; Hu and Stuckey, 2006; Huang *et al.*, 2008; Martinez-Sosa *et al.*, 2011). The paper by Hu and Stuckey (2006) is a key study in this area as they have shown that the operation of a SAMBR is possible with both low HRTs, and low feed strength (460 mg COD/l), while still achieving above 90% COD removal.

The treatment of low strength wastewater is particularly significant because it involves domestic wastewater which is usually classed as low strength, and therefore if the SAMBRs are to be used to replace existing WWTPs it is important to show their capabilities to treat low strength wastewater. In fact, Herrera-Robledo *et al.* (2011) and Martinez-Sosa *et. al.* (2011) have both demonstrated successful operation of a SAMBR treating domestic wastewater; however, in both cases they were still only lab scale reactors with a volume less than $1m^3$.

While the SAMBR has been demonstrated to have many advantages it is not without its problems. The benefits of using a membrane to retain all the bacteria within the membrane means that any contaminants that don't pass through the pores will also be retained. To alleviate this regular sludge wastage must be carried out to avoid contaminant buildup.

The membranes in the SAMBR are susceptible to fouling, for example dissolved metals in the wastewater feed can precipitate on the membrane. This type of irreversible fouling cannot be undone and requires the purchase on new membranes, which can be a significant drawback of this technology (Judd, 2006). There are many different types of fouling, and methods for mitigation, this will be discussed further in section 2.6.

2.2.3.1 Existing commercial anaerobic membrane systems

From Table 2-6 it can be seen that anaerobic membrane technology is a viable treatment option for a variety of waste feeds, with COD removals frequently in excess of 90%. In spite of this, however, there have been very few studies on SAMBR operation beyond lab scale. There are three major companies which supply most of the membrane modules used in MBR applications across the world, Kubota, Mitsubishi Rayon and GE Zenon. While these companies have previously focussed on the development of aerobic MBRs, they have started installing their membranes in anaerobic systems too. By the end of 2006 there were more than a dozen such systems in Japan alone with loadings ranging from 0.2 to 60 tonnes/day, the largest being for the stillage concentrate stream from *shochu* production (the aqueous by-product from the distillation of ethanol following fermentation of carbohydrates) (Judd, 2006).

Outside of Japan there have more recently been SAMBR installations provided by the Canadian company ADI Systems. The installation at Ken's Foods in Marlborough MA was commissioned in July 2008, for the treatment of waste from salad dressing and BBQ sauce manufacture. The project was a retrofit of an existing ADI proprietary sequencing batch reactor. In this case the lack of available space was a key factor in the choice of an SAMBR over other units. This installation at Ken's Foods was possibly the largest of its kind, treating an average of 325 m^3/day of high strength wastewater. The plant was successfully operated and in its first year achieved COD, BOD and TSS removals in

Table 2-6 Recent studies on anaerobic membrane bioreactors

Feed	Submerged or crossflow membrane	Feed strength COD mg/l	Volume (l)	COD removal	T °C	Pore size µm or MWCO	HRT (h)	VSS g/l	Reference
Food waste	Crossflow	2000-15000	400	81-94%	37	20000-70000	60+	-	(He *et al.*, 2005)
Meat extract/peptone	Submerged	450	3	95%	mesophilic	0.4	6	2.6-3.7	(Aquino *et al.*, 2006)
Synthetic wastewater (peptone based)	Submerged	460	3	>90%	mesophilic	0.4	3	3	(Hu and Stuckey, 2006)
Synthetic wastewater (vfa based)	Submerged	5000	3.7	-	30 and 55	0.2	30	17-35	(Jeison and van Lier, 2006)
Sucrose/meat extract	Submerged	4000	3	90%	mesophilic	0.4	80-20	5	(Akram, 2006)
Synthetic wastewater (glucose based)	Submerged	550	6	99%	25-30	0.45	30d-60d	5.6	(Huang *et al.*, 2008)
Food waste	Submerged	39000	103m^3	99%	30-35	0.4	5.2	23	(Christian *et al.*, 2011)
Methanol based	Submerged	10000	6.5	97-99%	37	70000	12d		(Lin *et al.*, 2009)
Organic fraction municipal solid waste	Submerged	-	3	95%	35	0.4	38-55	2.5	(Trzcinski and Stuckey, 2009)
Shochu distillery waste	Submerged	101000	pilot scale	73-92%	thermophilic	0.4	-	5.9	(Kanai *et al.*, 2010)
Municipal wastewater + glucose	Submerged	630	350	90%	35-20	-	-	10	(Martinez-Sosa *et al.*, 2011)
Domestic sewage	Crossflow	450	849	93%	22	100000	6	-	(Herrera-Robledo *et al.*, 2011)
Synthetic municipal wastewater	Crossflow	500	-	90%	25	10	18	7-8	(Ho and Sung, 2007)
Domestic wastewater	Membrane coupled EGSB	500	4.7	76-81%	11	0.1	5.7	15	(Chu *et al.*, 2005)

excess of 99.5%. The high strength feed had influent BOD and TSS concentrations of 18000 and 11500 mg/l, respectively, and in spite of the high solids concentration there was no foulant build up. In the first 18 months of operation there was no increase in TMP, and hence there was no need for an intensive membrane clean (Christian *et al.*, 2011; Judd, 2006).

The SAMBR at Ken's food was operated in the mesophilic temperature range, this involved significant heating cost since the influent wastewater had an average influent temperature of 14°C which is in the psychrophilic region (Christian *et al.*, 2011). While there are no current commercial SAMBRs operating in this psychrophilic temperature range, there is good reason to believe that such a unit would be successful. The final three studies in Table 2-6 show that at a lab scale high COD removals (in the region of 90%) is possible at temperatures below 25°C.

2.3 Reactor setup

2.3.1 Reactor Configurations

There are two main configurations for the design of MBRs; sidestream and submerged. In the sidestream (also called crossflow) configuration the membrane is contained in a separate unit to the bioreactor, and the reactor liquor is fed to the membrane unit via a recirculation pump. This pump also provides a crossflow velocity (CFV) past the membrane which is the major method of fouling reduction control for this setup. Further designs can compromise between the two configurations such as situating the membrane module in a separate tank so that it can be easily removed, while the biomass is gently circulated by another pump.

The sidestream configuration has in the past been favoured over the submerged setup, since membrane removal and cleaning can be achieved without interfering with the reactor (Huang *et al.*, 2008). In addition, the circulating pump applies a relatively high crossflow velocity (2-4ms^{-1}) which can scour the surface of the membrane, reducing fouling, and this scour means that a higher flux (in the region of 60 LMH) can be achieved (Stuckey, 2012).

However, in spite of the benefits of sidestream reactors there are some downfalls to this design. The pumps used to circulate the reactor liquor in the sidestream configuration have also been shown to shear the bioflocs, and this results in decreased reactor performance. Brockmann and Seyfield (1996) demonstrated a loss of activity between 50% and 90% when using an anaerobic sidestream reactor at various recirculation rates. In addition to this Kim *et al.* (2001) demonstrated, for an aerobic MBR, that the particular type of pump used for the crossflow stream can have an impact on the amount of shear experienced by the bioflocs. Padmasiri *et al.* (2007) also studied the effect of high shear on methanogens in a SAMBR treating swine manure; they found that the levels of methanogens in the reactor, monitored

by terminal restriction fragment length polymorphism, were not affected by the crossflow velocity. In spite of this the reactor performance decreased; this was thought to be due to an increase in fermentation products through faster hydrolysis which caused acidification of the reactor.

In addition to the problems caused by the increase in shear experienced in the crossflow configuration, the increased cost of the extra pumping requirements for the sidestream configuration suggests that a submerged membrane reactor may be the most favourable option (Le-Clech *et al.*, 2005).

The submerged configuration involves having the membrane unit submerged directly inside the main bioreactor unit. This means that there is no pumping requirement to pass the reactor liquor to the membrane, therefore the hydraulic shear on the biomass is much lower and the energy costs for the system are lower. To reduce the fouling on the membrane, bubbling is often applied to the membrane surface, which has the additional effect of ensuring the reactor is fully mixed, although this also requires significant amounts of energy.

Le-Clech *et al.* (2005) carried out a comparison between submerged and sidestream membrane bioreactors. The authors concluded that the submerged configuration showed less propensity to foul due to the gas flow over the surface, and consistently lower levels of soluble microbial product (SMP) were also found in the submerged reactor compared to the side stream which may explain the lower fouling. While this work was performed on an aerobic MBR it would seem logical that a similar principal would apply to an anaerobic system.

2.3.2 Membrane Types

There are two main types of membrane that can be used in MBRs; flat sheet and hollow fibre. Flat sheet membranes are the simplest as these are simply a supported sheet of the membrane material, while hollow fibre membranes are membranes with a small (<1mm) diameter. Each type of membrane configuration presents its own challenges; for example, with hollow fibres a high fibre density results in greater fouling since gassing does not penetrate to the central fibres (Sridang *et al.*, 2005). In addition, the conditions for an individual fibre in a bundle can vary a lot depending on its location within the bundle; therefore, analysis of a single fibre may give an inaccurate representation of the whole tube bundle. Hu (2006) compared a hollow fibre and flat sheet SAMBR, and both configurations had an acceptable COD removal rate. However, for the desired HRT the flat sheet membrane required a lower transmembrane pressure (TMP) and was therefore slightly preferable due to the lower energy requirements.

2.4 Operational parameters

There are a number of key parameters that must be set when operating a SAMBR, some of which can be controlled externally, while others are set by the biological/chemical system within the reactor. Some common variables are discussed below.

2.4.1 pH

Keeping the pH stable during operation is key to the successful running of a SAMBR. Anaerobic bacteria have an optimum operational pH at around neutral, in a range between 6.5 and 8.2. If the pH falls below 5 or above 8.5 then biomass growth will be severely inhibited (Speece, 1996). The presence of VFAs produced by the acidogenic bacteria can cause the pH to drop since they grow faster than the methanogenic bacteria that remove them. If this occurs it is necessary to buffer the reactor to avoid the pH falling too low. The most common method of buffering the anaerobic reactor is the addition of bicarbonate (HCO_3^-) usually in salt form; this increases the alkalinity (buffering capacity) of the system, and the equilibrium reaction is shown below.

$$HCO_3^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2 \qquad 2\text{-}5$$

Equation 2-5 demonstrates that the end result of this buffering reaction, when neutralizing with acid, is the production of CO_2. The CO_2 produced in this reaction enters the gas phase and therefore will dilute the concentration of methane in the gas stream, as such the over addition of HCO_3^- is undesirable.

2.4.2 Temperature

There are three temperature regions within which different groups of anaerobic bacteria operate optimally; psychrophilic (0-25°C, with an optimum around 17), mesophilic (25-45°C, with an optimum around 35) and thermophilic (>45°C, with an optimum around 55). Most studies tend to concentrate on either mesophilic or thermophilic operation since biomass growth is faster in these two regions. The graph in Figure 2-3 shows that psychrophilic methanogens have a maximum growth rate of about 25% that of thermophiles.

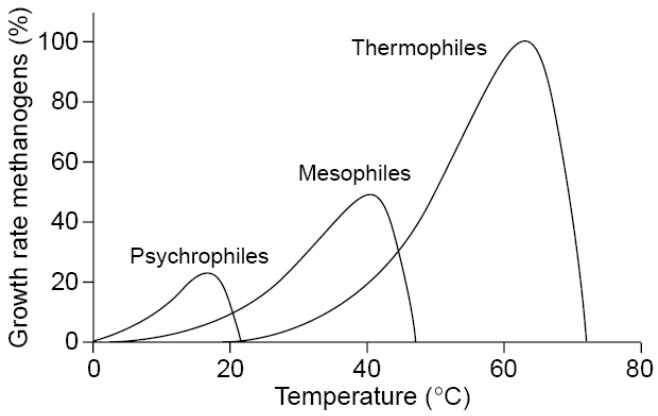


Figure 2-3 Relative growth rates for psychrophilic, mesophilic and thermophilic methanogens (Lettinga *et al.*, 2001)

2.4.2.1 Mesophilic

The majority of anaerobic reactors are operated in the mesophilic region between 30°C and 35°C since this region has a lower energy demand compared to thermophilic operation. Temperature fluctuations of up to 10°C in this region have little effect on the ability of the reactor to remove COD. However, acetic acid methanogenesis has an optimum conversion temperature between 40 and 45°C (Speece, 1996).

2.4.2.2 Thermophilic

Speece (1996) states that thermophilic anaerobic biomass can digest substrate 50% faster than mesophilic biomass, however this figure is highly dependent on factors such as the substrate type. For example Duran *et al.* (1997) found the substrate utilisation rate increased by only 26% between mesophilic and thermophilic reactors digesting dog food in basal medium. Jeison and van Lier (2008) assessed the feasibility of operating a thermophilic SAMBR, and they approximated a membrane cost of 0.5€ per cubic meter of permeate produced. They also found that cake formation was the limiting factor for critical flux, however, they observed very little irreversible fouling (Jeison and van Lier, 2008). Thermophilic operation does have some disadvantages, however, as it requires a long start up period to acclimatise the biomass, and cannot accommodate loading variations very easily. The hot temperatures used for thermophilic operation have the additional consequence that the cells can lyse easily, so it is important to operate in high growth conditions (Speece, 1996). Finally, in terms of energy it is only effective to operate at this temperature if the waste is hot (>60^{0}C).

2.4.2.3 Mesophilic vs Thermophilic

It has been shown that thermophilic operation (in a CSTR) has a higher initial substrate utilisation rate compared with mesophilic operation; this is most likely due to the increased reaction kinetics at higher temperatures. However, Duran *et al.* (1997) also found that the final effluent COD from a thermophilic reactor was higher than a mesophilic reactor, although they gave no reason as to why this might be. Duran *et al.* (1997) suggest that a two stage process of thermophilic reactor followed by a mesophilic reactor was better than a two stage at either temperature, however, as previously stated this is unlikely to be economically optimal unless the waste is hot. A further comparison between thermophilic and mesophilic conditions, using various types of tank reactors, was carried out by Moonil *et al.* (2002). They found that the fed batch thermophilic non-mixed reactor gave the best results with respect to volatile solids removal and gas production. The optimum reactor for mesophilic conditions was a continuously fed CSTR which had slightly lower performance than the best thermophilic reactors.

Jesion and van Lier (2006) compared SAMBRs under mesophilic (30°C) and thermophilic (55°C) conditions. It was found that under mesophilic conditions the biomass concentration in the reactor was directly related to critical flux; whereas under thermophilic conditions the biomass concentration had much less effect on the critical flux. It was also found that for a specific effluent outlet concentration, the thermophilic reactor required 50% less gas sparging than the mesophilic. However, both reactors had roughly the same effluent quality. While this data suggests that under thermophilic conditions fouling is easier to control, this needs to be offset against the extra cost of heating a reactor up to 55°C unless the inlet feed is very hot anyway.

2.4.2.4 Psychrophilic

It has been stated that psychrophilic anaerobic digestion is feasible for a wide variety wastewaters, yet compared to mesophilic and thermophilic operation there has been little research into the feasibility of psychrophilic anaerobic digestion in MBRs (Collins *et al.*, 2006). It has been suggested that psychrophilic MBRs is an area for further development (Mulder *et al.*, 2001). Anaerobic psychrophilic treatment was studied in an expanded granular sludge bed (EGSB) reactor format by Lettinga *et al.* (2001); they found that psychrophilic treatment was feasible for partially acidified wastewater including pre-settled domestic sewage. The researchers also state that the feasibility of psychrophilic treatment depends on 'an extremely high sludge retention under high hydraulic loading conditions….[and] an excellent contact between retained sludge and wastewater'. These conditions are easily met by the SAMBR since good mixing is provided through coarse bubbling, and 100% biomass retention is

achieved via the membrane. It is important to note that while anaerobic biomass generates less excess sludge than aerobic, some sludge wastage will still be necessary and therefore some biomass will be lost through this process.

Table 2-7 Summary of studies in psychrophilic anaerobic wastewater treatment

Reactor	Temp (°C)	COD removal efficiency (%)	Feed	Feed COD (mg l^{-1})	Reference
EGSB	8	90	VFAs	15500	(Lettinga *et al.*, 1999)
EGSB	15-9.5	80	VFA Based	10000-3500	(McKeown *et al.*, 2009)
EGSB	10-4.5	82	VFA Based	10000-3500	(McKeown *et al.*, 2009)
USAB	25	78	Black water	12311	(Luostarinen, 2007)
USAB	15	61	Black water	9503	(Luostarinen, 2007)
AnMBR	25	95	Synthetic wastewater	1000	(Ho and Sung, 2007)
AnMBR	25	90	Synthetic municipal wastewater	500	(Ho and Sung, 2007)
ABR	20	70	Milk	500	(Langenhoff and Stuckey, 2000)
EGSB (membrane coupled)	15	85-96	Domestic wastewater	500	(Chu *et al.*, 2005)
EGSB (membrane coupled)	11	76-81	Domestic wastewater	500	(Chu *et al.*, 2005)
ABR	10	60	Milk	500	(Langenhoff and Stuckey, 2000)
UASB	13	87	Pre-settled sewage	400	(Zandvoort *et al.*, 1999)
USAB	19	64	Municipal wastewater	281	(Álvarez *et al.*, 2008)
USAB	14	53	Municipal wastewater	118	(Álvarez *et al.*, 2008)

There have been a number of other investigations into the feasibility of anaerobic wastewater treatment in the psychrophilic region; a summary of the more recent studies is shown in Table 2-7, this table demonstrates that psychrophilic anaerobic digestion is viable for a variety of scenarios. All of the studies documented in Table 2-7 show a COD removal of 60% or higher, with the exception of the 14°C USAB studied by Álverez (2008) where the average COD removal was 53%. The reason for this low removal is partly due to the low level of COD in the influent that was diluted by rainfall. This report also suggested that a higher COD removal was

possible if better biomass retention could be achieved (Álvarez *et al.*, 2008). The authors also concluded that 'self-inoculation resulted in high COD and TS removal and biogas production, but required periods in excess of three months to become fully effective'. This may also explain the unusually low COD removals reported, if the UASB had been allowed to acclimatise for longer periods a higher COD removal may have been achieved.

2.4.2.5 Psychrophilic vs mesophilic operation

Connaughton *et al.* (2006) carried out a direct comparison between mesophilic and psychrophilic EGSB reactors for brewery wastewater with a high inlet COD. They concluded that the economic benefits from psychrophilic anaerobic digestion make it a promising technology for the future. They found that the COD removal was similar for both reactors, at 80-90%; however, biogas yields were up to 50% lower in the psychrophilic reactor. This drop is to be expected since methane is more soluble at low temperatures; Yamamoto *et al.* (1976) noted an 70% increase in methane solubility between 30°C and 11°C.

More recently several studies have shown that the methane dissolved in the effluent is often supersaturated. *Souza et al.* (2011) found that at 25°C, the concentration of methane in the effluent was 1.37 - 1.67 times greater that the saturation predicted by Henry's law. While Bandara *et al.* (2011) found that the amount of dissolved methane in the effluent increased by 65% as the temperature fell from 35 to 15°C.

The increased solubility of methane in the effluent at lower temperatures is an important factor when considering the environmental benefits of psychrophilic operation. While lower temperature mean that no excess energy is require to heat the waste stream, the methane lost in the effluent is not only a loss in terms of energy recovery, but also in terms of environmental impact. Methane has a greenhouse gas effect 23 times greater that CO_2 and therefore releasing supersaturated effluent will increase the amount of methane being released into the atmosphere. Recently there has been several published works demonstrating the recovery of dissolved methane via stripping (Giménez *et al.*, 2010), advanced membranes (Cookney *et al.*, 2012; Wasala *et al.*, 2011) and down hanging sponge (DHS) post treatment (Matsuura *et al.*2010).

Whilst there has been little study into the psychrophilic operation of SAMBRs, several authors who have operated other reactor setups at low temperatures commented that operational performance could be increased if greater biomass retention could be achieved (McKeown *et*

al., 2009; Lettinga *et al.*, 2001; Álvarez *et al.*, 2008). Since the SAMBR has 100% biomass retention by design, it therefore has considerable potential for use in low temperature wastewater treatment.

2.4.2.6 Psychrophilic biomass adaptation

As shown in Table 2-7, McKeown *et al.* (2009) operated an EGSB reactor under psychrophilic conditions as low as 4°C with an initial inocula of mesophilic biomass. McKeown *et al.* (2009) found that their biomass adapted quickly to the low temperature conditions, and believed the conditions selectively enriched a psychrophilic methanogenic consortia over a long period of time. Lettinga *et al.* (1999) operated a similar reactor seeded with mesophilic biomass at temperatures between 3°C-8°C, however, they did not believe they produced a truly psychrophilic biomass. After extended psychrophilic operation their biomass still showed an optimum performance between 30°C -40°C.

In general most psychrophilic reactors use a mesophilic biomass which then adapts to low temperatures, and these conditions will favour the growth of mesophiles that are psychrotolerant but will still show an optimum operating temperature in the mesophilic range (Cavicchioli, 2006). True psychrophiles (stenopsychrophiles) cannot tolerate mesophilic temperatures, and therefore will not appear in the psychrophilic biomass adapted from mesophilic sludge. It has been suggested that efforts to isolate and characterise true psychrophilic species from the bioreactor sludge will be important for the future of low temperature reactor operation (McKeown *et al.*, 2009).

Currently, the number of psychrophilic microorganisms isolated from methanogenic processes is very low, reflecting the lack of knowledge regarding the microbiology of anaerobic reactors operated at low temperatures (Lettinga *et al.*, 2001). Several authors have shown that adaptation of mesophilic communities to low temperatures (10-12°C) takes place, but that no selection of psychrophilic organisms occurs (Akram, 2007; Connaughton *et al.*, 2006; Langenhoff and Stuckey, 2000; Rebac *et al.*, 1999; McKeown *et al.*, 2009). Methanogenic activity of microorganisms adapted to low temperatures (11°C) was observed to be up to 7 times higher than non-adapted biomass (Kettunen and Rintala, 1997). A number of specifically psychrophilic anaerobic Archaea have been identified, which raises the possibility of bio-augmentation for future projects (Cavicchioli, 2006).

2.5 Membrane fundamentals

The key property of a membrane is its ability to create a difference in permeation rate between two or more different substances (both soluble and insoluble), hence its usefulness as a method of separation. Since 1960 there has been a rapid increase in the development of membrane technologies, and membranes are now used for applications as diverse as drug delivery, gas extraction and of coarse wastewater treatment (Barker, 2004). In the SAMBR the membrane acts as a barrier to the biomass exiting in the effluent, removing the need for a settler. The membrane also retains high molecular weight soluble organics, and bacterial and viral pathogens that exist in the reactor, and this will be discussed later in this review. An initial barrier to the introduction of membrane technologies has been the capital cost of the membrane. However as technology develops membrane cost has been falling, from 1994 to 2004 the cost of purchasing a membrane for wastewater treatment has fallen fivefold, from €300 to €60 per m^2 of membrane (APAN, 2012).

2.5.1 Membrane characterisation

Membranes are usually characterised by their average pore size, or the minimum particle size in the permeate. Table 2-8 shows the main types of membrane filtration used for water treatment.

The membranes used in wastewater treatment are usually microfiltration membranes that work on a size exclusion principal, with a pore size of around 0.1-1µm, since these provide adequate filtration and are cheaper to produce and operate than ultrafiltration membranes.

2.5.2 Materials

The material which a membrane is made of can help determine the nature of the reactor (Stephenson *et al.*, 2000).

Table 2-9 shows the common materials that membranes are made out of, and some of the attributes of each material.

Choi and Ng (2008) studied the difference between three different types of membranes made of phase inverted polytetraflouroethylene (PTFE), track-etched polycarbonate (PCTE) and track-etched polyester (PETE). The PETE showed the most rapid flux decline, which is likely to be due to the lower porosity and hydrophobicity of the PETE membrane, and the fact that it was operated at a flux above the recommended design flux.

Table 2-8 Membrane uses for water treatment (Meunier, 2009)

Membrane type	*Microfiltration*	*Ultrafiltration*	*Nanofiltration*	*Reverse Osmosis*
Smallest species removed	Bacteria and colloids	Viruses, large organic molecules	Small organic molecules, divalent Ions	All dissolved species
Operating pressure (bar)	0.2-2	1-5	5-20	20-80
Typical flux (LMH)	100-1000	50-200	20-50	10-50
Treatment applications	Clarification	Clarification	Water softening, micro pollutant removal	Desalination

Table 2-9- Membrane material and their properties (Stephenson *et al.*, 2000)

Material	**Advantages**	**Disadvantages**
Ceramics	Good mechanical resistance Good thermal resistance	Very expensive
Titanium dioxide/ Zirconium dioxide	Good thermal resistance Good chemical resistance Good mechanical resistance	Very expensive Limited to MF and UF Brittle materials
Cellulose acetate	Inexpensive Chloride resistance Solvent cast	Poor thermal stability Poor chemical stability Poor mechanical stability
Polysulphone	Steam sterilisable pH resistant Solvent cast	Poor resistance to hydrocarbons
Polypropylene	Chemically resistant	Hydrophobic unless surface treated
PTFE	Very hydrophobic Excellent organic resistance Excellent chemical stability Sterilisable	Very hydrophobic Expensive
Polyamide	Good chemical stability Good thermal stability	Sensitive to Chloride

2.5.3 Membrane throughput and selectivity

There are many important factors to consider when selecting a membrane to use in an MBR, aside from cost, the two major ones are throughput (flux) and selectivity:High throughput is important to minimise the required surface area, thereby reducing cost and footprint; high selectivity, so that the biomass and many other impurities such as low molecular weight solutes are retained. Combining these two factors is challenging, since smaller pores required

for high selectivity mean a higher resistance that decreases throughput. Membrane thickness also has an effect on throughput; the thicker the membrane the higher the transmembrane pressure (TMP) required to achieve the same flux. A thicker membrane, however, will also have a higher strength; this is an important attribute so that the membrane will not break under pressure.

2.5.4 Critical Membrane Flux

The membrane flux (J) is the volume of fluid that passes through the membrane in a certain time per unit of the membrane area; this is often given in units of L $m^{-2}h^{-1}$ (LMH). Field *et al.* (1995) introduced the concept of critical flux (J_c); the authors state that 'on start up there exists a flux below which a decline of flux with time does not occur; above it fouling is observed'. Once critical flux has been achieved, if the TMP is increased, the flux will increase for a short period of time before decreasing back to the critical value (Howell, 1995). Therefore, operating just below critical flux will be more energy efficient, since a lower TMP can be employed to generate flux. SAMBRs are usually operated with a flux between 10-15 LMH (Liao *et al.*, 2006).

Le Clech *et al.* (2003) proposed a widely used method for determining critical flux in an MBR called the 'flux step method'. This method does not find critical flux in its strictest form since a zero dP/dt was not achieved during tests, however, the point at which fouling becomes significant is easily determined (Le Clech *et al.*, 2003). Studies have shown that over a longer term fouling does occur even at subcritical fluxes. In MBRs, however, the critical flux is often so low that it is impractical to operate within the critical flux region (Jefferson *et al.*, 2004).

Less flux decline has been observed in hydrophilic membranes compared to hydrophobic membranes, and therefore it is desirable to operate with a hydrophilic membrane (Ramesh *et al.*, 2006). Using graft polymerisation it is possible to alter the surface of a membrane to make it more hydrophilic; Choo *et al.* (2000) found significant flux improvements using this method, however, too much grafting saw a decrease in flux due to steric hindrance. Ho *et al.* (2007) assessed the suitability of PTFE membranes for anaerobic treatment and found that when the cake foulant became denser the effluent quality increased; however, flux could be easily restored through back flushing. Work by Gu *at el.* (2009) assessed the benefits of changing the hydrophobicity of a membrane used in an aerobic MBR. The authors found that the modified membrane had 9.97% higher flux compared to the unmodified membrane. The associated costs involved with surface modification, however, make this unlikely to be economical for a 10% flux increase.

Critical flux has been determined for many SAMBR setups (Choo *et al.*, 2000; Choo and Lee, 1998; Jeison and van Lier, 2006; Jeison and van Lier, 2007; Liao *et al.*, 2006; Spagni *et al.*, 2010). The design flux for most units tends to be between 10-40 LMH (Liao *et al.*, 2006), however, critical fluxes as low as 2 LMH have been reported in the literature (Spagni *et al.*, 2010). Jeison *et al.* (2006) demonstrated a first order relationship between biomass concentration and critical flux within the SAMBR. It has also been reported that the critical flux for anaerobic units tends to be lower than for their aerobic counterparts (Spagni *et al.*, 2010). Over time the critical flux for the SAMBR will drop due to increased deposition of colloids and organics on the membrane surface. Jeison *et al.* (2007) reported a significant drop in critical flux measured during long term operation (200 days) from 20 LMH down to 3 LMH.

2.6 Membrane Fouling

Membrane fouling is the main challenge surrounding MBR technology; it can reduce the flux, require increased energy inputs and means that greater membrane area is required in the design (at increased capital cost). The issue of fouling is clearly of significant importance, and this can be seen in the number of papers published about MBRs where the most common research keyword associated with MBR papers is 'fouling' (Santos *et al.*, 2011).

Membrane fouling is a complex process caused by a multitude of factors, and fouling occurs when material builds up on the membrane surface; this inhibits the flux through the membrane and makes the process less efficient. While the build-up of a cake layer on the membrane surface restricts flow through the membrane, it also increases the selectivity of the membrane since the pore size is effectively reduced. Almost every parameter involved in SAMBR operation will affect membrane fouling to some degree; the major causes of fouling are from the feed and biomass characteristics, and these will be discussed below.

2.6.1 Fouling classifications

There are several classifications and definitions for different types of fouling in MBRs, in this work the definitions used are those defined by Meng *et al.* (2009), removable, irremovable and irreversible fouling. Removable fouling, as the name suggests, is the fouling layer that can be easily removed by manipulating the physical parameters, such as gas sparging or back washing. Irremovable fouling is defined as the deposits on the membrane that cannot be removed using physical methods, but can be removed using a chemical cleaning process. Irreversible fouling is defined as the fouling on the membrane that cannot be removed without damaging the membrane (Meng *et al.*, 2009).

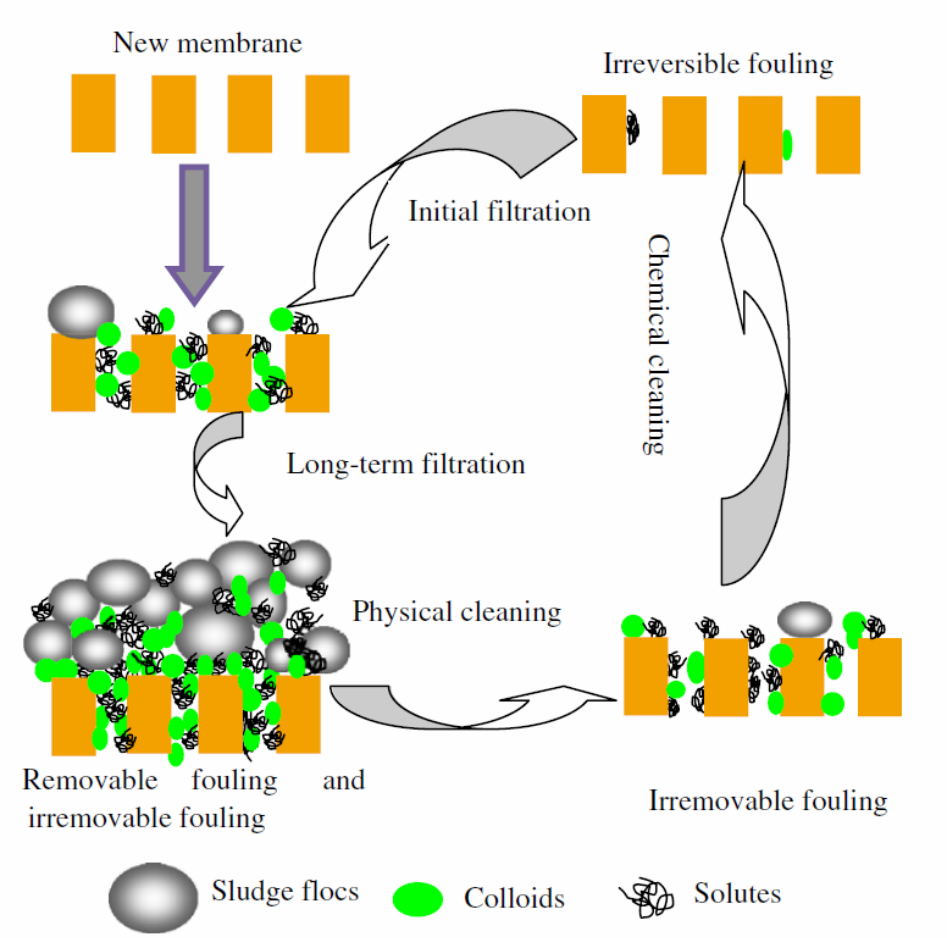


Figure 2-4 Fouling mechanisms in an MBR (Meng *et al.*, 2009)

As demonstrated in Figure 2-4, sludge floc colloids and solutes can deposit on the membrane surface (called the cake layer), while the smaller solutes and colloids can enter the membrane pore and attach to the inside of the membrane in a process called pore blocking. Removable fouling tends to be associated with the cake layer on the membrane surface, while irremovable fouling is often more likely to be associated with pore blocking. Irreversible fouling can be associated with both the membrane surface and pore blocking, however, the foulants are much more strongly attached to the membrane.

2.6.2 EPS/SMP definitions

Membrane fouling is often attributed to the deposition of microbial products on the membrane surface, and inside the pores. The exact definitions of extracellular polymeric substances (EPS or ECP) and soluble microbial products (SMP) have been subject to much debate (Barker and Stuckey, 1999). One of the reasons for this is that the definitions of EPS and SMP are dependent on the methods used to extract them, and currently there is no standardised method. In general EPS are the polymeric material bound to the cell surface, and

these need to be extracted using physical and chemical methods (Aquino *et al.*, 2006). SMP are defined as microbial products released into the bulk solution from substrate metabolism, cell lysis, as well as products released for quorum sensing (Aquino and Stuckey, 2008; Barker and Stuckey, 1999). Through the process of cell lysis some of the EPS will be released into solution as SMP meaning there is an overlap between these two fractions, however, Aquino found EPS contributed only 7% of the total SMP under steady state (Aquino and Stuckey, 2008). There have been many studies on the effects of EPS and SMPs on membrane fouling (Al-Halbouni *et al.*, 2008; Chu and Li, 2005; Germain, 2005; Ho, 2006; Jarusutthirak and Amy, 2006; Rosenberger *et al.*, 2006) which have all demonstrated that EPS and SMP are important foulants.

2.6.3 Extracellular polymeric substances (EPS)

The EPS encompasses several types of macromolecules found in the intercellular space between microbial flocs. These macromolecules are mostly polysaccharides, but also include nucleic acids, lipids and proteins (Flemming and Wingender, 2001). The EPS macromolecules form a matrix causing the biomass to form aggregates. Another function of the EPS matrix is to help control the immediate environment for the cells. By affecting porosity, water content, charge and hydrophobicity, the EPS allows the biomass to survive in less favourable conditions (Flemming *et al.*, 2007). In MBRs, however, it has been shown that EPS contributes significantly to fouling. The EPS can cause bioflocs to attach to the surface of the membrane, and also allows biofilms to generate, thus reducing porosity. In addition to this, digestion in large bioflocs can be significantly slowed by mass transfer restrictions (Flemming and Wingender, 2001).

2.6.4 Soluble microbial products (SMP)

In the filtration process SMPs can attach to the membrane surface to form a gel matrix or block individual pores. When on the membrane surface they can provide a nutrient source for biofilm growth, further exacerbating fouling (Rosenberger *et al.*, 2005; Le-Clech *et al.*, 2006). It has been shown that the soluble fraction of a biological suspension can contribute up to 50% of the fouling (Ho, 2006).

Barker and Stuckey (1999) comprehensively reviewed the literature on SMP production, and summarised the following 7 factors believed to cause SMP production:

1. Concentration equilibrium: organisms excrete soluble organic materials to establish a concentration equilibrium across the cell membrane;
2. Starvation: bacteria excrete organic materials during starvation because they must obtain energy for maintenance by endogenous respiration or metabolism of intracellular components when the substrate is essentially absent;

3. Presence of energy source: the presence of an increased concentration of exogenous energy source can stimulate the excretion of SMP;
4. Substrate-accelerated death: sudden addition of a carbon and energy source to bacteria starved for carbon and energy may accelerate the death of some bacteria. SMP may be produced as a result of this process;
5. Availability of required nutrient: if essential nutrients are present in very low concentrations, SMP may be produced to scavenge the required nutrient;
6. Relieving environmental stress: SMP are produced in response to environmental stress, such as extreme temperature changes and osmotic shocks. Kuo (1993) also speculates that SMP are produced in response to toxic substances;
7. Normal bacterial growth and metabolism: SMP, such as exocellular enzymes, are not only produced during stressed conditions, but also during normal growth and metabolism.

Further to these factors, other reviews on the subject of SMPs have been published with varying definitions of the precise nature of SMPs and improvements on modelling SMP production (Aquino and Stuckey, 2008; Menniti and Morgenroth, 2010; Laspidou and Rittman, 2002).

2.6.5 Feed/biomass characteristics

The nature of the feed will have a direct effect on membrane fouling, as well as affecting EPS and SMP production, and it has been shown that increasing feed concentration increases fouling (Choi *et al.*, 2005). The feed is usually considered alongside the characterisation of the biomass in terms of fouling propensity (Le-Clech *et al.*, 2006). The biomass can be considered to consist of three components: suspended solids, colloids and solutes. In general colloidal materials are responsible for pore blockages in the membrane, while suspended solids accounts for the cake layer resistance (Itonaga *et al.*, 2004). The mixed liquor suspended solids (MLSS) are often the major fouling parameter since an increase in MLSS results in an obvious increase in the cake layer. Some researchers have observed a 'critical' MLSS below which the MLSS appear to have little effect on the fouling (Liao *et al.*, 2006).

2.6.6 Anaerobic biomass rheology

The rheological parameters of biomass are closely related to the fouling properties, while performance increase is often discussed in terms of fouling reduction. It is the resistance across the membrane that determines the reactor performance, and this is partially governed by the rheology of the reactor contents (see equation 2-6). Where R_t is the resistance across the total membrane unit (m^{-1}), TMP is the transmembrane pressure (Pa), J is the flux (m^3/m^2) and η is the viscosity (Pa.s);

$$R_t = \frac{TMP}{J\eta} \qquad \text{2-6}$$

In general anaerobic biomass has been found to be a non Newtonian shear thinning fluid (Mu and Yu, 2006; Pevere *et al.*, 2006), meaning that the more stress is exerted upon the biomass the lowers its apparent viscosity.

Moreau *et al.* (2009) investigated the significance of biomass viscosity in relation to aerobic MBR operation. The authors found a direct correlation between the total suspended solids and the overall viscosity, while temperature had very little effect. Interestingly, they determined no correlation between the viscosity of the biomass and the reversible fouling potential of the biomass. It seems that there is very little information in the literature on this topic, and that research into the effect of anaerobic biomass rheology on the SAMBR is lacking.

2.7 Fouling mitigation

In addition to a clear insight into the causes of fouling, it is also important to understand the various methods of fouling mitigation. Several methods have been suggested to try and combat the effects of fouling or reduce its impact on MBRs, and this section summarises the main fouling control techniques with a focus on gas scouring.

2.7.1 Relaxation

Relaxation is a method of fouling mitigation, where permeate is not continuously pumped through the membrane, so that for short periods the membrane is 'relaxed'. During this relaxation period the cake layer that has built up can diffuse back into the mixture by back transport mechanisms. This method has been shown to be most effective if used in combination with gas sparging (this method is discussed below), so that the extra shear induced by the gas sparging can enhance cake dispersion (Le-Clech *et al.*, 2006).

2.7.2 Backflushing

A further progression from relaxation is backflushing, where the permeate pump is reversed so that for short periods permeate is pumped back through the membrane. This method more actively forces the cake layer to mix back into the liquor. Psoch and Schiewer (2005) have shown that a combination of air sparging and backflushing produces the most sustainable flux in an aerobic MBR (for low TMP). The key variables of this process are the frequency and duration of the back wash; it has been found that less frequent longer washing cycles (600s filtration, 45s backwashing) was more efficient than frequent short backwashing cycles (Le-Clech *et al.*, 2006). However, backflushing does have the disadvantage of requiring additional energy to reverse the pressure difference to impose a back flow. This method also causes a loss of permeate which lowers the efficiency of the reactor (Psoch and Schiewer, 2005). In addition, backflushing cannot remove a consolidated cake that has built up of a long term

operation (Jeison and van Lier, 2007). Gas can also be used as a backflushing fluid and has shown flux improvements of up to 371% for a 15min backwash for every 15mins of normal operation (Visvanathan *et al.*, 1997); of course this significantly reduces the operating time. As well as the above stated problems with backflushing, using gas also has a greater potential for tearing the membrane.

2.7.3 Activated Carbon

Fouling can also be reduced by the addition of activated carbon to the reactor. Activated carbon reduces fouling by the adsorption of organic polymers to its surface, and this increases the mean particle size within the reactor (Choo *et al.*, 2000). The powdered activated carbon (PAC) particles also scour the surface of the membrane further reducing cake formation (Akram and Stuckey, 2008; Park *et al.*, 1999). Hu and Stuckey (2007) showed that PAC has a greater effect on cake formation than granular activated carbon due to the larger surface to volume ratio of PAC. As well as mitigating fouling, PAC addition has also been shown to increase COD removal within the reactor (Choo *et al.*, 2000; Hu and Stuckey, 2007), and improve performance during start up and hydraulic shock (Akram and Stuckey, 2008). Adding PAC to the reactor can only improve flux up to a certain concentration, after this the maximum possible flux decreases due to increased suspended solid concentrations. Akram and Stuckey (2008) found that a PAC concentration of 1.67 $g.l^{-1}$ led to an increase in maximum flux, but when the PAC concentration was increased to 3.4$g.l^{-1}$ the maximum flux decreased due to the increase in viscosity.

Activated carbon has also been used in hybrid reactors in relation to reducing fouling in MBRs, whereby the carbon is added in a pre-treatment unit. Guo *et al.* (2004) found that an powered activated carbon pre-treatment, provided improvements in both organic removal and filtration flux for a microfiltration-hybrid system. The author's state that the optimum does is 1g/l PAC, however this will be a system dependant parameter and the optimum PAC dose will likely change depending on the organic loading rate.

2.7.4 Chemical additives

While PAC is the most common additive to prevent fouling, other chemicals have been investigated for their flux enhancing properties. These additives are usually dosed on their ability to remove SMP; however, this does not always directly correspond to a reduction in fouling. An improvement in critical flux of up to 46% was achieved with the cationic polymer MPE50 with a corresponding SMP removal of 45%. In general all cationic polymers show good and steady performance in preventing fouling (Koseoglu *et al.*, 2008).

2.7.5 Bubbling or Gas Sparging

Bubbling is the most commonly used method of fouling control; as well as reducing fouling it also aerates the reactor for aerobic MBRs, hence its popularity. In the SAMBR the gas bubbled through the reactor is a fraction of the gas produced by the biomass, and therefore is mostly composed of methane, carbon dioxide and nitrogen. The use of bubbles to enhance membrane bioreactor processes was first introduced by Imasaka *et al.* (1989) who developed a two phase microfiltration process coupled with an anaerobic digester. The methane injected into the ceramic membrane module proved to be an effective mitigation technique (Cui *et al.*, 2003).

There are three main benefits to sparging in the SAMBR; the bubbles promote mixing in the reactor, reducing the likelihood of dead spots. The individual bubbles scour the surface of the membrane to remove the cake layer. Finally, the turbulence created by the bubbles also reduces concentration polarization occurring across the membrane. An extensive review of the use of gas bubbling on membrane processes has been carried out by Cui *et al.* (2003), this section will deal with the aspects of gas sparging relevant to the operation of a SAMBR.

The extent of gas sparging in an MBR is reported in many ways, most frequently it is recorded either as the exact flow rate i.e. litres per minute (LPM); or it is recorded relative to the membrane area i.e. cubic meters of gas per square meter of membrane area per hour; or the gassing rate is given as a superficial upflow velocity. While the latter measurement has wider applications for unit design. it is still flawed because the amount of scour any membrane achieved is highly dependent on reactor geometry. For example, three flat sheet membranes stacked vertically would receive a more intense gas scour than the same membrane situated side-by-side, for the same gas rate (whether reported as LPM or $m^3m^2h^{-1}$). Therefore it may be more widely applicable to consider flow regime rather than flow rate when investigating gas sparging.

2.7.5.1 Flow regimes

The dynamics of gas flow in liquid (often referred to as two phase flow) can be split into many different types, and there are a plethora of different ways of categorising the different flow types; here just the major categories are considered. In Figure 2-5, sketches a and b show bubble flow, this occurs when the bubbles are significantly smaller than the channel diameter. Within the SAMBR this would mean that the bubbles are free to flow through the reactor and are unlikely to have much scouring effect on the membrane. Sketches c and d show slug (or plug flow); in this case the gas flow rate is slightly increased and therefore the walls in the channel start to have an effect. Since the bubble size is impeded by the channel walls these

bubbles will have a scouring effect on the walls of the membrane. Sketch e shows the transition phase between slug and churn flow, where small 'satellite' bubbles trail the main slugs. Sketch f shows fully developed churn flow, in this case the gas flow rates is high enough that the flow pattern becomes entirely chaotic. Sketches g and h show film and annular flow respectively, these two cases are less relevant to MBRs because the flows of gas involved are so high that operation in this region would be completely impractical. While there are some methods in development for determining the nature of two-phase flow by calculating void fraction, the type of flow is usually determined by visual observation and can often be subjective; results are usually backed up with representative pictures of the flow regime (Kreutzer *et al.*, 2005).

Slug (also called plug) flow is most commonly used for gas sparging in MBRs (Cui *et al.*, 2003); to achieve this flow regime the bubbles must occupy 60% or greater of the channel. Because membrane reactors are rarely cylindrical the channel width is assumed to be the narrowest dimension. To increase the effect of slug flow, baffles are often included in the reactor design to restrict the channel width and force the bubbles over the membrane surface.

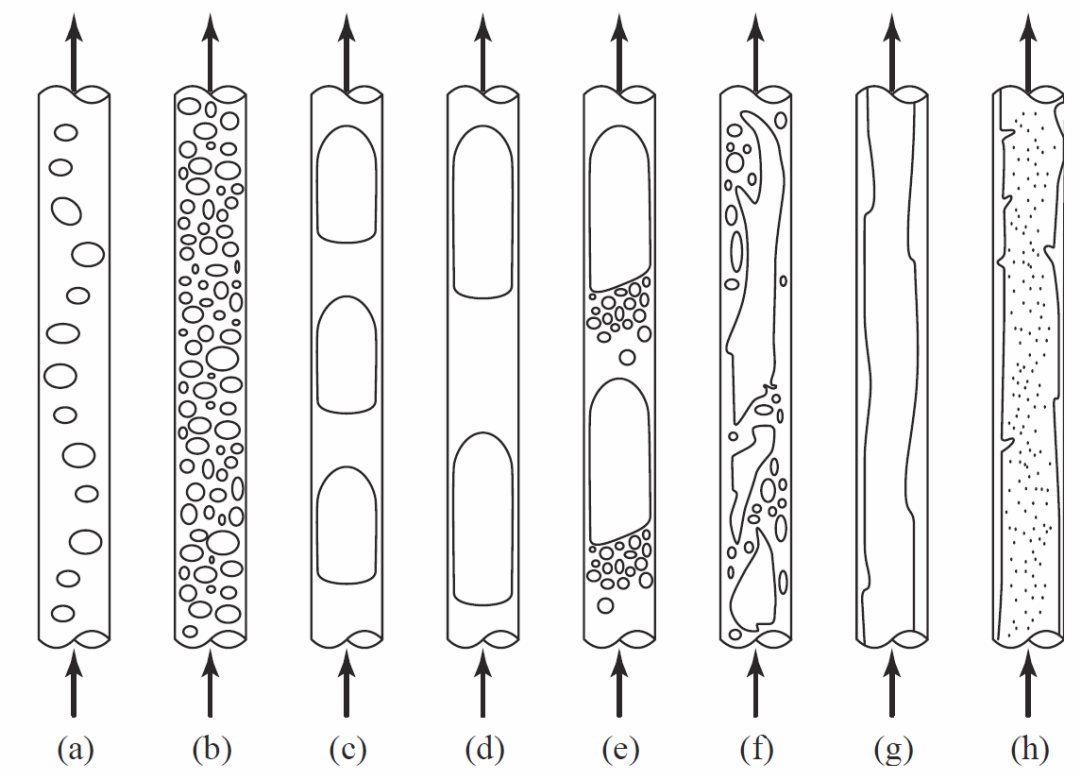


Figure 2-5 Two phase flow patterns (Kreutzer *et al.*, 2005)

Bubble flow is also used in some MBRs, however, bubble flow creates much less turbulence than slug flow and therefore less mixing is promoted along with lower scouring. Faster gas flows like churn flow can also be used as they would promote further turbulence, however, because a higher gas flow is required, it makes them less economically viable (Cui *et al.*, 2003).

The gas flow cases considered here strictly apply only to flow though hollow tubes, although the flow patterns can be observed in other geometries as well. The purpose of gas bubbling is to introduce turbulence at the membrane wall thus increasing the mass transport process: to further this effect inserts can be placed near the membrane surface to further disrupt gas flow and promote turbulence (Yang *et al.*, 2011). Yang *et al.* (2011) achieved a 200% increase in membrane flux using turbulence promoting inserts for a slug flow regime.

2.7.5.2 Flux enhancement

The effect of gas sparging on the membrane can cause an increase in flux across the membrane (for constant pressure systems). The measurement of this increase in flux is called the flux enhancement ratio, as shown in equation 2-7, where ϕ is the flux enhancement ratio, and J_{gas} and $J_{no\ gas}$ are the respective fluxes achieved with and without a gas scour.

$$\varphi = \frac{J_{gas}}{J_{no\ gas}} \qquad \text{2-7}$$

In the MBR reactors, without a gas scour the extent of the fouling can mean that without a gas scour the flux can drop to zero, in a constant pressure situation; therefore the flux enhancement ratio can essentially be infinite in this circumstance. The gas bubbling across the membrane has the effect of increasing mass transport processes, as well as removing deposits on the membrane surface that cause pore blocking; the additional mass transport effect helps to reduce concentration polarisation.

Concentration polarisation is defined as the build-up of solutes (often ions) on the surface of a membrane, such that the concentration at the membrane surface is greater than in the bulk solution. The effect of concentration polarisation in the SAMBR reactors is important to consider: if the concentration of small particles or solutes on the membrane surface is greater than in the bulk solution there will be an increase in the throughput in cases where the particles are smaller than the pore size. As such it would be desirable to reduce concentration polarisation where possible.

Bacchin *et al.* (2002) developed a unifying model combining concentration polarisation, gel layer and particle deposition on a cross flow membrane. This model is a little too complex for

use on a SAMBR as it is not possible to measure all the parameters required, and many parameters such as the range of particle sizes present would change over time. In spite of this, it is interesting to note that they found the transition between reversible and irreversible fouling become more defined as the size of the colloids increased. The researchers also found a relationship between colloid size, driving force (TMP) and the controlling type of fouling mechanism, as illustrated in Figure 2-6. At low TMPs concentration polarisation is the controlling factor for all particle sizes, however, as the TMP increases the fouling tends towards gel formation or deposition depending on the particle size. Since the SAMBR will contain a range of particle sizes it is expected that both fouling mechanisms will have a part to play.

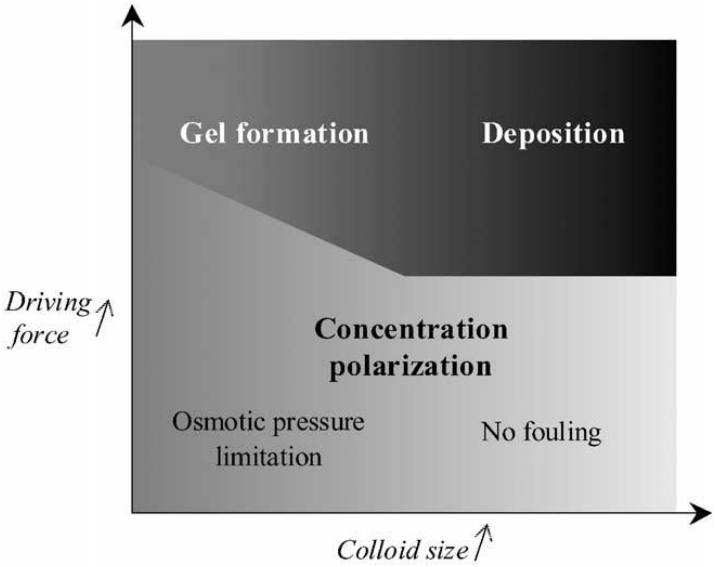


Figure 2-6 mechanisms controlling membrane transport processes (Bacchin *et al.*, 2002)

2.7.5.3 Bubbling and effect on TMP

The first investigations on the effect of gassing rate on the TMP in the MBR system was by Ueda *et al.* (1997). These researchers investigated the upper limits of the aeration rate on an MBR operated for an extended period of time. The main conclusion of this work was that there existed a maximum aeration rate beyond which there was little further effect on cake removal, and this result was further confirmed by Bouhabila *et al.* (2001). Ueda *et al.* (1997) also investigated the long term effect of gassing rate alteration on MBR operation. After 330 days of operation the researchers dropped the aeration gassing rate by 50% for 24 hours, during this time the TMP doubled; however, once the aeration was increased back to the original value the TMP stayed in the high range. This indicates that once fouling occurs on the membrane surface it cannot be removed simply by increasing the gassing rate.

Simulations of the MBR process have found the gassing rate cost to be high, and this is because it has not been optimized (Maere *et al.*, 2011). This demonstrates the importance of a proper study on the rates of gassing in the MBR process with regards to making it as economical as possible. When the aeration was modelled as a standalone parameter, Verrecht *et al.* (2008) found that the lower fluxes and lower mixed liquor suspended solids (MLSS) had a significant effect on reducing the aeration demand in an MBR. While the membrane aeration requirements of the MBR depend on MLSS, Howell *et al.* (2004) have shown this is not a simple relationship.

The existence of a critical aeration rate for aerobic MBRs similar to the critical flux proposed by Field *et al.* (1995) was introduced by Monclus *et al.* (2010). These researchers compared the flux step method with a proposed aeration step method. In this case the authors determined a critical specific aeration rate, beyond which there was a sharp rise in TMP, thus confirming the existence of a critical aeration rate similar to the critical flux. Monclus *et al.* (2010) also claim that their results imply that the flux step experiments determine an overly conservative flux, and that aeration stepping is a more representative method for identifying sustainable conditions. However, this is only the case if the aeration rate used in full scale operation is higher than that used in the critical flux experiments. Guglielmi (2008) demonstrated a linear relationship between critical flux and aeration rate. There are many parameters in MBR operation that can affect the TMP, and Gugliemi *et al.* (2008) investigated the effect of various cleaning protocols on the TMP and fouling build up, and found that whatever the cleaning method used, the aeration demand in the MBR remained the same.

All the cases discussed in this section above deal with aeration rates in MBRs, however, there has been little work done on the importance of gassing rate in anaerobic membrane systems. In fact Lee *et al.* (2001) proved that reasonably high fluxes could be maintained in a SAMBR using intermittent air sparging. In most cases, however, the anaerobic MBRs were sparged with a fraction of the gas produced by the biomass (a mixture of CH_4 and CO_2). In all cases gas sparging was reported to be beneficial, but little attention has been paid to the optimisation of this parameter for anaerobic systems (Brub *et al.*, 2006; Hu and Stuckey, 2006; Imasaka *et al.*, 1989; Kayawake *et al.*, 1991).

2.7.5.4 Practical issues issue surrounding bubbling

While gas sparging in MBRs is generally regarded as an effective method for fouling control, there are a few practical issues surrounding gas bubbling that need to be addressed. The major problem with gas sparging is the potential for foaming within the reactor. Excessive foaming can cause problems with the level control mechanisms. Foaming in WWTPs can be attributed

to various factors, however, when bubbling is involved it is usually due to protein denaturation (Clarkson *et al.*, 1999). For anaerobic reactors an additional factor for concern is the gas solubility. Since the reactors will be operated at pressures slightly above atmospheric, the gas in suspension will tend to dissolve into the reactors, and therefore on release to the environment greenhouse gasses CH_4 and CO_2 will be released to the atmosphere (Cui *et al.*, 2003).

2.8 Viral removal in wastewater treatment systems

One of the many challenges of modern wastewater treatment is to produce an effluent that is free of pathogenic contaminants such as waterborne viruses. Many wastewater treatment plants (WWTPs) around the world discharge their effluents directly into a river system. It is often the case that this same water is used as a raw water supply for towns further downstream; therefore discharging enteric viruses in the effluent can constitute a significant health risk (Leong, 1983).

In practice the issue of virus removal in wastewater effluents is usually tied to that issue of general pathogen control. In the USA for example, easily detectable bacteria such as Escherichia coli and fecal coliforms are used as standard indicators for assessing the amount of pathogens in a wastewater effluent. These microorganisms do not directly cause disease but serve as an indicator for the likely presence of other pathogens including viruses. Methods for indentifying and quantifying fecal coliforms and E. Coli in water and wastewater are well established (this is not the case for viruses), and as such direct sampling for viruses is not often carried out (APHA, 1999; U.S. Department of the Interior and U.S. Geological Survey, 2011).

Viruses have been shown to be more resistant to environmental factors compared to bacteria, therefore water that shows little sign of bacterial pollution may still contain significant amounts of viruses, in some cases an ingestion of a single virus can be enough to cause infection (WHO, 1980). It is important therefore to consider the removal viruses in wastewater treatment separately to pathogenic bacteria. Additionally in the case of MBRs however, the concentration of viruses in the effluent cannot be directly linked to the concentration of bacteria, because the membrane can theoretically screen out all bacteria and protozoa but not viruses.

The viral content of domestic sewage varies greatly depending on factors ranging from the time of year to the socioeconomic conditions. While the concentration of viruses in raw sewage has been detected at concentrations as low as a few hundred viral units per litre, to 500,000 viral units per litre (Leong, 1983), a typical concentration of enteric viruses in

wastewater is thought to be in the region of 10^4-10^5viral units per litre (Feachem *et al.*, 1983). An average infectious dose is in the region of 1-10 viral units, although this number can vary greatly depending on the viral strain and an individual's general health (Tchobanoglous *et al.*, 2003). Therefore a typical WWTP would need to achieve at least a 6 log removal of viruses; in cases where water recycling is being considered the viral removal would need to be even greater than this.

2.8.1 Viral removal in conventional WWTP units

Leong (1983) has extensively reviewed the removal of virus units in the various process units commonly found the WWTPs. A summary of the report's main findings is displayed in Table 2-10. From this data it can be concluded that the standard units alone do not remove enough of the viral content for an acceptable effluent to be produced. Therefore various tertiary disinfectant methods have been developed.

Wastewater treatment plants that chose to install tertiary treatment to remove viral pathogens have three main options open to them: chlorination, ozonation and UV radiation. Chlorination can be split into two further options: free chlorine or chlorine dioxide. Free chlorination is a well established technology; in this process liquid or gaseous Cl_2 is mixed with the waste water. The chlorine reacts with the water to form hyperchlorous acid; the total concentration of hyperchlorous acid (HOCl) and hyperchlorite ions (OCl^-) are together termed the 'free available chlorine'. These chemicals attack the protein capsid in viruses rendering them inactive.

Table 2-10 Viral removal percentages for standard WWTP units (Leong, 1983)

Treatment unit	Median removal percentage
Primary settling	6.6%
Trickling filters	54%
Activated sludge	94%
Coagulation/flocculation/sedimentation	95-99.5%
Filtration	73%
Granular activated carbon	90%

Chlorine dioxide disinfection works on a similar principal to the addition of free chlorine. In this case chlorine dioxide is added to the effluent shortly after its generation, (ClO_2 is an unstable compound). On addition to water the chlorine reacts to form free Cl^- ions, and these ions

inactivate the viruses in much the same way as the free chlorine process. In fact, chlorine dioxide has proved more effective than free chlorine at virus inactivation.

Ozonation as with chlorine dioxide must be generated onsite, where the ozone rich gas is pumped into contact basins through porus diffusers. The ozone molecules oxidise the protein coating of the viruses causing them to become inactive.

The final traditional option for disinfection is ultraviolet radiation, this is a physical rather than a chemical disinfecting method. At specific wavelengths the UV radiation breaks the molecular bonds within the viral DNA/RNA, thus rendering the virus inactive. In this method a bank of UV bulbs are placed into a flow channel set at a specific flow rate to ensure that enough of a radiation dose is applied to the effluent. In a typical WWTP, disinfection via UV radiation has an energy cost of 0.093kWh/m3, (Anglian Water, 2013).

A summary of advantages and disadvantages of each type of disinfection is shown in

Table 2-11. It can be seen that while all the 4 main technologies are effective viral disinfectants, there are significant disadvantages mostly centring on safety concerns for all the methods. Therefore, any innovation in WWTP design that can reduce or remove the need for tertiary disinfection would be an important step.

2.8.2 Bacteriophages as viral indicators

Bacteriophages or phages are simply viruses that infect bacteria rather than higher organisms; the most common types are coliphages which use Escherichia coli (E. Coli) bacteria for propagation. Due to the difficulties and safety concerns involved in using enteric viruses, bacteriophages are frequently used as viral indicators because of their similarities with viruses in terms of structural morphology, size, and behaviour (Shang *et al.*, 2005). As with viruses, phages come in a wide range of shapes and sizes. The smallest phages such as MS-2 with a diameter of 24nm, have been widely used as a model for the Polio virus which has a diameter of 27nm (Meng and Gerba, 1996; Powell *et al.*, 2000; Springthorpe *et al.*, 1993) Similarly, the T4 phage (longest dimension 200nm) has been used as an indicator for larger viruses such as SARS virus (Lv *et al.*, 2006).

Table 2-11 Advantages and disadvantages of the 4 main methods of disinfection adapted from Tchobanoglous (2003).

Advantages	*Disadvantages*
Chlorine (free)	
well established technology	hazardous chemical requiring strict safety measures
chlorine residual monitoring can give an indication of effectiveness	less effective on viruses than other disinfectants
	hazardous disinfection by-products(DBP) like trihalomethanes
	possible release of volatile organic compounds
	chloride content of effluent is increased
	chemical scrubbing facilities may be required
	Increased dissolved solids
Chlorine dioxide	
more effective than free chlorine at inactivating viruses	unstable compound; must be produced onsite
under proper operation no halogen by products	formation of chlorite and chlorate DBP
	increased TDS in effluent
	high operating cost
	can form odours
Ozone	
more effective than Chlorine disinfection on viruses	no immediate measure of successful disinfection
requires less space	highly corrosive
	relatively expensive
	energy intensive
	maintenance sensitive
	significant safety concerns with oxygen storage
	formation of some DBP
UV radiation	
no residual toxicity	no immediate measure of successful disinfection
no DBP formation	expensive to operate
no TDS formation	hydraulic design is critical (bad design will result in non effective disinfection)
no chemical storage safety concerns	large numbers of UV lamps required
	energy intensive

2.8.3 Phage removal in MBRs

In membrane reactors, the membrane provides a direct barrier that stops bacteria passing through the membrane through physical size exclusion, i.e. the membrane pore size is smaller than the bacteria. For viruses this is not often the case since viruses vary in size from approximately 20nm up to 200nm, while the microfiltration membranes used in MBRs range from pore sizes of 200-400nm. Therefore, most viruses should be able to pass through the membrane without restriction, however, due to the surface biofilm on the membrane this may not always be the case. In recent years there have been several publications on work done on viral removal within membrane bioreactors, and in most cases bacteriophages rather than enteric viruses have been used (Shang *et al.*, 2005).

The studies detailed in this section use a variety of different phages, membrane types, and experimental conditions. A summary of this data is displayed in Table 2-12. While the log removal value (LRV) of the phages varies from study to study, in every case the LRV has been found to be higher than that demonstrated by Leong (1983) for the activated sludge unit that the MBR would replace. Interestingly none of the studies listed in Table 2-12 considered the potential effect of phage adsorption to the biomass or any other material.

2.8.3.1 Studies using Qβ

The first study detailing phage removal in an MBR was conducted by Chiemchaisri *et al.* (1992), the results of this study are shown in Table 2-12. These researchers investigated the log removal of Qβ coliphages through a membrane bioreactor treating domestic wastewater. The Qβ phage is very similar to the MS-2 phage in size and morphology; both are icosahedral, F specific phages less than 30nm across. Chiemchaisri el at (1992) reported a 4-6 log removal of the Qβ phage. They suggested that the reason for the high removal reported was due to the gel layer formed on the membrane. Urase *el al.* (1994) performed a similar experiment and found a 3-4 log removal of the Qβ phage. While this result is slightly lower than the original experiment, they still found a significant removal of the phage which they also attributed to the cake/gel layer. The discrepancy between the two sets of results could probably be explained by the work of Herath *el al.* (1999) who demonstrated a 25% change in rejection of Qβ in the 7-5.5 pH range. This pH range is the region in which an MBR would operate, and therefore a slight difference in the pH of the MBR could have a significant effect on the log removal of viruses.

2.8.3.2 Studies on MS-2 removal

Shang *et al.* (2005) studied the removal potential of the MS-2 bacteriophage in an aerobic MBR using a 0.4 μm hollow fibre membrane. They found that the membrane alone showed a poor

log removal of the phage (0.4 log). However, when operated in the presence of activated sludge the phage removal increased, and they found that the suspended biomass contributed 0.8log to the overall phage removal. The authors also agreed with the Qβ studies that the development of a biofilm on the surface of the membrane was the cause of the increased phage removal. This is because they found the phage removal to increase over a period of three weeks as the biofilm developed from an initial LRV of 0.8 up to 2.5 LRV after 3 weeks of operation. In the case of MS-2, Herath *et al.* (1999) found that the effect of pH only became significant at pH values less than 6. Above this pH MS-2 viral rejection remained constant, and since most WWTPs are operated close to neutral pH, it is assumed that pH variation will have little effect on the studies discussed above.

2.8.3.3 Studies on T4 removal

Several researchers have reported on the promising removal of the T4 phage in aerobic membrane bioreactors; the T4 coliphage is one of the largest coliphages at 200nm in its longest dimensions. The first work on this phage in a membrane bioreactor was reported by Ueda *et al.* (2000). In this study the authors used a T-even-like coliphage, and while they do not identify the exact phage, they do identify the size (200nm) which is the same as that of the T4 phage. As with the MS-2 studies, Ueda *et al.* (2000) found that the membrane alone demonstrated poor phage removal (between 0 and 0.7 LRV) for a Kubota membrane with a 400nm pore size. On the addition of aerobic biomass to the MBR they achieved an average log removal of 2.3 when treating settled sewage.

Further to this Lv *et al.* (2006) also investigated the removal performance of T4 in a lab scale 12l MBR; in this case the authors used a finer membrane with a 0.22 µm average pore size. With the smaller membrane pore size, the effect of the membrane alone starts to become significant, and the membrane alone was found to contribute 1.7 log to the overall removal. When the MBR was operating stably the authors found a 6.3 LRV demonstrating almost complete removal of the T4 phage. The authors attributed most of this removal figure to a combination of the cake and gel layer on the membrane surface.

The results of Lv *et al.*'s study are also backed up by the work of Zheng *et al.* (2005). They demonstrated T4 removals in excess of 5.5LRV in an MBR under steady state operation with a membrane of 0.22µm average pore size. In this case the authors attributed the rejection to be mostly due to the cake layer on the surface of the membrane. The authors operated the MBR reactor under a gravity drain, and therefore the TMP remained low throughout the course of operation at 8.5kPa.

Lv *et al.* (2006) also investigated the effect of membrane cleaning on the log removal. They found that just after membrane cleaning (using an hydroxide and hypochlorite solution) the phage removal dropped by approximately 4 log, and this has significant implications for full scale operation as it means that just after membrane cleaning the effluent may require further disinfection processes to prevent a contaminated effluent being released. In addition, Lv *et al.* (2006) also investigated the effect of using an even finer 0.1 µm membrane- in this case no T4 was detected in the effluent. This indicates that choosing a membrane with a mean pore size smaller than the virus to be removed will result in complete rejection.

Finally, Herath *et al.* (1999) found that pH had a moderate effect on the removal of T4 by the membrane. Above pH7 the rejection of T4 remained constant, while between the pH values of 7 and 6 the phage rejection increased by 10%. Between pH 6 and 4 the rejection increases by a further 30%, however, this is beyond the operational pH for most bioreactors. The effect of pH on the phage solution is discussed further in section 2.8.4.

2.8.3.4 Studies on somatic and F-specific phages

In addition to the previously mentioned studies on specific phages there have been a number of studies performed on membrane bioreactors where, rather than investigate the removal of a specific phage, the analysis is given in terms of the removal of F specific and somatic coliphages (Marti *et al.*, 2011; Oota *et al.*, 2005; Ottoson *et al.*, 2006; Wong *et al.*, 2009). These data are of limited usefulness because both somatic and F specific phages come in many different sizes. Therefore, no direct conclusion can be drawn on the size cut-off or specific rejection of the membranes, however, an overview of the viral removal by the process can be observed.

Phage removal is often analysed in this manner in full and pilot scale operation where it is not straight forward to spike the reactor with a single type of phage, since domestic wastewater can already contain a significant amount of phage (US EPA, 2001). From studies that use this method of phage detection, the LRV in membrane reactors has been found to be between 2.6 and 5.6 LRV, with most of the data between 3 and 4 LRV (Marti *et al.*, 2011; Oota *et al.*, 2005; Ottoson *et al.*, 2006; Wong *et al.*, 2009). In the study by Marti *et al.* (2011), the researchers found that the log removal of coliphage increased in accordance with the irremovable fouling layer (fouling that can only be removed with a chemical wash, not by any physical parameters). In contrast, the authors found no connection between the removable cake layer and phage rejection.

Table 2-12 Summary phage removals in MBRs

Reactor type	Reactor volume litres	Feed	Membrane type and configuration	Pore size µm	Phage type	Log removal	Length of operation before sampling	Author
MBR (crossflow)	62	synthetic wastewater	polyethylene hollow fibre (Mitshibushi Rayon)	0.1	Qβ	4-6	30-140	(Chiemchaisri *et al.*, 1992)
MBR (crossflow)	-	activated sludge	PVF (polyvinylidene flouride) flat sheet	0.1	Qβ	3	-	(Urase *et al.*, 1994)
MBR	27	settled sewage	Polyethylene flat sheet (Kubota)	0.4	T-even-like	2.3-5.9	1-14	(Ueda and Horan, 2000)
MBR	12	municipal wastewater	PVDF hollow fibre	0.22	T4	6.3	34	(Lv *et al.*, 2006)
MBR	12	municipal wastewater	PP(polypropylene) hollow fibre	0.1	T4	complete removal	34	(Lv *et al.*, 2006)
MBR	12	municipal wastewater	PVDF hollow fibre	0.22	T4	5.5	21	(Zheng *et al.*, 2005)
MBR	19	synthetic wastewater	Polyethylene flat sheet (Mitshibushi Rayon)	0.4	MS-2	0.8-2.5	1-21	(Shang *et al.*, 2005)
MBR	250	screened sewage	Flat plate	0.4	coliphage	5	-	(Oota *et al.*, 2005)
MBR	14900	coarse filtered sewage	Polyethylene flat sheet (Kubota)	0.4	somatic/F specific coliphage	3.08/ 3.78	some months	(Ottoson *et al.*, 2006)
AnMBR (crossflow)	100	sand separated, part digested dairy manure	PVDF hollow fibre	0.03	coliphage	3.7	1-3 months	(Wong *et al.*, 2009)
MBR	2260	settled and raw sewage	Polyethylene flat sheet (Kubota)	0.4	coliphages (F specific & somatic)	2.6-5.6	-	(Marti *et al.*, 2011)

2.8.3.5 Phage removal in anaerobic reactors

To date there has been little research into the removal of phages in anaerobic membrane reactors. Wong *el al.* (2009) investigated the removal of coliphages in an anaerobic membrane reactor treating agricultural waste; in this case they used a sidestream membrane reactor configuration with a PVDF hollow fibre membrane with an average pore size of 0.03µm. The authors found a coliphage removal of 3.7 log removal in the anaerobic MBR. A direct comparison with a complete mix anaerobic digester (CMAD) was made, and as expected the CMAD showed a poor phage removal of 0.5 log.

It is interesting that the authors report a phage removal of just 3.7 LRV, because the membrane is reported to have a pore size of just 0.03µm. Looking at the data reported by other researchers (Table 2-12) a membrane this fine would be expected to completely remove all but the smallest viruses through size exclusion alone, even before the effect of a biofilm is considered. Wong *et al*.'s results are therefore inconsistent with the rest of the literature.

2.8.3.6 Phage removal in clean membrane systems

There are variations in the literature for data reported for clean membranes operated without the presence of biomass. Shang *et al.* (2005) reported a 0.4 LRV for MS-2 phage passing through a clean membrane system before the biomass was introduced. While Ueda and Horan (2000) reported a negligible removal of T4 (a much larger phage) in a 'clean system', this discrepancy is likely to be due to the high fluxes used by the Ueda and Horan.

2.8.3.7 Summary of phage removal in MBR studies

All of the studies on phage removal in MBRs (summarised in Table 2-12), suggest that the removal of phages in the MBR is due to the fouling layer on the membrane. There is, however, disagreement over what type of fouling (removable/irremovable/irreversible) is the major factor. Membrane fouling is affected by the length of operation, and the amount of time between cleaning cycles, therefore Table 2-12 lists the length of operation before the phage removal analysis was undertaken. In full scale operation the membranes are typically removed for chemical cleaning every 6-12 months (Judd and Judd, 2006b). It is therefore important to consider how long each MBR unit had been running before the phage rejection was analysed. For example Chiemchaisri *et al.* (1992) did their phage analysis on days 30 to 140 and broadly found the phage log removal increased as time went along. Whereas Zheng *et al.* (2005) worked on a similar system but did short term experiments over a few hours, thus the fouling layer may not have developed as extensively explaining the authors lower log removal values compared to the work by Chiemchaisri *et al.* (1992).

2.8.4 Viral and phage behaviour at different pHs

As well as looking at the rejection of viruses and phages with the membrane reactor, it is important to understand the behaviour of such particles under the conditions found in membrane bioreactors. An extensive review of virus- surface interactions was completed by Gerba in 1984.

Viruses are colloidal and as such can be modelled using double layer theory; this states that while the individual particles may carry an electrostatic charge, the solution as a whole remains neutral (Verwey, 1947). This is possible due to a tight layer of oppositely charged particles on the surface of the virus particles called the Stern layer, and further out a less tightly attached layer is a diffuse layer of counter ions called the Gouy layer. A diagrammatic representation of this is shown in Figure 2-7; the extent of the double layer determines the interaction with other particles, and the greater the extent of the double layer the greater the repulsive force between particles. The extent of this double layer is dependent on the ionic concentration of the solution and the pH. If the available ions in solution increase then the extent of the double layer decreases because less volume is required to hold the ions needed for the double layer. Eventually the double layer shrinks such that the attractive van der Waals forces overcome the repulsive double layer at this point the phage particles will coagulate.

In addition to this, the charge on the surface of the membrane will alter depending on the pH of the solution; this is because the zwitterions in the amino acid on the phage surface layer can change pH. At a certain pH the phage particle will have a neutral surface charge- this is called the isoelectric point or pI.

At the isoelectric point the surface charge on the phage particle is neutralised and so the phage has a zero overall charge increasing the chances of coagulation. In terms of phage removal in the membranes this is important because if the phages coagulate then their overall 'size' is greater and they are more likely to be screened out by the membrane.

Viruses and phages have a variety of isoelectric points ranging from 8.2 to 2.6 (Gerba, 1984). In this work the phages that were used are MS-2 and T4, the pI of bacteriophage MS-2 was determined to be 3.9 by Zerda *et al.* (1985), and the pI of phage T4 has been found to be between 4 and 5 (Childs and Birnboim, 1975). The isoelectric point of both these phages is well below the operational pH range for anaerobic biomass, and therefore phage coagulation is not expected to occur in the SAMBR reactors. In general low pH will favour adsorbed or coagulated phages, while high pH will result in free phages, although the pI of the individual phage involved and the pI of any other particles in solution can have an effect.

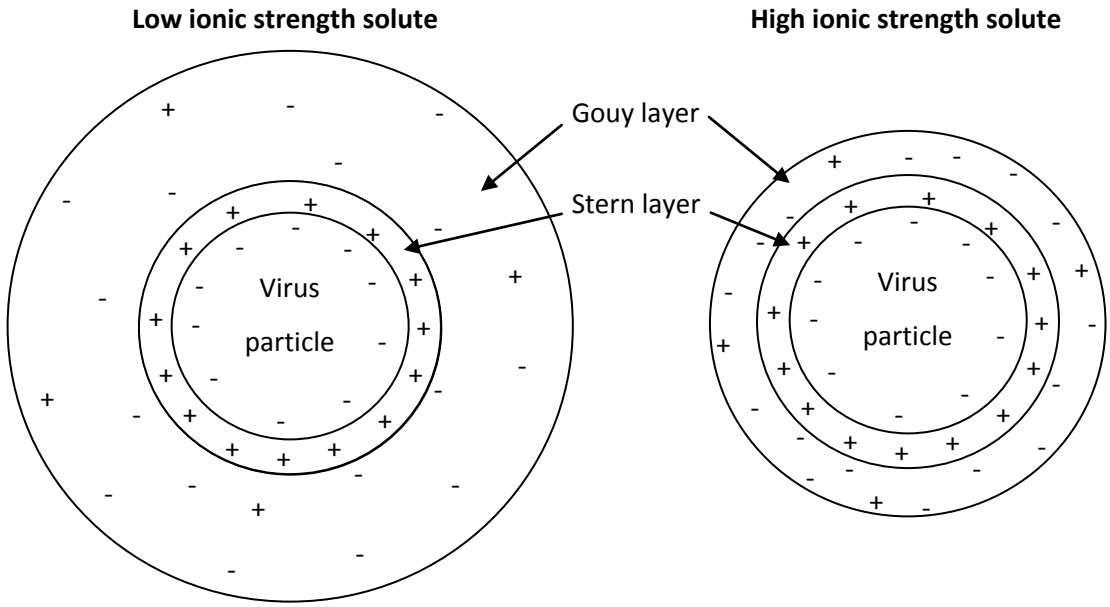


Figure 2-7 Schematic of the ionic double layer on virus particles at different ionic strengths

As stated in section 2.8.2, MS-2 is frequently used as an indicator organism to model the behaviour of the polio virus, however, when looking at pI values it is interesting to note that the pI of the polio virus has been measured between 8.2 and 4.5 (the average pI for polio is 6.1). Given the difference in isoelectric point between MS-2 and poliovirus, it is possible that MS-2 may not be a good model for polio in situations where virus coagulation has an impact on removal, because the poliovirus is likely to coagulate in anaerobic operation, whereas MS-2 is not.

2.8.5 Phage interaction with activated carbon

Activated carbon is widely used in tertiary wastewater treatment to remove trace organics, and in drinking water treatment to remove unpleasant odours and taste (Gerba, 1984). The use of activated carbon to adsorb viruses has been studied by several researchers. Cookson *et al.* (1967) studied the adsorption of bacteriophage T4 onto both granular and powdered activated carbons. The researchers found a standard adsorption isotherm for the T4 removal, however, the overall removal was poor. This is thought to be due to the size of the virus because the T4 particles are larger than the pores on the carbon, and hence they cannot penetrate the carbon surface. Thus most of the active sites on the carbon surface remain unused as they are 'shielded' inside the pores. The researchers also found that the adsorption of T4 onto the carbon surface did not affect its activity.

More recently, Powell *et al.* (2000) investigated the adsorption of MS-2 particles onto different shapes of activated carbon. They theorised that an activated carbon fibre composite would show greater adsorption compared to granular activated carbon (GAC) due to the increased contact time afforded by the carbon fibre composite. While the researchers did find increased adsorption on the composite fibre, this could also be due to the greater surface area on the outside of the fibre compared to the GAC where much of the surface area was unavailable to the MS-2 particles because they could not fit into the pores.

In recent years there has been a wealth of research into the beneficial effects of activated carbon in SAMBRs (Akram and Stuckey, 2008; Hu and Stuckey, 2007; Kim and Lee, 2003; Park *et al.*, 1999; Vyrides and Stuckey, 2009; Yang *et al.*, 2011). These studies have shown the benefits of activated carbon including increased membrane scouring, lowering the TMP and increased COD performance. However, the effect that adding PAC to the SAMBR has on its virus removal potential has not been reported.

2.9 Wastewater treatment modelling and decision making

While there has been a great deal of published work on the feasibility of individual anaerobic reactor types, it is important to be able to form an overall comparison between different treatment options so that an optimum flowsheet can be designed. With tightening legislation on effluent standards, (Directive 2006/44/EC) and targets set for carbon footprints, new methods for wastewater treatment are being investigated, however, there is a need for a simple decision making analysis tool to effectively compare the available options. Guest *et al.* (2009), in a study on wastewater resource recovery, identified that the primary issue faced was not the availability of suitable technologies, but the lack of a design methodology to select the ideal solution for any particular geographic context.

Wastewater treatment is comprised of many sequential unit operations, and for any given situation there will be different constraints upon which a design must be based, e.g. space constraints, cost, solids disposal, and emissions. To design a plant that is optimal for all constraints a rigorous methodology is required for wastewater treatment plants. Such a methodology would need to take into account all the parameters involved in the design of a wastewater treatment process, from effluent requirements and carbon footprint to capital expenditure location restrictions, and return an optimal WWTP design for the individual constraints. Existing methodologies range from a life cycle assessment in which a comprehensive data set is required to a simpler flowsheeting analysis combined with a decision making algorithm.

Garrido-Baserba *et al.* (2012) published a knowledge based methodology for medium and small wastewater treatment plants. They identify two main types of knowledge bases; a 'specifications knowledge base' which details the technological aspects of treatment, and a 'compatibility knowledge base' which analyses how well different treatment units fit together. While the authors provide a comprehensive methodology for the design of a wastewater treatment plant, they focused mostly on very established technologies and did not consider the possibilities for resource recovery or biofuel generation within their model.

Puchongkawarin *et al.* (2011) demonstrated the need for a simple, reliable, model for the comparison of wastewater treatment units. The authors published a simple model for the comparison of various resource recovery models. In this case the authors were looking at a variety of novel units, and therefore identifying accurate performance and costing data for different units proved to be the biggest challenge to creating a useful methodology. Costing data is usually split into two categories, capital expenditure (CAPEX) and operational expenditure (OPEX). CAPEX is the total cost to build the physical unit while OPEX is the yearly cost required to keep the unit running. Due to the confidential nature of company finances the exact CAPEX and OPEX costs for different treatment processes are difficult to find. There is software available to acquire preliminary costing data for standard units it can be quite inaccurate and does not include the more novel units (Puchongkawarin *et al.* (2011).

In contrast Giliot *et al*. (1999) propose a standardized cost procedure that allows a cost comparison of several different treatment scenarios. While this method would certainly produce the most cost effective plant, it did not fully consider other parameters involved in plant design, such as plant greenhouse emissions or stricter effluent control. So if the EEC tightened regulations on effluent standards the 'optimum process' described by this model may not be able to produce effluent that was up to the required standard.

2.9.1 Life cycle assessment

Life cycle assessment (LCA) is a 'cradle to grave' approach for considering the environmental impact of processes or products. The LCA method has in recent times been recommended by NATO as a newly emerging technique with applications for the field of wastewater treatment (Ahmed, 2007). The LCA approach requires four steps; firstly the goal and scope of process including process boundaries and assumptions must be defined. Secondly an inventory analysis is carried out, for a WWTP this includes not only mass and energy balances of the wastewater but also the material used during construction operation and demolition. Thirdly an impact assessment of all the data collected from the inventory analysis is performed to analyse the environmental consequence of

each of the inputs and outputs of the process. The final step is the interpretation, which involves a final comparison of the environmental issues presented; this is converted to an environmental index such that it can be compared with other options. The full principals and framework for LCA are set out in ISO 14040 (2006).

In wastewater treatment LCA studies have been carried out on well established processes; because of the volume of data required there are difficulties in performing this analysis on more novel processes since the required data is not available. Emmerson *et al.* (1995) and Dixon *et al.* (2003) both published LCA studies to examine and compare the sustainability of small-scale conventional wastewater systems, while both studies provide a comprehensive overview of the environmental impacts of the process, they do not consider the financial or social implications of the process, which are likely to be a key design factors in a real world scenario.

Machado *et al.* (2007) used LCA to investigate and compare wastewater treatment options for small and decentralized communities; in this case the LCA was used partially as a decision tool. The researchers demonstrated that energy saving treatment processes such as slow rate infiltration and constructed wetlands have a lower environmental impact compared to a conventional environmental sludge plant. Additionally the researchers found that replacing steel and concrete with high density polyethylene (HDPE) resulted in reduced CO_2 emissions and abiotic depletion.

To date there has not been a life cycle assessment of wastewater treatment processes centred on anaerobic digestion; this is likely to be due to the complex nature of LCA and the many unknowns presented by these more novel processes and treatment units.

2.9.2 Anaerobic treatment flowsheet comparisons

Schafer *et al.* (2002) compared the performance on various types of advanced anaerobic digestion processes, however, they focused mainly on the anaerobic digestion of aerobic sludge, rather than using anaerobic treatment as the main form of wastewater treatment. Chrobak and Ryder (2009) compared anaerobic treatment alternatives for distillery wastewater. In this case the authors reviewed only two types of anaerobic unit: the upflow anaerobic sludge blanket (UASB) reactor and a low rate anaerobic lagoon. The UASB reactor was found, in this case, to be the favourable treatment option over a variety of process constraints, such as effluent quality, cost effectiveness and footprint; however, the authors did not consider other types of anaerobic reactor such as the anaerobic baffled reactor (ABR) or the SAMBR. There was little mention in the literature of any overall comparison between different types of anaerobic treatment in relation to domestic wastewater treatment, and how the different reactor types available might fit into an overall

wastewater treatment design. Therefore, there is a clear need for a design methodology to allow for the comparison and selection of anaerobic wastewater treatment technology designed to meet specific objectives such as energy recovery, low solids disposal, and reducing Greenhouse Gas emissions.

2.10 Summary

The overall picture gleaned from the literature is as follows:

1) Anaerobic digestion is a complex process using many pathways to break down complex organic molecules into methane and carbon dioxide, without the presence of oxygen. The growth of anaerobic organisms can be modelled using the Monod equation. The anaerobic digestion process has been shown to be a viable treatment alternative to conventional aerobic wastewater treatment for both strong and dilute wastewaters.
2) The submerged anaerobic membrane bioreactor has been widely demonstrated on a lab scale. Benefits of this technology include 100% solids retention, biogas production and low solids production. The capacity of the SAMBR to treat low strength wastewater has been shown with COD removals in the region of 90%.
3) There are many important parameters to consider when setting up a SAMBR, and the temperature of operation will determine the rate of digestion. While anaerobic digestion works best in the mesophilic range, several researchers have shown good operation of anaerobic reactors at temperatures as low as 5°C.
4) The major barrier to wider implementation of membrane bioreactor technology is one of fouling. The major component of membrane fouling is thought to be the deposition of extracellular polymers on the membrane surface. The deposits cause pore blocking and pore restriction, which can cause a reduction in flux.
5) Membrane fouling can be broadly classified into 3 types: removable, irremovable and irreversible. Removable fouling can be alleviated by manipulating the parameters of the SAMBR operation such as gas scouring rate and membrane relaxation. Irremovable fouling cannot be mitigated simply by changing the physical parameters, but can be removed through chemical cleaning. Irreversible fouling cannot be removed, even with chemical cleaning.
6) There are many parameters involved in SAMBR operation that can be controlled to mitigate or alleviate fouling. The most common method is the use of a gas bubbling to scour the surface of the membrane. Within gas scouring there are several different flow regimes, the most common used in membrane reactors is the slug flow regime. As well as controlling deposition on the membrane surface, gas bubbling also increases gas transport processes such that the effects of

concentration polarisation are minimised. While there has been much research into the issue of membrane fouling, there are still gaps in the knowledge surrounding the effect of rheology and the criticality of the gassing rate.

7) A big advantage of MBR technology is that due to the pore size of the membrane the biomass and other particulates are retained within the reactor, therefore bacterial pathogens are not released in the effluent. While the pore size in the membrane is usually greater than the size of the average virus, there have many reports in the literature that aerobic MBRs have shown significant removals of various viruses.
8) Research on virus removal in MBRs is frequently done using bacteriophages as viral indicators. The reason for this is that the bacteriophage are non pathogenic but share many similar properties to viruses as well as being easy to detect; the main phages used in experimentation are MS-2, Qβ and T4. Most of the work in this area has been done on aerobic MBRs, and very little work has been carried out on anaerobic reactors, and none on the SAMBR reactors.
9) Recently there has been a wealth of research into the use of activated carbon as an adsorbent to remove trace organics from wastewater, and also the use of activated carbon to improve SAMBR performance. In addition it has been reported that activated carbon is an ineffective method for removing viruses from wastewater, because the size of the virus particles means they cannot fully utilize the internal active sites on the carbon.

2.11 Research objectives

While the feasibility of the SAMBR to treat wastewater has been widely shown, there are still some gaps in the knowledge that would benefit further study. The aims of this thesis can be split into three sections; fouling and small particle throughput; virus removal; and, flowsheet modelling.

2.11.1 Fouling and small particle throughput

It is important to gain an understanding of the parameters involved in the fouling process in order to analyse the removal potential of the SAMBR reactors. In this section the parameters that affect membrane resistance were investigated. The aims of this section were:

- To investigate the parameters that affect the membrane resistance, including critical flux, permeability gas scour and biomass rheometry.
- To investigate the possibility of a critical gas scouring rate.
- To investigate small particle rejection in the SAMBR, and the effect fouling has on this.

2.11.2 Virus removal

There has been very little research in the area of virus removal in anaerobic membrane reactors. To investigate this phages were used as model organisms to indicate the passage of large and small viruses in the SAMBR. The aims of this section were:

- To monitor the log removal of bacteriophages MS-2 and T4 in a SAMBR
- To analyse the impact of the gas scouring rate on phage removal,
- To assess the impact of adding activated carbon to the SAMBR on phage removal.

2.11.3 Flowsheet modelling

The viability of many different types of anaerobic reactors has been proven in the literature. As such a direct comparison between the different anaerobic treatment options is required. In a holistic approach the entire wastewater treatment process is to be modelled with an anaerobic theme. The aims of this section were:

- To investigate the potential anaerobic treatment options that could be utilized in the UK today.
- To model a short-list of potential flowsheets with a focus on anaerobic digestion and contrast this to a conventional aerobic treatment option.
- To use a decision making process to recommend an optimal anaerobic flowsheet for waste water treatment.

Chapter 3. Methods

This chapter details the design of the Submerged Anaerobic Membrane Bioreactor (SAMBR) system. The operational parameters for the treatment of synthetic wastewater are also given. Finally, the analytical techniques for monitoring the reactor's performance are documented.

3.1 SAMBR design and build

The reactors used in this study were built by the departmental workshop, and consisted of two plates of cast acrylic plastic screwed together with an O-ring seal; the design drawings are shown in Figure 3-2 and Figure 3-3. The reactor can hold three litres of biomass with a one litre head space for the gas to be collected. The membrane envelope is submerged inside the main reactor unit and the effluent pumped out through it. The gas collected at the top of the reactor is pumped around the system and into a long stainless steel diffuser at the bottom of the reactor. The coarse bubbles from the diffuser are forced over the membrane due to the baffle in the unit to provide scouring. This scouring action is intended to minimise the build-up of foulants on the membrane. To ensure the SAMBR was digesting the feed, the influent and effluent COD were monitored at least every 48 hours along with the gas concentration in the head space.

The feed and effluent are pumped by variable-speed Watson-Marlow peristaltic pumps (model 101U). The effluent from the membrane was drawn off by a larger peristaltic pump (Watson-Marlow model 500 REH), due to the increased pressure demands of this line. The gas line uses a vacuum pump (Charles Austin, UK, model B100 SEC) to create the scouring bubbles in the reactor. The design also incorporated a pressure transducer to allow for the measurement of TMP, a level controller, and a flow sensor to determine the flux. A process flow diagram of the general reactor set up is shown in Figure 3-1.

3.1.1 Sampling

Samples were taken from the reactor using the sample ports shown in Figure 3-1. The influent and effluent sample ports (S1 and S4 in Figure 3-1) were t-valves. Under normal operation the flow continued along the pipe, during sampling the flow was switched such that all the flow was directed out of the pipe. Biomass samples from inside the reactor were taken from sample port S2, these samples were removed using suction via a plastic syringe and under normal operation this pipe was closed with a pinch clamp. The gas samples were taken through sample port S3, a PTFE septum, this was pierced with a fine needle syringe and the sample taken for analysis.

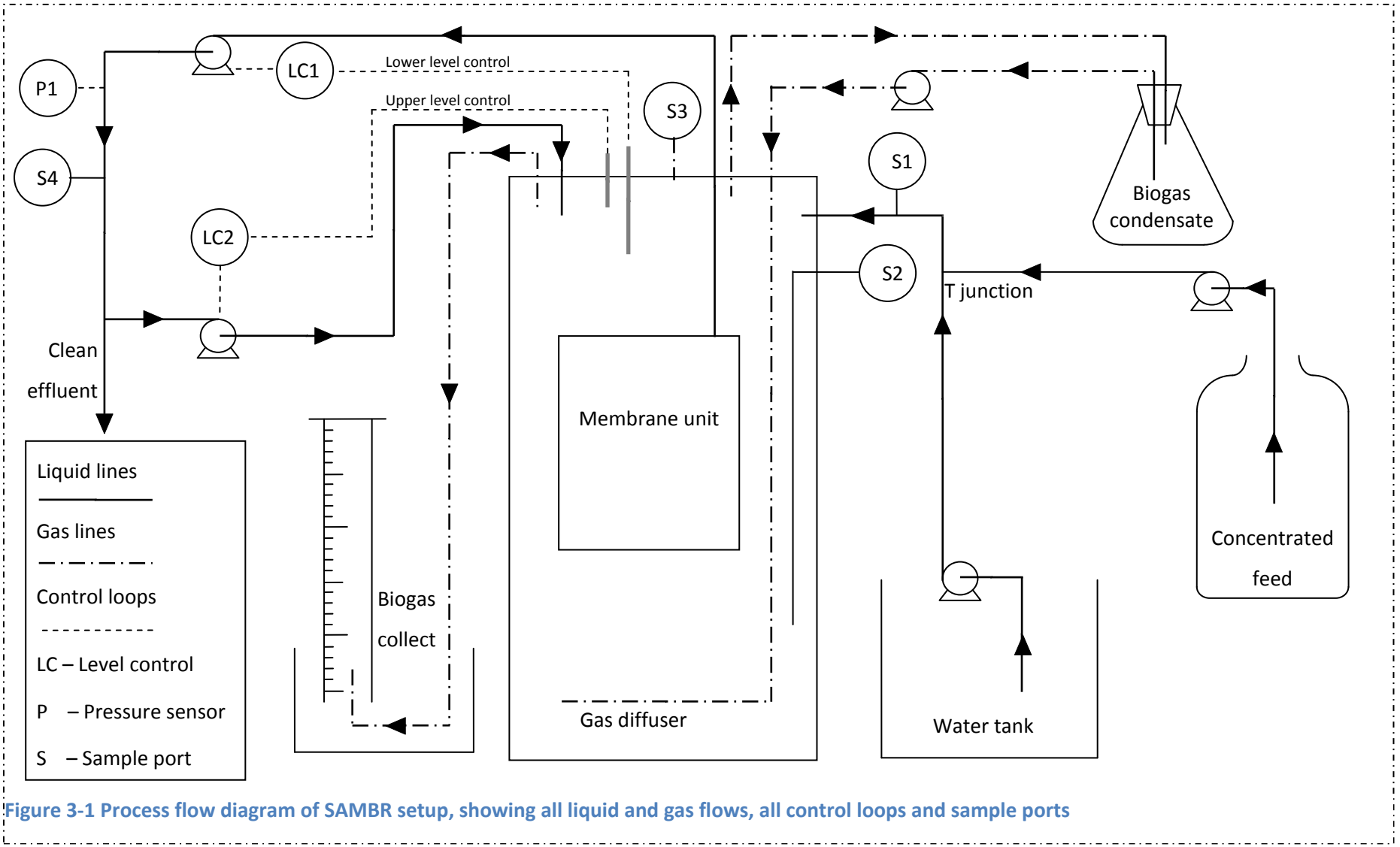


Figure 3-1 Process flow diagram of SAMBR setup, showing all liquid and gas flows, all control loops and sample ports

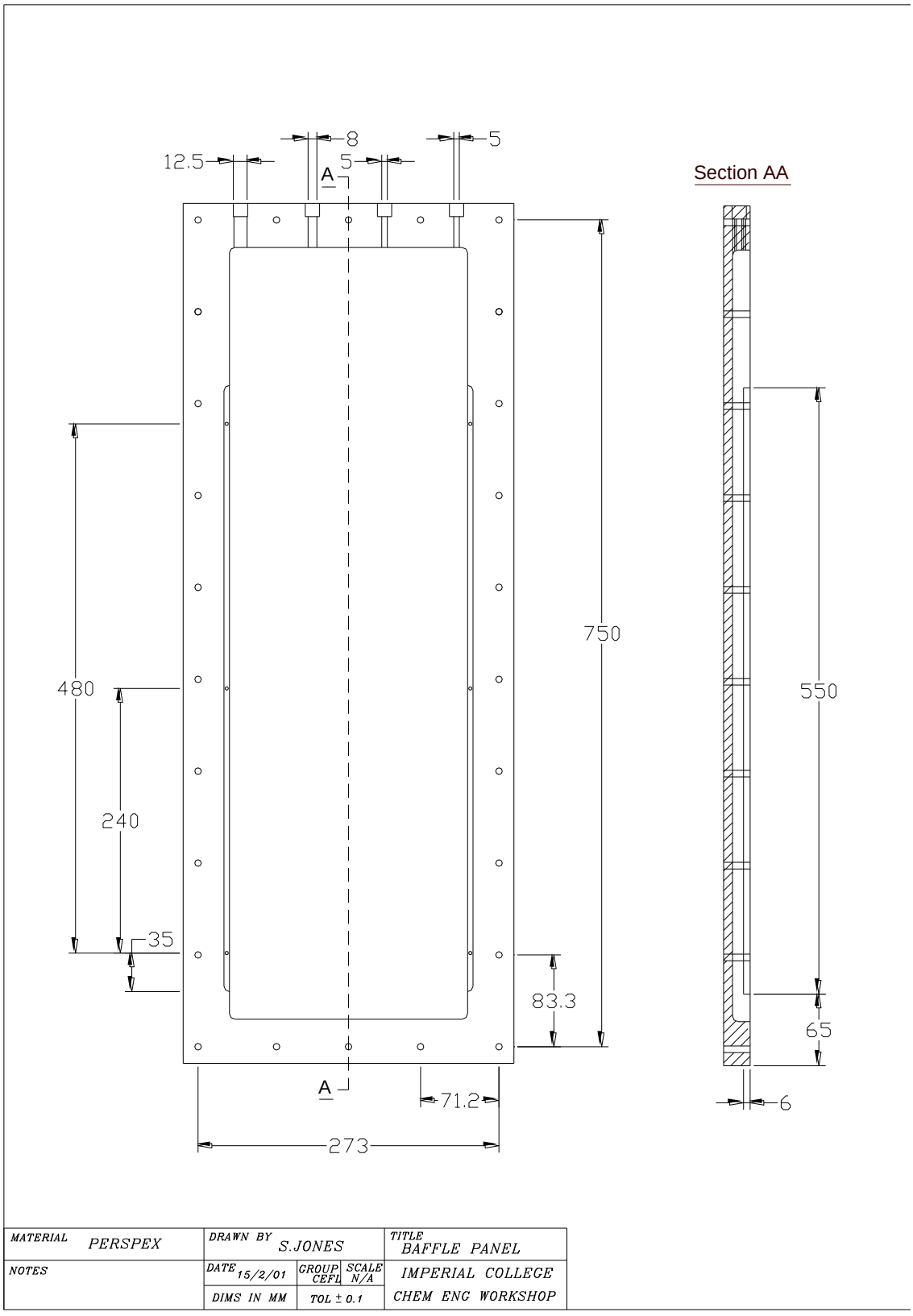


Figure 3-2 Detailed drawings of the SAMBR baffle panel designed by A. Hu drawn by S. Jones

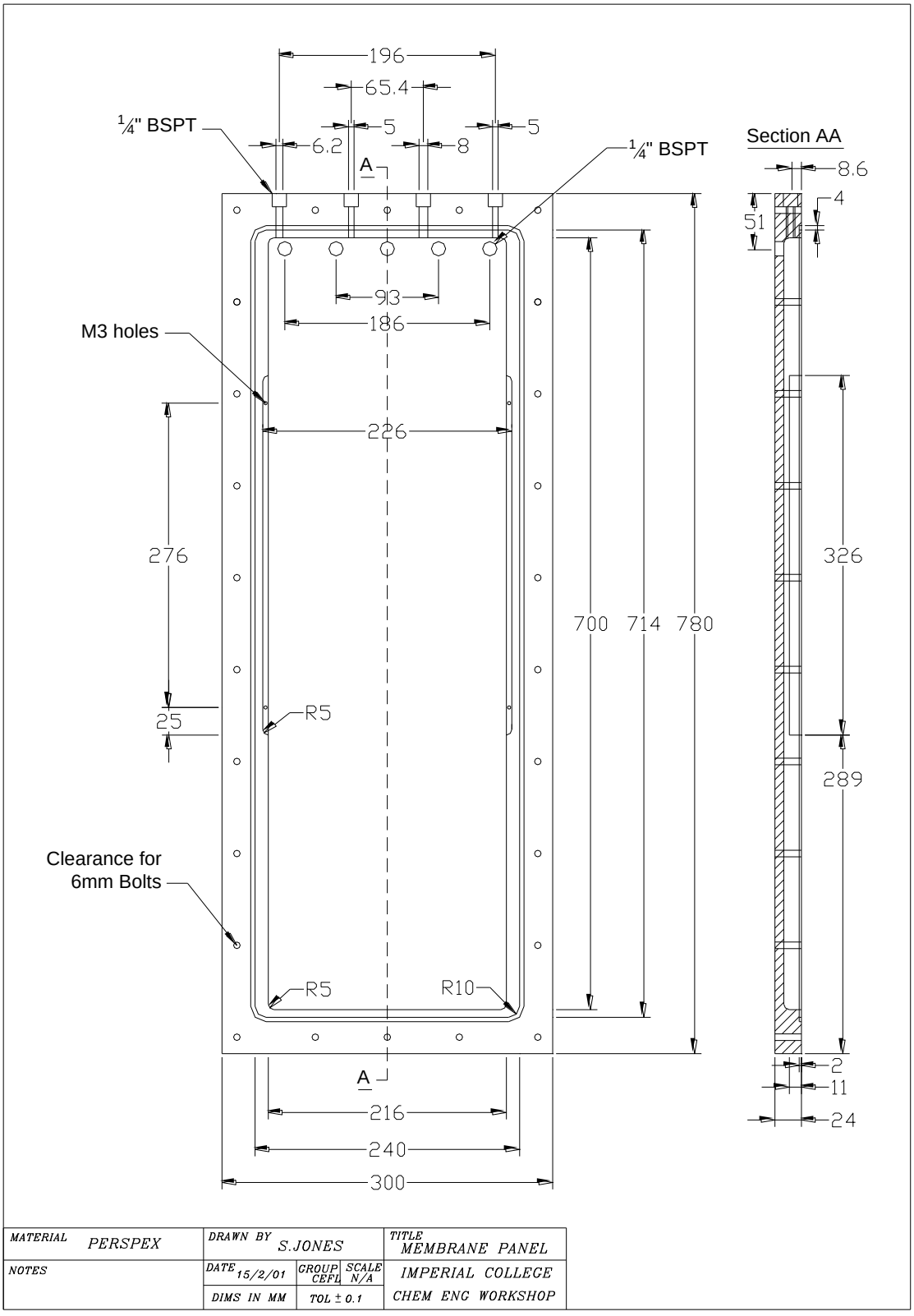


Figure 3-3 Detailed drawings of the SAMBR membrane panel designed by A. Hu drawn by S. Jones

3.1.2 Membrane

The membranes used in this project were Kubota type 203 modules which were kindly donated by Kubota, UK. The membrane module consisted of a solid acrylonitrile butadiene styrene support plate welded to the polyethylene flat sheet membrane. The pore size was 0.4 μm with a total membrane surface area of 0.11m^2.

3.1.3 Membrane access

Two slightly different reactor designs were used in this research, the original reactor design completed by A. Hu is shown in Figure 3-2 and Figure 3-3. In this case the membrane was encased inside the reactor, and to remove it for analysis the whole reactor unit had to be taken apart.

In order to allow for easier access to the membrane a second reactor was built. In this reactor a new top section was designed such that a segment could be removed from the top of the reactor which was wide enough for the membrane to be pulled out, as shown in **Error! Reference source not found.**. The segment was sealed into the reactor with an O-ring, and weighted down during operation to prevent high pressure inside the reactor causing a gas leak. To access the membrane the sparging pump was first switched off, and a mixture of 70% N_2 and 30% CO_2 was bubbled into the reactor through the sample port to maintain anaerobic conditions in the biomass when the membrane was lifted out.

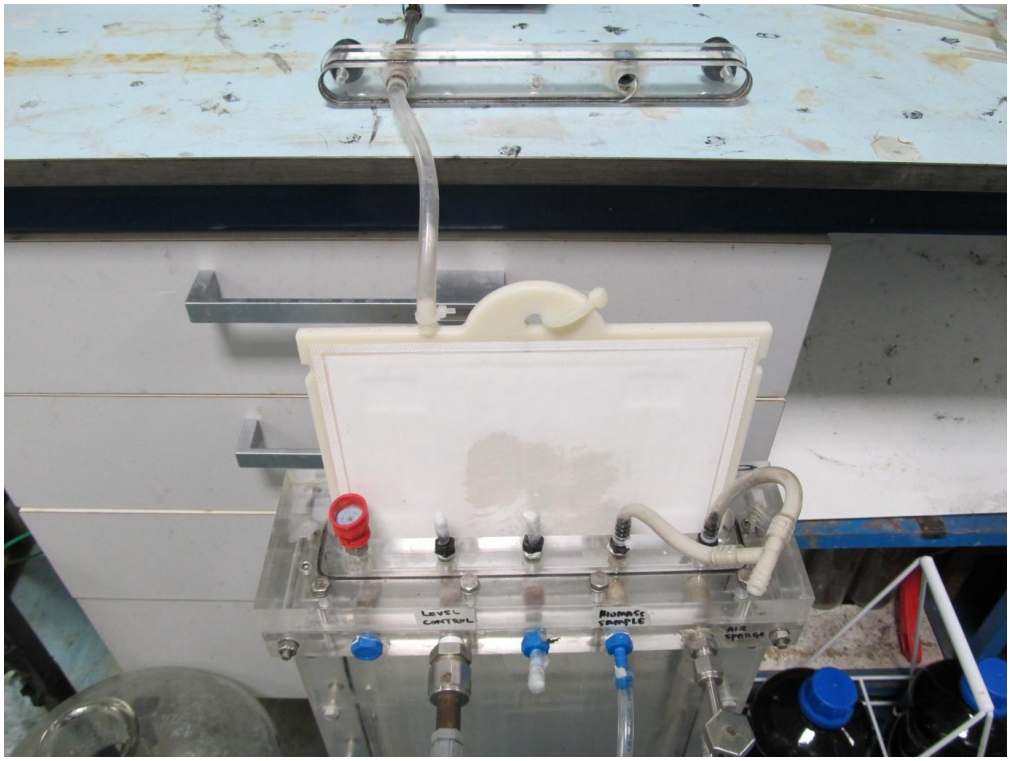


Figure 3-4 SAMBR design with membrane access photograph

3.1.4 Sparging control

The gas in the scouring line was on a constant recycle through the bulk liquid in the reactor, and consequently this gas was always close to its liquid saturation point. As a result, the condensation of water in the sparging line was a significant challenge during operation. To mitigate this, a condensate collector was added to the system which can be seen in Figure 3-1. This solution alleviated most of the condensation occurring in the gas line, however, a small amount of condensation built up inside the gas flow meter, and therefore a bypass system was setup as shown in Figure 3-5 . During normal operation the gas flow bypasses the flow meter (a 101 Flo-Sen, Cole Palmer). When the gas sparging rate needs to be checked the T junction valves are switched so all the gas is diverted through the flow meter and a reading can be taken before condensation forms on the ball causing errors.

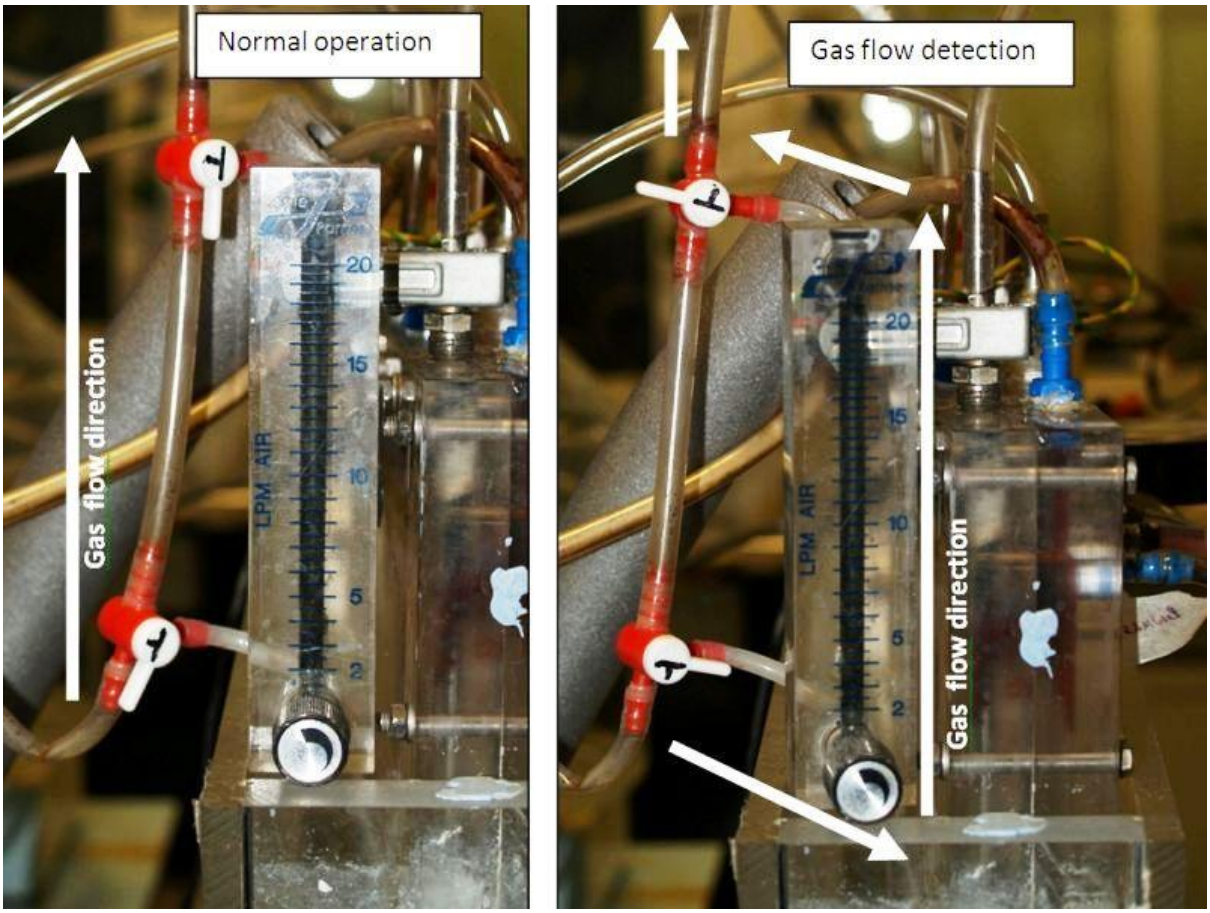


Figure 3-5 flow diversion for gas line to avoid condensation in the flowmeter

The sparging rate itself was set using a pinch valve just before the sensor setup which can be seen in Figure 3-5. The gassing rate was monitored using the flow meter which could be set accurately to ±0.25 litres per minute (LPM).

3.1.5 Biomass

The seed biomass was collected from the anaerobic digesters at Mogden sewage treatment works in West London. The sludge was screened through a 400µm screen and mixed with a biomedia solution (see Table 3-4 and Table 3-5). Stocks of biomass were kept in 2 and 5 litre batch reactors at 30°C and regularly fed with glucose, peptone and meat extract.

3.2 Synthetic feed

The synthetic feed used in the reactor was designed to mimic the organic makeup of municipal wastewater. The OECD (1993) synthetic sewage solution shown in Table 3-1 was used as a feed. The solution was made five times concentrated and diluted with deionised water before being pumped into the reactor- 300mg/l of $NaCO_3$ was also added to keep the pH around neutral. The feed strength was 460± 20 mg/l COD. The concentrated feed solution was autoclaved to stop it degrading in the storage container before it was required, and the COD was sampled at least every 48 hours to check the influent concentration.

Table 3-1 OECD synthetic wastewater makeup (OECD, 1993)

Chemical	***Concentration (mg/l)***
Peptone	200
Meat extract	140
$CaCl_2.2H_2O$	4
Urea	10
$MgSO_4.7H_2O$	2
K_2HPO_4	11
NaCl	7

3.2.1 Operational details

Throughout operation the HRT was set to 12 hours, this was set by measuring the flowrate of the continuous influent pump, along with the feed concentration of 460± 20 mg/l COD this corresponds to an organic loading rate (OLR) of 2.76 $gCOD.l^{-1}d^{-1}$.

3.3 Analytical Methods

3.3.1 Total and volatile suspended solids (TSS/VSS)

The measurement of Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) were performed as given in Standard Methods (APHA, 1999). The coefficient of variance for 5 identical samples was within ± 6%

3.3.2 pH

The pH was measured using a pH meter (Jenway, Model 3020) calibrated with standard pH solutions at pH 4, 7 and 10. Values obtained were accurate to within ± 0.02 units.

3.3.3 Gas composition

The composition of the gas produced by the reactor was analysed using a Shimadzu GC-TCD fitted with a Porapak N column (1500 × 6.35 mm). The carrier gas used was Helium with a flow rate of 16 ml/min, while the column temperature was set at 28°C, the detector temperature at 38°C and the injection temperature was 128°C. The peak areas were calculated and printed out on a Shimazdu Chromatopac C-R6A integrator.

3.3.4 Chemical oxygen demand (COD)

COD measurements were based on the Standard Closed Reflux Colorimetric Method described in section 5220-D of Standard Methods, the digestion solution, and the sulphuric acid reagent were made up according to the directions in Standard Methods (APHA, 1999). 1 ml samples were added to Hach reflux tubes along with 0.6ml of the digestion solution and 1.4ml of the sulphuric acid reagent. The tubes were sealed tightly and inverted several times to aid mixing. The tubes were then placed in a Hach COD reactor (Model 45600) and left to reflux at 150 °C for 2 hours. When cooled the absorbance of each sample at 600nm was measured using a Shimadzu Spectrophotometer (Model UV-2101/3101 PC). A calibration curve was constructed using standard solutions made using potassium hydrogen phthalate (KHP)- this chemical has a stable COD of 1.18 mgO_2/mg a calibration curve is shown in appendix A. Each COD sample was analysed in triplicate to give an average value. The coefficient of variance for 5 identical samples was ± 4%

3.3.5 Extra cellular polymeric substances (ECP)

There are several methods available for the measurement of ECP, and these have been assessed by Zhang *et al.* (1999). The steaming method will be used in this report since it had the highest protein yields, and the method is as follows:

10 ml samples of anaerobic sludge were centrifuged at 3500 rpm (Biofuge Stratos, Heraeus Instruments) for 20 minutes at 4°C. The pellets obtained were re-suspended in deionised water. This suspension was then steamed in a water bath at 80^0C for 10 minutes to release the ECP into the supernatant. Once steamed, the samples were centrifuged again at 13000 rpm for 20 minutes immediately while the samples are warm. The supernatant was then filtered through a 0.45 µm filter to ensure complete separation of the cells from the supernatant. The filtered supernatant solution contained the extracted soluble ECP. Samples were analysed using either the COD test (to determine the relative amount of ECP present) or size exclusion chromatography (to determine the particle size fraction).

3.3.6 Volatile fatty acids (VFA)

Volatile fatty acids, formic, acetic, propionic, isobutyric, butyric, isovaleric and valeric acids were measured on a Shimadzu (model 10A) HPLC system with an auto-sampler using an Aminex HPX-ion exclusion column (300 mm x 7.8 mm). The sample volume was 50 µl, while the column was maintained at 55°C, and the eluent was 0.005 M H_2SO_4 at a flow rate of 0.68 ml/min. VFAs were detected with ultra-violet (UV) light at 210 nm. The detection limit was 5 mg l^{-1} for VFAs, and the coefficient of variance for each VFA is shown in

Table 3-2. Each sample was assessed in triplicate and the calibration data for each acid across the range used is shown in Appendix A.

Table 3-2 VFA coefficients of variance

VFA	Coefficent of variance (%)
acetate	2.09
propionate	1.64
isobutyrate	3.28

butyrate	5.48
isovalerate	3.59
valerate	16.86

3.3.7 Phage and E. Coli solutions

Table 3-3 Phage media solutions

Nutrient Broth	Phage buffer	Overlay agar	Blood agar base
10g/l peptone 10g/l meat extract 5g/l NaCl	50ml - 1M tris/HCl 2g - $MgSO_4$ 5ml - 2% gelatine + distilled water up to 1000ml	10g/l peptone 10g/l meat extract 5g/l NaCl 4g/l Agar	10g/l peptone 10g/l meat extract 5g/l NaCl 15g/l agar

3.3.8 E coli host propagation

A freeze dried culture of Escherichia Coli NCIMB accession number 9481, (ATCC12435 or DSM5695) was obtained from the NCIMB culture collection. This is a non-pathogenic strain that can be used as a host for both MS-2 and T4 phages. 5ml of the nutrient broth was pipetted into a 10ml conical flask which was sealed with a non-absorbent cotton wool bung and a foil wrap over the top. The broth was then autoclaved for 20 minutes at 121°C.

When the broth had cooled, it was inoculated with a colony of E. coli from a solid agar plate. The inoculated solution was left to grow overnight (12-16 hours) at 30°C on a shaking tray at 200rpm to allow the culture to reach the log-stationary phase. Using an inoculation loop, the E. coli culture was streaked onto a solid agar plate and left to grow overnight; this plate was then sealed with Parafilm and kept at 4°C to provide the initial colonies for further cultures. At 4°C these plates can be stored for up to 6 weeks.

After streaking, the rest of the E. coli culture was centrifuged at 3000g for 15 minutes, the supernatant was removed and the E. coli pellet resuspended in 10ml of phage buffer. The optical density of this E. coli solution was in the region of 1.8. This E. coli suspension was kept at 4°C and remade on a weekly basis.

3.3.9 Phage Enumeration (double layer method)

The Phage enumeration assay was performed using the double layer method (Kropinski *et al.*, 2009). Phage buffer, overlay agar and blood agar base media solutions were made up according to the concentrations displayed in Table 3-3; all solutions were sterilised by autoclaving at 121°C for 15

minutes. All samples to be assayed were centrifuged at 3000g for 15 minutes, and the supernatant was filtered through a 0.45µm syringe filter.

100µl of each sample was pipetted into 900µl of phage buffer solution, and vortexed to mix. Serial dilutions were made using the phage buffer up to 10^{-7}. Meanwhile the blood agar base was melted and approximately 10ml of the agar was spread evenly onto individual petri dishes; these were left to set and dry before use. The agar overlay solution was also melted and 3ml samples of the soft agar solution were pipetted into snap cap tubes and held in a water bath at 46°C.

100µl of the diluted phage solution and 100µl of the E.coli suspension was added to a warm agar overlay tube. The tubes were gently vortexed to avoid bubble formation, and then poured over the solid agar on the petri dishes. The plates were left for 5 minutes to set and then inverted and placed in a 35°C incubator overnight. A control plate containing only E. coli solution and no phage sample was also set up to check that the E. coli suspension had not become infected with phage. Where a phage had infected the E. coli a clear plaque appeared on the E. coli lawn, plates containing 10-300 plaques were selected for counting. The coefficient of variance for 5 samples at a 10^{-7} dilution was ± 2.3% (in log form) or ± 53% in decimal numbers.

3.3.10 Phage propagation

After the E. coli suspension was grown, the phages were propagated. A liquid sample of phage was obtained from the NCIMB (accession number 10108 for MS-2 and 10423 for Phage T4). 100 µl of the phage sample was added to 900µl of phage buffer, and serial dilutions of the phage sample were made up to 10^{-9}. The phages were then plated using the same double layer method described above for enumeration. When the E. coli and phage had grown, those plates showing confluent lysis were selected for propagation. 5ml of phage buffer was pipetted onto the plates, and these plates were placed onto an orbital shaker at 100rpm for 1 hour to elute the phage.

The eluted phage solution was then centrifuged at 3000g for 15 minutes to separate the phages from any cell debris. The supernatant was filtered through 0.45µm cellulose acetate syringe filters to create a concentrated phage solution. If kept sterile this phage solution can be stored at 4°C for several months.

3.3.11 Biochemical methane potential

The biochemical methane potential test was based on the media and serum bottle method developed by Owen *et al.* (1979). The media solution was made up according to the solution data in Table 3-4 and Table 3-5, while biomass was taken from a stock solution of known VSS kept at 30°C. The stock of biomass was fed regularly with glucose to keep it active. The solution in the serum bottles contained a concentration of 1g/l VSS and 1g/l of the substrate COD. Once filled the serum bottles were purged with a gas mixture of 70% N_2 30%CO_2 at approximately 0.5l/min to remove any oxygen from the headspace. The serum bottles were sealed with a PTFE septum and aluminium crimp cap.

Table 3-4 stock solutions made up for biomedia

Solution number	Compound	concentration g/l
S1	Sample	1 g/l COD
S2	reasazurin	1
S3	$(NH_4)2HPO_4$	26.7
S3 trace elements	$CaCl_2.2H_2O$	16.7
	NH_4Cl	26.6
	$MgCl_2.6H_2O$	120
	KCl	86.7
	$MnCl_2.4H_2O$	1.33
	$CoCl_2.6H_2O$	2
	H_3BO_3	0.38
	$CuCl_2.2H_2O$	0.18
	$Na.MoO_4.2H_2O$	0.17
	$ZnCl_2$	0.14
S5	$FeCl_2.4H_2O$	370
S6	$NaS.9H_2O$	500
S7 trace vitamins	Biotin	0.002
	Folic acid	0.002
	Pyridoxine hydrochloride	0.01
	Riboflavin	0.005
	Thiamin	0.005
	Nicotinic acid	0.005
	Pantothenic acid	0.005
	B12	0.0001
	p-aminobenzoic acid	0.005
	Thioctic acid	0.005

Table 3-5 biomedia made-up from stock solutions

Stock solution	volume ml
S2	1.8
S3	5.4
S4	27
S5	1.8
S6	1.8
S7	18

The samples were triplicated and set on a 180rpm shaking tray in a 30 ±0.5°C room. Methanogenic activity was measured by the amount of gas produced in the head space of the serum bottle. Gas production was measured using pressure equalisation with glass syringes, the syringes were handled as briefly as possible to reduce any heat transfer to the syringe. The gas composition was also analysed using the method detailed in Section 3.3.3. Triplicate samples were assessed, and the coefficient of variation for the production of methane was ±7%.

3.3.12 Size exclusion chromatography

Size exclusion chromatography was performed in a Shimadzu HPLC (model 10-A) using an Aquagel OH-30 column (polymer labs). 18.2MΩ deionised water was used as the eluent at a flow rate of 1ml per minute. The column was maintained at ambient temperature, and a Refractive Index (RI) detector was used to detect the separated compounds. Standards of polyethylene glycol and polyethylene oxide were used to identify the retention times of certain molecular weight compounds, and hence the results are quoted relative to these compounds.

3.3.13 Viscosity

Samples were measured using a Physica UDS 200 rheometer using a double gap attachment. Samples were filtered through a 400µm sieve to remove any large particles that could block the machine. The sample volume was 22.5 ml and the measurements were taken at 30°C; the system temperature was maintained using a Physica VT2 thermostat. The shear stress was increased in increments of 10 s^{-1} every ten seconds.

Chapter 4. Membrane Fouling and Rejection

4.1 Introduction

One of the main barriers to full-scale implementation of the MBR process is the challenges associated with membrane fouling. Once fouling has occurred on the membrane, either the flux is reduced, or the suction pressure required to achieve a constant flux increases; in either case more energy is required to generate the product. To this end membrane fouling has been widely studied in an effort to understand the fouling mechanisms, and to determine the optimal operational parameters to minimise its occurrence.

Membrane fouling in aerobic wastewater treatment has been extensively reviewed by Le-Clech *et al.* (2006). While there are certainly similarities between anaerobic and aerobic membrane reactors, the microbial ecology of anaerobic biomass is quite different to aerobic biomass, and therefore it is important to fully evaluate the effects of anaerobic biomass on fouling. There have been studies into the effects of fouling in anaerobic MBRs, and the key details of these studies are reviewed in Chapter 2. In essence the main problem caused by fouling is the increase in resistance across the membrane. The formula for resistance (equation 2-6) shows that the resistance is governed by three factors; TMP, flux and viscosity. Therefore, it is important to consider each of these factors in turn to gain an understanding of the critical parameters that control the overall resistance, as well as methods of mitigation.

With a greater understanding of the membrane fouling process, and mitigating factors involved in membrane fouling, it will be possible to optimise the membrane reactor process to maximise the output with minimum energy input. The aims of this study were to investigate the major parameters involved in membrane fouling, including critical flux, gassing rate, biomass rheometry and membrane permeability with respect to SAMBR operation. Once these critical parameters have been established the effect of small particle removal in the membrane was also considered.

4.2 Methods

Two reactors, SAMBR B and C, were operated to obtain the results in this chapter. The reactors were fed with the OECD feed listed in Chapter 3 at a constant influent COD of 460 $\pm$ 30mg/l. Both reactors were started with a membrane which had been cleaned according to the protocol for Kubota membranes listed by Le-Clech *et al.* (2006). The two reactors were both started up in the same manner with a biomass concentration of 3 g/l. One SAMBR was operated with a scouring rate of

10LPM and a flux at 7.2 LMH, while the other SAMBR was operated with the same flux but at a varying gassing rate.

The analytical methods were carried out as described in Chapter 3, while the trans-membrane pressure was monitored using a pressure transducer connected to a computer data logger. The pressure in the headspace of the SAMBR was determined using a simple manometer constructed from some 5mm diameter PVC tubing fixed to a stiff background.

Membrane permeability was calculated using equation 4-2 Where L_p is the permeability, J is the flux (LMH) and TMP is the transmembrane pressure in bar. In order to maintain a constant flux the membrane effluent pump was set to a constant value, while the corresponding flux was checked manually to check for any deviation from this figure.

$$L_p = \frac{J}{TMP} \qquad 4\text{-}1$$

4.3 Results

4.3.1 Critical flux

The critical flux of any membrane process is an important parameter to determine as it allows the maximum output (effluent flowrate) to be achieved for the minimum input (pumping pressure). Critical flux is defined as: 'the flux below which a decline of flux with time does not occur' (Field *et al.*, 1995). In this case the pragmatic critical flux was determined using the flux step method proposed by Le Clech *et al.* (2003).

4.3.1.1 Pragmatic critical flux

The effluent flux was controlled using the membrane peristaltic pump which was increased in steps of 2 rpm every 20 minutes. The corresponding TMP at each flux step was monitored on line every 4 minutes. This experiment was performed on a SAMBR that had been operating for 2 weeks to ensure steady state operation had been reached; the flux was kept constant in the initial run at 7.2 LMH. The gassing rate was maintained at 6LPM throughout and the VSS was 4.5±0.5 g/l. The operational data for the SAMBR during these experiments is shown in appendix B.

The data (Figure 4-1) shows that there was virtually no increase in TMP at pump speeds of 4-8 rpm for fluxes from 4.8 to 9.5 LMH. This indicates that there was little or no fouling occurring on the membrane surface during this time. When the pump speed was increased to 10 rpm, (a flux of 11.8 LMH) a slight increase in TMP was observed, suggesting some fouling build-up had occurred on the membrane surface; however, the TMP remained constant throughout the experiment. After this the

pump speed was further increased to 12 rpm (a flux of 14.6 LMH), here the TMP increases gradually throughout the run, suggesting that at this point gradual fouling was occurring. At higher pump speeds the TMP increased rapidly, as the fouling layer built up quickly. Therefore, in this study the critical flux was determined at 11.8 LMH, as marked on Figure 4-1.

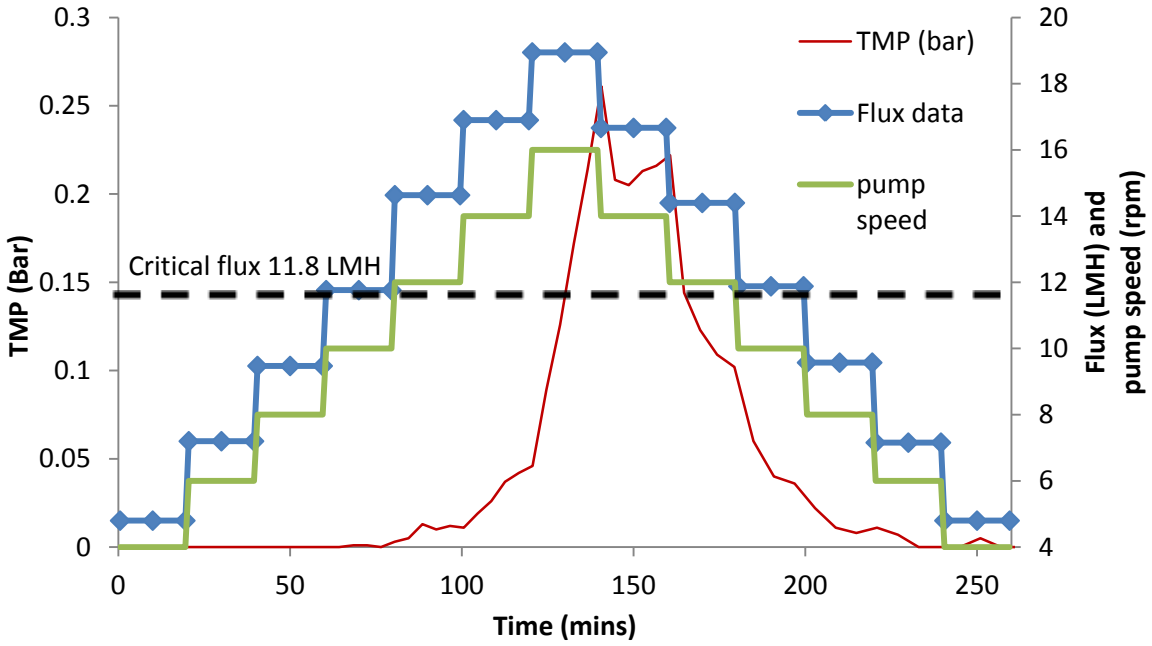


Figure 4-1 Critical flux experiment performed on a clean membrane at start up showing critical flux to be 11.8 LMH

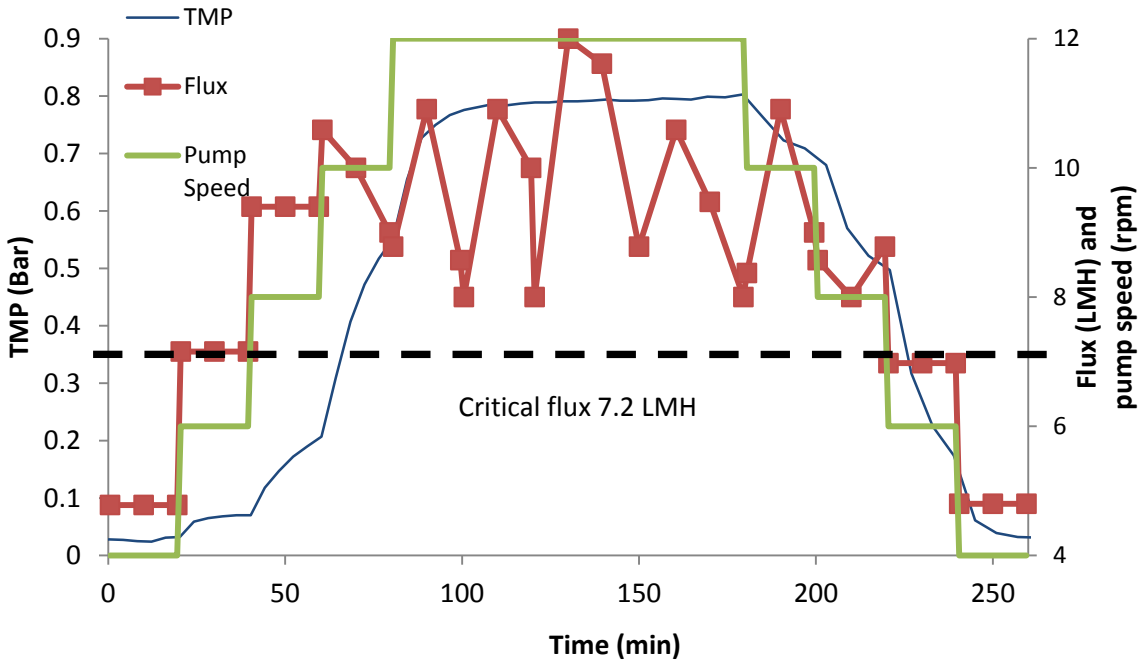


Figure 4-2 Critical flux after operation at low gassing rates showing critical flux to be 7.2 LMH

This is within the range determined by Liao *et al.* (2006) for the operation of anaerobic membrane bioreactors. It is, however, a much lower critical flux than that determined by Hu (2004) who determined a critical flux of 17.5 LMH on a similar SAMBR. Hu used a higher VSS concentration (7 g/l compared to 5 g/l in this work) and therefore it would be expected that the critical flux in this case would be higher than in Hu's work. Since this is not that case it must be assumed that another factor was having a controlling effect on the critical flux parameter. Hu's work involved the use of fresh biomass, in this case the biomass was from an established culture that had been running in the reactor for over a month. This difference in critical fluxes is most likely due to the increased colloid faction and non volatile suspended solids in the reactor, similar to that observed by Chen *et al.* (1997).

When the membrane flux was reduced the TMP did not fall back to its original value, and this effect has been demonstrated by many other researchers (Chen *et al.*, 1997; Judd and Judd, 2006a; Le Clech *et al.*, 2003). This observation points towards some irremovable fouling on the membrane that cannot be removed by gas scouring.

4.3.1.2 Critical flux after operation at low gassing rates

Critical flux is commonly determined for a clean membrane under steady state conditions at the beginning of an MBR's setup. As stated in the previous section, some irremovable fouling builds up on the membrane when operation beyond the critical flux has occurred. This suggests a degree of hysteresis in the critical flux value for individual membranes.

For this experiment the SAMBR had spent the 10 days prior to the critical flux test with the gassing rate at 2LPM, causing a marked increase in TMP. This allowed any irremovable fouling to occur. The gassing rate was reset to 6 LPM 3 days prior to the experiment, so that any removable fouling would be lifted off by the gas scour.

Figure 4-2 shows that even at the beginning of the experiment the TMP had not quite returned to zero, although the TMP remained steady at the lowest pump speeds of 4 rpm (4.8 LMH). When the pump speed was increased to 6 rpm the TMP jumped slightly to 0.07 bar, but remained stable for the period at that flux (7.2 LMH). After the pump speed was increased again to 8 and 10 rpm the TMP increases dramatically, while at 12rpm the TMP appears to level off. This is due to the maximum possible suction on the membrane pump being achieved, and hence the pump speed was increased no further. In this experiment the critical flux was determined to be 7.2 LMH, which is a 40% reduction on the previous experiment. This drop in critical flux is similar to that observed by

Jeison and Van Lier (2007), in their work the authors found that over long term operation (300 days) the critical flux fell to between 7 and 3 LMH.

It can also be seen in Figure 4-2 that once the TMP had passed 0.45 bar the flux no longer remains constant. This flux variation is due to the stress exhibited on the effluent pump; because the pump was operated beyond its suction capacity the required flux step increases could not be maintained, and hence the flux varies on the graph. The high stress on the membrane pump means that the flux does not reach a stable value until the pump speed is reduced back to 6 rpm.

This critical flux experiment is interesting because it shows that there is a degree of hysteresis in the membrane properties. While the reactor had been operated under the same flux conditions as the previous experiment, the gassing rate prior to experimentation was much lower. This suggests that operation at low gassing rates has caused some irremovable fouling that the increase in gassing rate cannot alleviate. The effect of gassing rate on membrane fouling is considered further in the next section.

From an operational perspective it is not desirable for this drop in critical flux to occur because it would result in a drop in plant output capacity, or an increase in energy demand. To ensure the TMP remains low in MBR operation, other parameters which can cause a similar effect should be considered.

4.3.2 Critical gassing rate

On introducing critical flux Field *et al.* (1995) proposed: "*The critical-flux hypothesis is that on start-up there exists a flux below which a decline of flux with time does not occur.*" Since then there have been several variants in the definition of critical flux, most notably that by Defrance and Jaffrin (1999), who state that "*Fouling is present below the critical flux but changes dramatically when critical flux is reached, leading to a steep rise in TMP".* It can be supposed that there is large number of other parameters for which a similar statement can be made; here it is considered that there also exists a critical gassing rate which when reached causes a steep rise in TMP. While there has been some previous work on this area, it has not been studied for anaerobic systems. Previous works on gassing rate (or aeration demand) have been focussed on its effect on the critical flux (Bouhabila *et al*., 1998; Ueda *et al,*. 1997; Guglielmi *et al*., 2007), rather than as a standalone parameter, as discussed in section 2.7.5.3. The idea of a critical aeration rate was proposed by Monclus *et al*. (2010), and they confirmed the existence of a critical aeration demand for an aerobic hollow fibre MBR.

The experiments for this section were carried out in a similar manner to the flux step method proposed by Le Clech *et al.* (2003) for the determination of critical flux. The gassing rate was set initially at the highest tolerable flow rate of 10 LPM (beyond this gassing rate foaming significantly interfered with operation). The flux during this operation was kept constant at 7.2 LMH which was the lowest critical flux determined in section 4.3.1. Once stable operation was achieved the gassing rate was decreased in a stepwise manner down to the lowest rate of 2 LPM. Re-setting the gassing rate in the SAMBR causes a change in the level sensor, so there was some pressure fluctuation each time the gassing rate was reset while the SAMBR settled into a new equilibrium; therefore, at each gassing rate the SAMBR was run for 24 hours to ensure an accurate TMP recording was achieved. The critical gassing rate experiment was carried out on a SAMBR under continuous operation with an established biomass; before the experiment was started the membrane was removed from the reactor and cleaned. The COD removal data and operational parameters for the SAMBR during this experiment are shown in appendix B.

It can be seen in Figure 4-3 that the TMP remained negligible for gassing rates between 10 and 4 LPM, except at the point of change where the resetting of the gassing rate caused a fluctuation of the internal pressure of the system. This suggests that at this particular flux there was no significant deposition on the membrane for gassing rates as low as 4 LPM. As soon as the gassing rate was lowered to 2 LPM the TMP started to rise dramatically. This fast increase in TMP suggests that 2 LPM is well past the point of any critical gassing rate. For any practical application of this technology the gassing rate would need to be kept above this value to avoid excessive fouling.

For each gassing rate a line of best fit was plotted, and the gradient displayed in Table 4-1, in the literature critical flux can be defined as the last flux (in this case gassing rate) before dTMP/dt raises above an arbitrary value, usually given as 0.1 mbar/min (Le Clech *et al.* 2003; Monclus *et al.* 2010). From Table 4-1, it is clear to see that there is sharp jump in dTMP/dt between gassing rates of 4LPM and 2LPM; from 0.003 mbar/min at 4LPM (below the critical threashold) up to 1 mbar/min at 2LPM (well above the critical threashold). From this data the critical gassing rate for this experiment can be said to be 4 LPM.

After the SAMBR operations at 2LPM had been concluded the gassing rate was once again raised in a stepwise fashion in-keeping with the previously discussed experimental protocols. It can be observed that at the end of the operation at 2 LPM in Figure 4-3 the TMP had dropped to around 0.51 bar from a maximum of 0.65 bar; at the beginning of Figure 4-5 the TMP at 2 LPM has dropped further to 0.45 bar. This is thought to be due to some settling occurring; at such low gassing rates, not enough turbulence is introduced to prevent settling (of the bacterial flocs) from occurring.

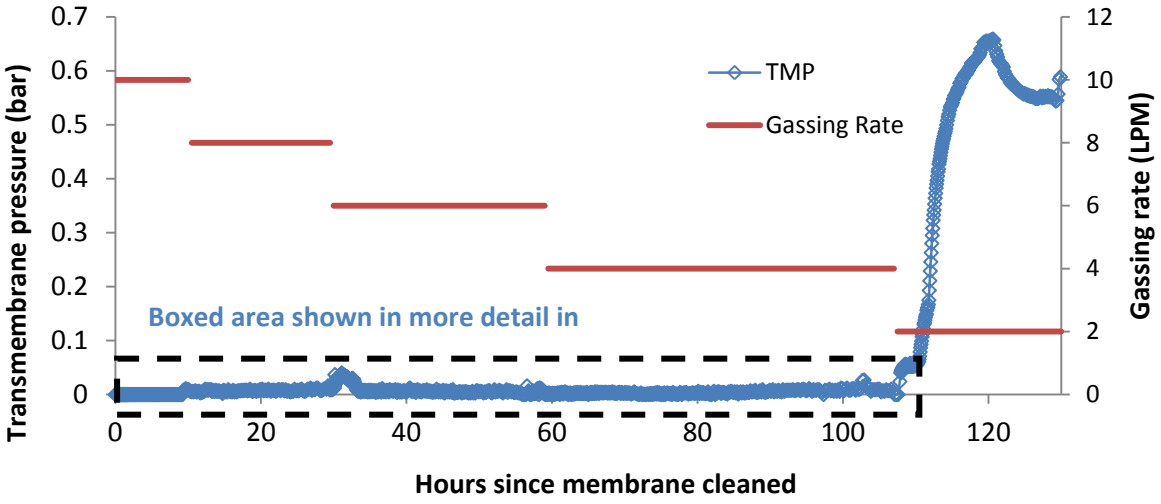


Figure 4-3 Critical gassing rate decreasing gassing rate from 10LMH to 2LMH over 120 hours showing the corresponding increase in TMP, such that the critical gassing rate is observed at 2LPM

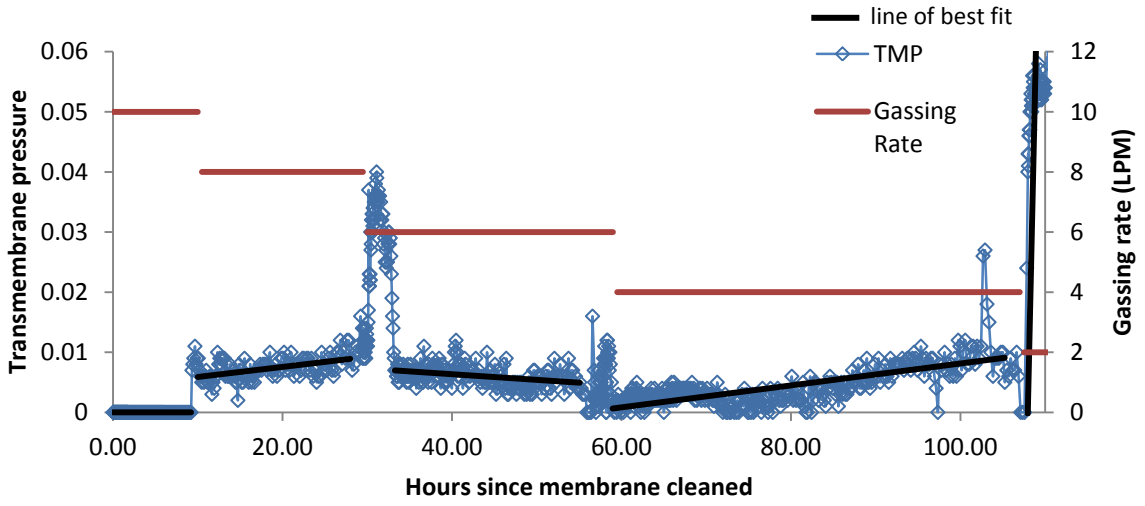


Figure 4-4 Critical gassing rate data showing the low TMP, this is a zoomed in graph of the data in the dashed box in Figure 4-3, with the plotted lines of best fit for each gassing rate

Table 4-1 dTMP/dt data for each gassing rate (relating to Figure 4-4), demonstrating the critical gassing rate to be at 4LPM becasue it is the last gassing rate before the rate of TMP increase exceeds 0.1 mbar/min

LMH	dTMP/dt (mbar/min)
10	0.0000
8	0.0028
6	-0.0016
4	0.0030
2	1.0504

When the gassing rate was increased to 4 LPM the SAMBR developed a problem with foaming; this caused the level control mechanism to fail which resulted in the TMP fluctuations circled in the figure. By manually overriding the level control system, the foaming layer was removed and standard reactor operation continued. Once the foaming had been alleviated the reactor settled down to a steady TMP of 0.22 bar. The TMP continued to fall as the gassing rate was increased the exception between 465 and 490 hours where the gas pump was mistakenly set to 4LPM). the TMP continued to fall, it did not drop down to the low TMPs observed in

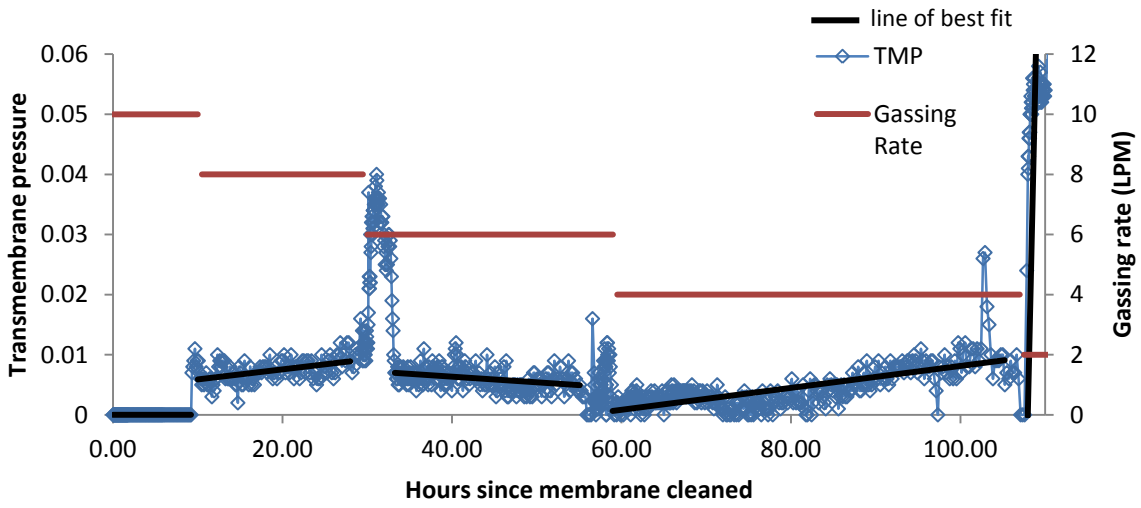


Figure 4-4; this suggests that while the gas scouring does remove fouling, it does not remove the entire fouling layer similar to the results found by Ueda *et al*. (1997) for an aerobic MBR. Therefore, it is better from a practical stand point to operate above the critical gassing rate to avoid any fouling build-up that cannot be alleviated by an increased gas scour.

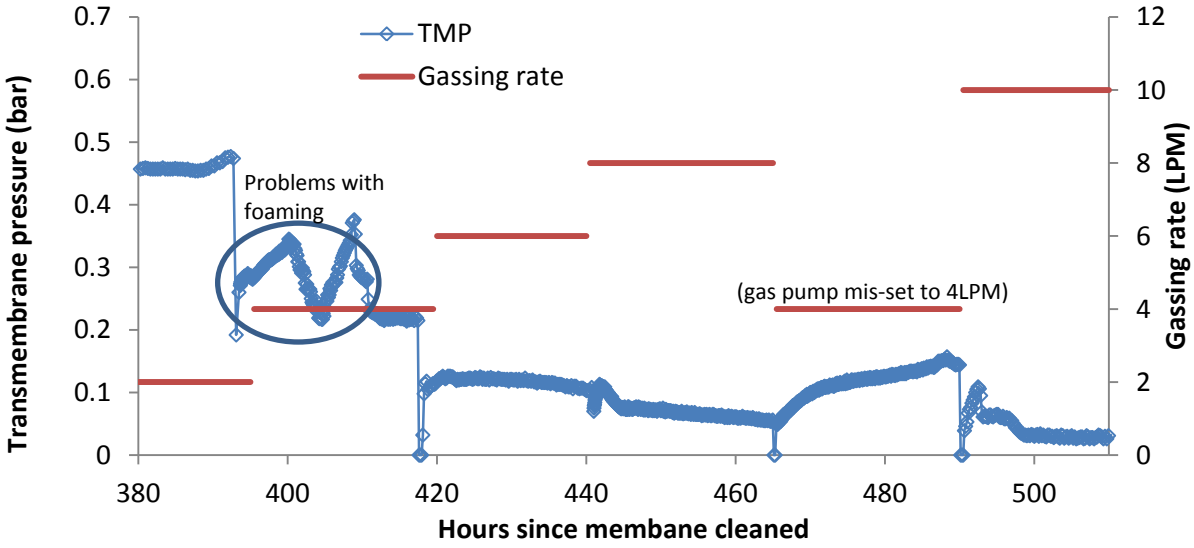
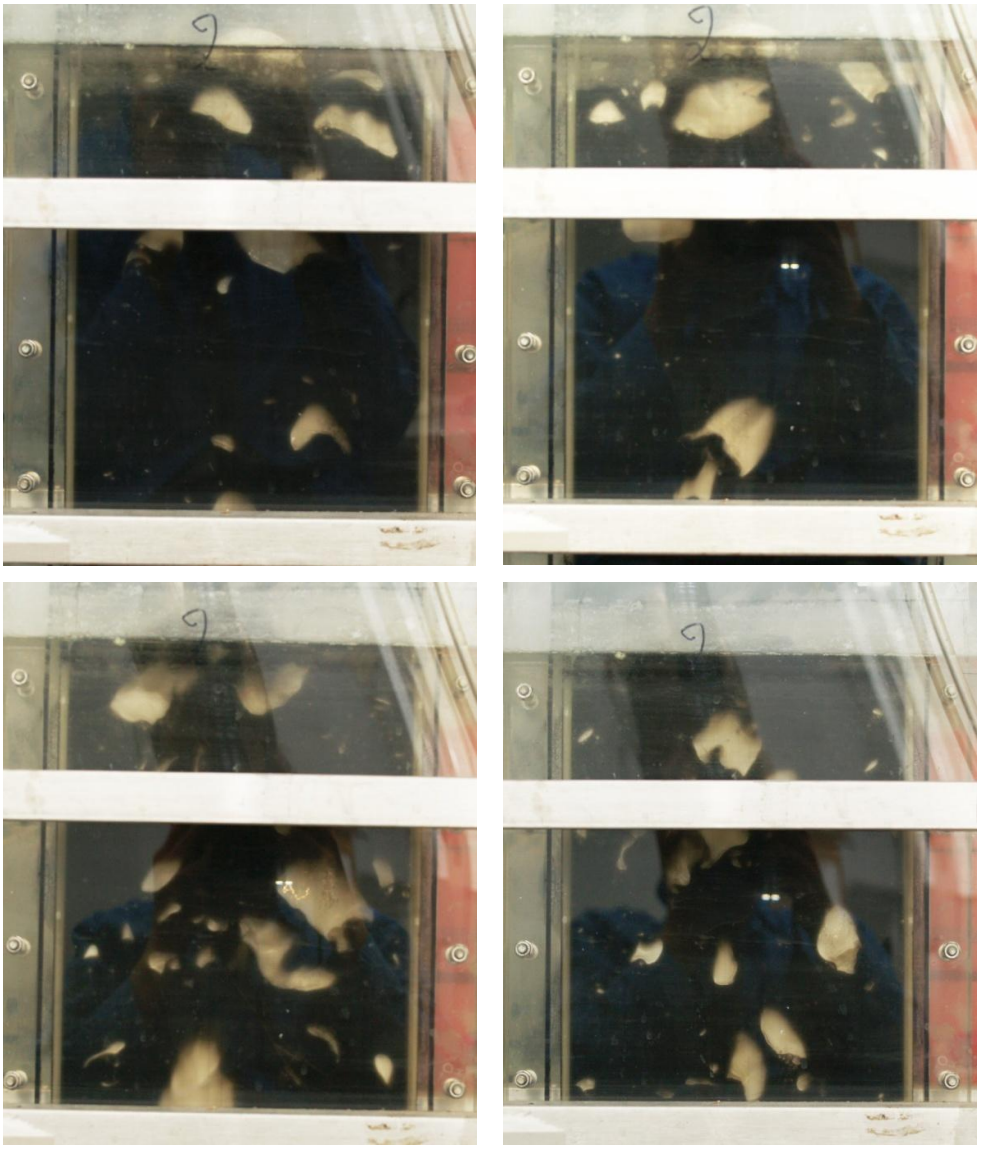


Figure 4-5 Critical gassing rate TMP recorded during the raising of the gasing rate

During experimentation it was noted that operation at 2LPM appeared to be in the 'bubble' flow regime, while operation at 4-10 LPM was in the recommended slug flow pattern. Example photographs of the gas flow pattern at 2, 5 and 10 LPM are shown in Figure 4-6. In fact, at the highest flow rate of 10 LPM the flow was tending more towards the transitional flow region. This suggests that where gassing rate is the controlling parameter, the transition from bubble to slug flow is the important limit. The dependence on flow regime to critical gassing rate would allow for better integration with future work in this area. This is because the flow regime can be spotted visually, whereas the gassing rate required for critical operation will depend greatly on the size and configuration of the reactor used.

2LPM bubble flow



5 LPM slug flow

10 LPM Slug/transitional flow

Figure 4-6 Gas flow pattern photographs for 2, 5 and 10LPM showing the different flow regiemes

4.3.3 Membrane permeability

Permeability is an important factor to consider when optimising the SAMBR process, and permeability is defined as: the ability of a membrane barrier to allow the passage or diffusion of a substance (Allgeier *et al.*, 2005; Allen *et al.*, 2009). The permeability in the SAMBRs was calculated by the following equation:

$$L_p = \frac{J}{TMP} \qquad 4\text{-}2$$

Where L_p is the permeability, J is the flux (LMH) and TMP is the transmembrane pressure in bar. During SAMBR operation the permeability of the membrane was measured at different gassing rates. After the membrane was chemically cleaned the permeability was then measured in deionised water to determine the clean water permeability. This measurement varied according to the flux

being drawn, possibly because at low TMPs the pressure transducer was less accurate, and the clean water permeability was found to be 1650 ± 280 LMH/bar.

From Figure 4-7 it can be seen that the membrane permeability decreases with gassing rate to a minimum value. The initial clean membrane flux decreases to a permeability of 20.8 LMH/bar at 2 LPM. The SAMBR left to operate at 2 LPM for an extended period of time showed a slight further decrease in permeability to 13.0 LMH/bar. However, when the gassing rate was then increased the initial permeabilities are not achieved, and a degree of hysteresis is observed.

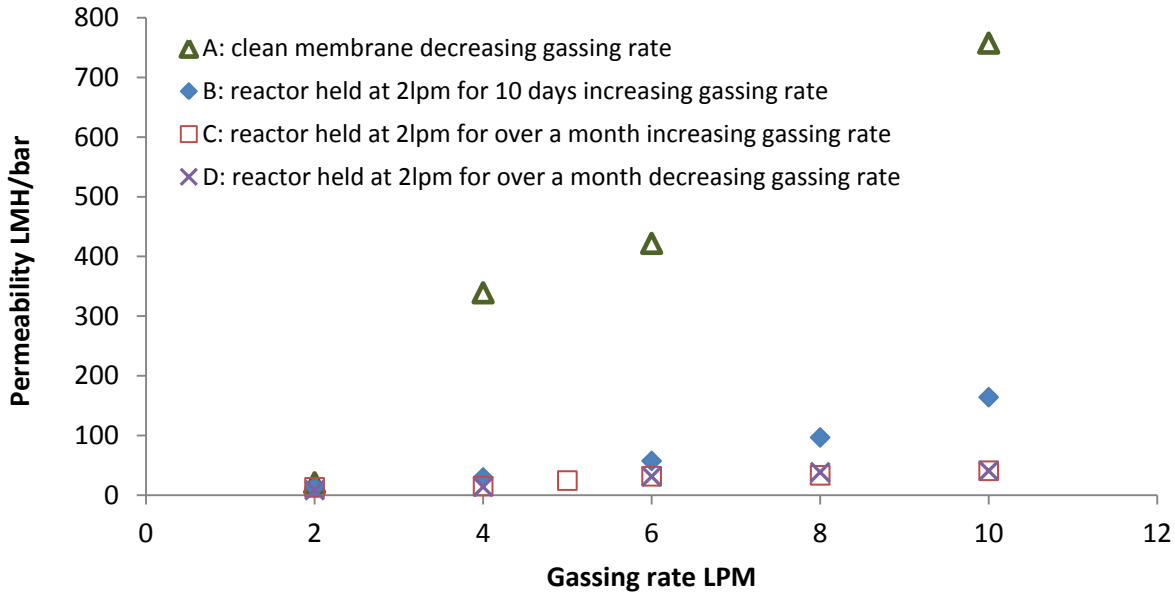


Figure 4-7 Membrane permeabilites at different gassing rates, demonstrating a degree of hystersis.

The membrane left to operate at 2 LPM for the longest showed the lowest level of permeability increase when the gassing rate was increased. Once this reactor had achieved a stable permeability at 10 LPM the gassing rate was dropped again, and the permeability closely followed those achieved when the gassing rate was increasing, demonstrating complete reversibility. This suggests that operating at the lowest gassing rates causes the greatest build-up of irremovable fouling. The small increase in permeability when the gassing rate is increased shows that there is some removable fouling deposited on the membrane surface which the gas scour removes, but this is not the major factor in limiting membrane permeability.

Permeability is an important factor to consider when setting the gas scour parameter. While it is desirable to keep the gas scour as low as possible so as to reduce operational cost, the resulting drop

in permeability means that in the long term the lowest possible gas scour may not be the most economical option. For data sets A and B inFigure 4-7, the membrane was also removed from the reactor so that a sample could be taken from the membrane surface; pictures were also taken of the membrane shown in Figure 4-8 and **Error! Reference source not found.**. The pictures show that the deposits on the membrane surface are removed back to almost the initial clean membrane by the time the reactor had been operated at 10LPM. However, there was still some increased fouling on the edges of the membrane where less gas scouring occurs due to channelling of the gas flow.

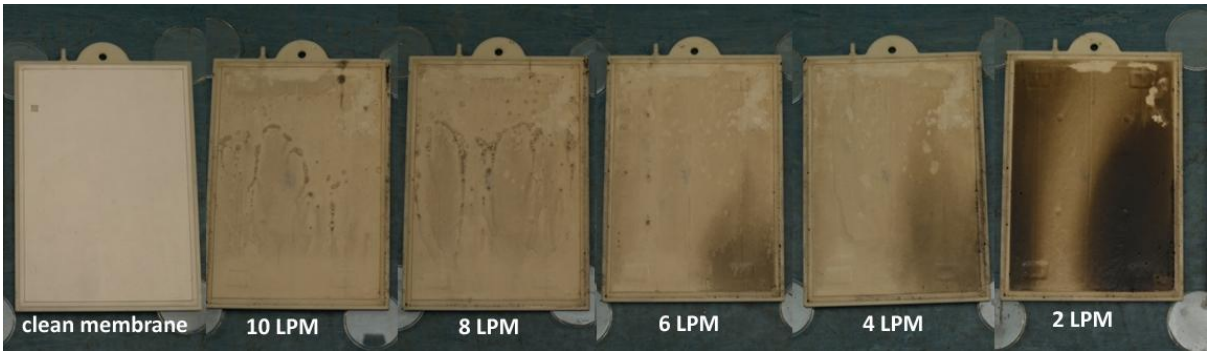


Figure 4-8 Membrane pictures during operation with decreasing gassing rate

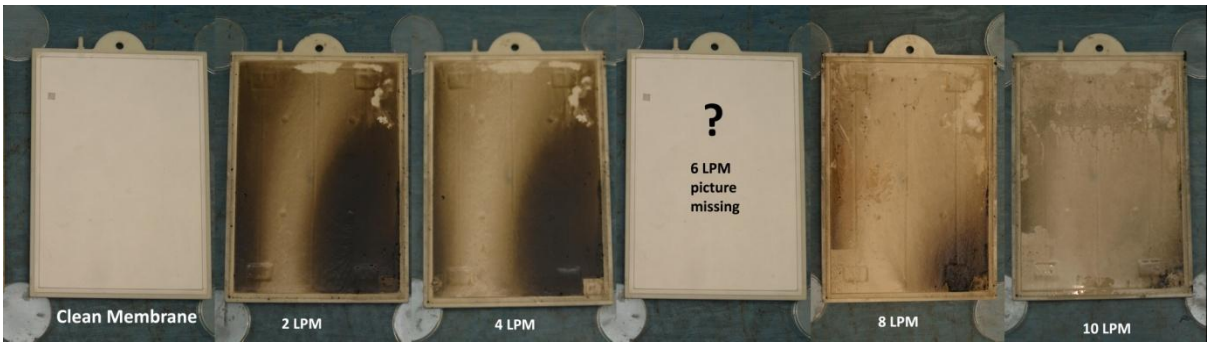


Figure 4-9 Membrane pictures during operation with re-increasing the gassing rate

The majority of the visible fouling on the membrane surface appears be removed as the gassing rate is raised; therefore the loss in permeability cannot be due to a thick biofilm layer on the surface. Therefore, it can either be contributed primarily by the initial thin biofilm layer, or by some form of irremovable internal pore blocking, as suggested by Meng *et al.* (2009).

4.3.4 Permeability at low sparging rates

Previous results on critical gassing have indicated that the operation at low gas sparging rates (2LPM) is responsible for the bulk of the irremovable fouling on the membrane. To investigate the extent of the fouling build up a clean membrane was submerged into the reactor and operated at 2 LPM, and the permeability monitored over time.

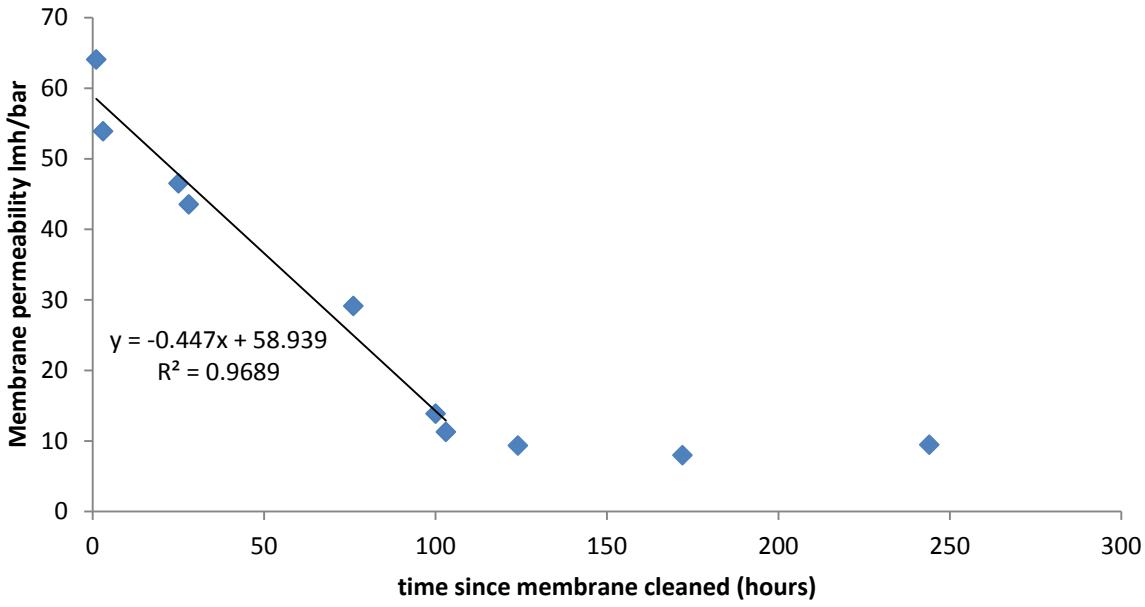


Figure 4-10 -Permeability drop at 2LPM gassing rate, showing the maximum buildup of irremovable fouling occurs at 100 hours. The initial permeability drops at a rate of 0.45 per hour.

The membranes were cleaned using the protocol for Kubota membranes outlined by Le-Clech *et al.* (2006). The clean membrane regained its original clean water permeability, which indicates that no irreversible fouling had occurred. Immediately after the clean membrane was submerged in the SAMBR the permeability dropped to 65 LPM/bar (Figure 4-10). Over time the permeability continued to drop sharply for the first 100 hours of operation; after this time the permeability of the reactor continued to fall, but at a much slower rate. Figure 4-10 suggests that the maximum build up of irremovable fouling occurs after 100h. The initial drop in permeability from 1600 LMH/bar in clean water to 65LMH/bar in the biomass suggests that the cake layer formation occurs almost instantaneously. This linear permeability drop is similar to the effect observed by Bouhabila *et al.*(2001) for an aerobic MBR; however in Bouhabila's work the irremovable took 20 days to reach a maximum value, this longer duration is likely to be because the researchers employed backflushing to slow the rate of irremovable fouling build up.

4.3.5 Viscosity measurements

The rheometry of the biomass solution in the SAMBR is a key factor in determining the resistance across the membrane, see equation 4-3.

$$R_t = \frac{TMP}{J\eta} \qquad 4\text{-}3$$

Where R_t is the resistance across the total membrane unit (m^{-1}), TMP is the transmembrane pressure (Pa), J is the flux (m^3/m^2) and η is the viscosity (Pa.s). Along with the permeability, critical flux and

TMP, the viscosity of the bulk solution requires investigation, and thus far all constituent parts of the resistance have been considered except viscosity. To this end two samples of biomass were analysed in a double gap attachment in a rheometer, as described in section 3.3.13. One biomass sample was taken straight from the SAMBR which had been operating for over 2 months without any significant sludge wastage; the other sample came from a stock of biomass that had been fed with glucose. Both sludge samples originated from a collection of sludge taken from the anaerobic sludge digesters at Mogden STW; before analysis both samples were filtered through a 300 µm sieve to remove any large particulates. The VSS content of the reactor and stock biomass samples was 4.2 and 4.4 g/l, respectively.

Error! Reference source not found. shows the results of this experiment; there is slightly more variation in the initial readings (low shear rate) for the stock biomass sample, and this is thought to be due to errors within the machine.

In cases where the viscosity of anaerobic biomass has previously been measured, it has been for sludges with a much higher suspended solids concentration (Pevere *et al.* 2009; Mu and Yu, 2005), so a comparison with these results is not possible. However, the results do agree with other researchers, that the sludge is a non Newtonian fluid, i.e. the viscosity does not remain constant with increasing shear rate. For both samples the sludge appears to be slightly shear thinning, so the samples become less viscous with increasing agitation.

These data have interesting implications for application in the SAMBR because the biomass will undergo higher shear rates with an increased gassing rate. For the established SAMBR biomass there is a 20% difference between the low shear rate viscosity and the viscosity at the highest shear rate. In theory, at high gassing rates the biomass viscosity will be slightly reduced, further decreasing the membrane resistance, although in practice raising the gassing rate to increase flux is unlikely to be economically viable, but this effect should be investigated further.

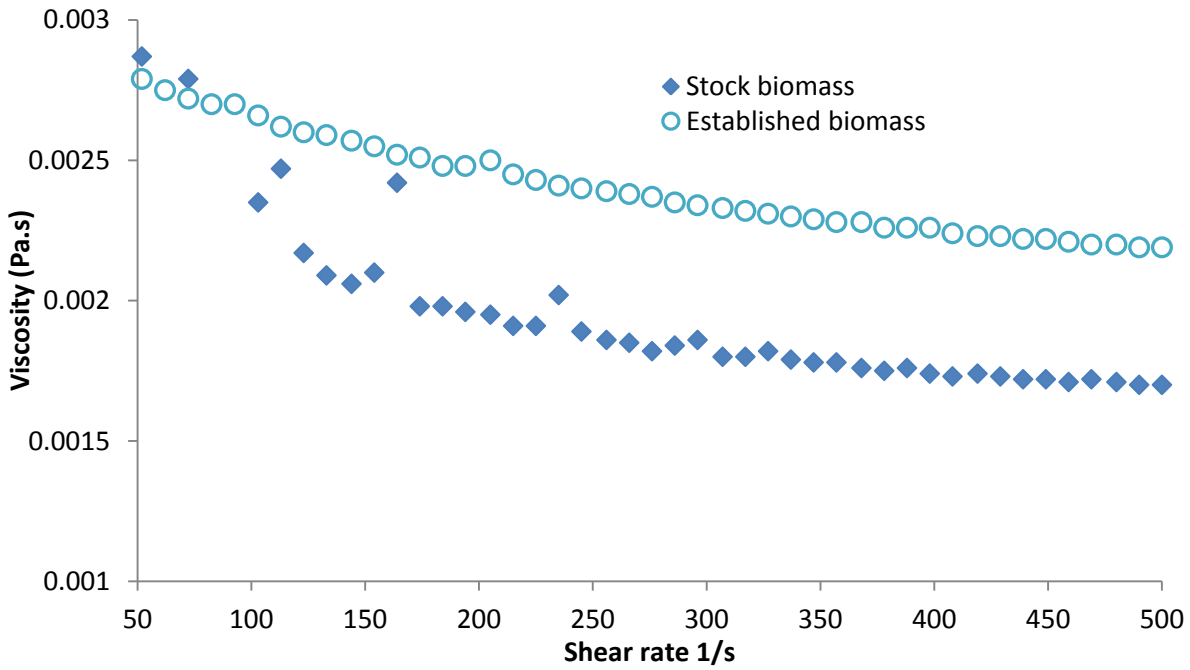


Figure 4-11 Viscosity measurements for stock and established biomass samples at varied shear rates

In both cases, as the shear rate is increased the biomass viscosity reaches a limit value of 0.0017 Pa.s for the stock biomass and 0.0022 Pa.s for the established SAMBR sample. For comparison the viscosity of water (a Newtonian fluid) under the same conditions is 0.00089 Pa.s, so the stock biomass and SAMBR sample are 1.9 and 2.5 times more viscous than water at their respective limit viscosities. Comparing these results with that from an aerobic MBR, Sweity *et al.* (2011) found that the viscosity of aerobic biomass with a suspended solid concentration of 4g/l to be 0.0018 Pa.s which is very similar to that of the stock biomass. The sludge age of the aerobic biomass in Sweity's case was 30 days, whereas in the SAMBR the SRT will be in the region of 150 days (Hu, 2004). This extended SRT is likely to be the cause of the increased viscosity of the established biomass. Since the concentration of SMPs in the reactor will increase with SRT, however, further research into this area is required.

While the stock and the established biomass samples had very similar VSS contents (5% different), there was a significant difference between the two viscosity measurements. For aerobic sludge the VSS content has been shown to be the major factor affecting viscosity (Moreau *et al.*, 2009), however, these data suggests that there appear to be more factors than just bacterial concentration contributing to the membrane resistance in terms of viscosity. It has been suggested that the interstitial matter, colloids and solutes, have an important effect on membrane fouling in aerobic MBRs (Bouhabila *et al.*, 2001). To investigate this effect on anaerobic biomass the samples were centrifuged at 4500rpm for 1 minute to remove the biomass, and the resulting supernatant containing the colloids and solutes was analysed for viscosity. The colloids and supernatant samples

were then treated with aluminium sulphate to coagulate the colloids; the sample was then centrifuged again at 4500 rpm for 10 minutes to remove the colloid faction. The remaining supernatant containing only the solutes was also analysed for viscosity. The results from these experiments are shown in Figure 4-12 and Figure 4-13.

Figure 4-12 shows the viscosity variations for the stock biomass; here it can be seen that the biomass is the major contributor to viscosity above that of water. The viscosity of the colloid and solute sample (0.00118 Pa.s) was very similar to that of the sample containing only solutes (0.00116 Pa.s); this suggests that for this sample the colloid content was very low and did not have a significant effect on the sample's overall viscosity. As mentioned previously, the biomass sample shows shear thinning properties, however, once the suspended solids are removed, the remaining two samples show Newtonian behaviour as the viscosity remains constant for all shear rates. It is assumed that the colloids and solutes data point at 429 s^{-1} is anomalous, since each data point represents a single measurement.

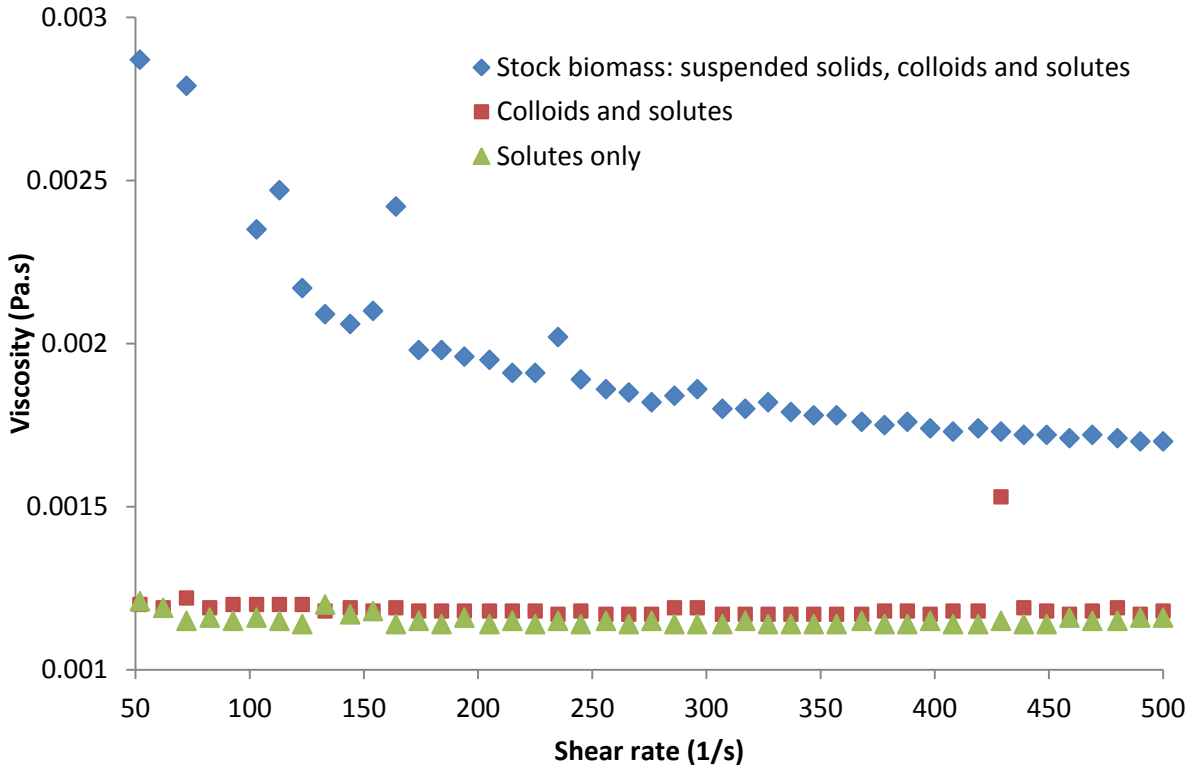


Figure 4-12 Viscosity variations for different sludge fractions for the stock biomass sample.

The results for the established SAMBR biomass are shown in Figure 4-13- here it can be seen that the colloid fraction has a significant influence on the overall viscosity. Also in this sample the colloids

as well as the biomass show a slight shear thinning behaviour. The solute-only fraction shows Newtonian behaviour with a constant viscosity of 0.00117 Pa.s.

Table 4-2 shows a comparison between the viscosity data displayed in Figure 4-12 and Figure 4-13; the contribution to the total viscosity of each individual fraction is displayed, and where the viscosity showed shear thinning behaviour the limit viscosity is used. The most notable difference between the two samples is the part that the colloid fraction played. In the stock sample there was very little colloid fraction present, hence the colloid fraction contributed very little to the overall viscosity. However, in the SAMBR sample the colloid fraction had the largest contribution to the overall viscosity (after water), therefore in order to keep the viscosity in the SAMBR as low as possible, it is important to keep the colloid fraction down.

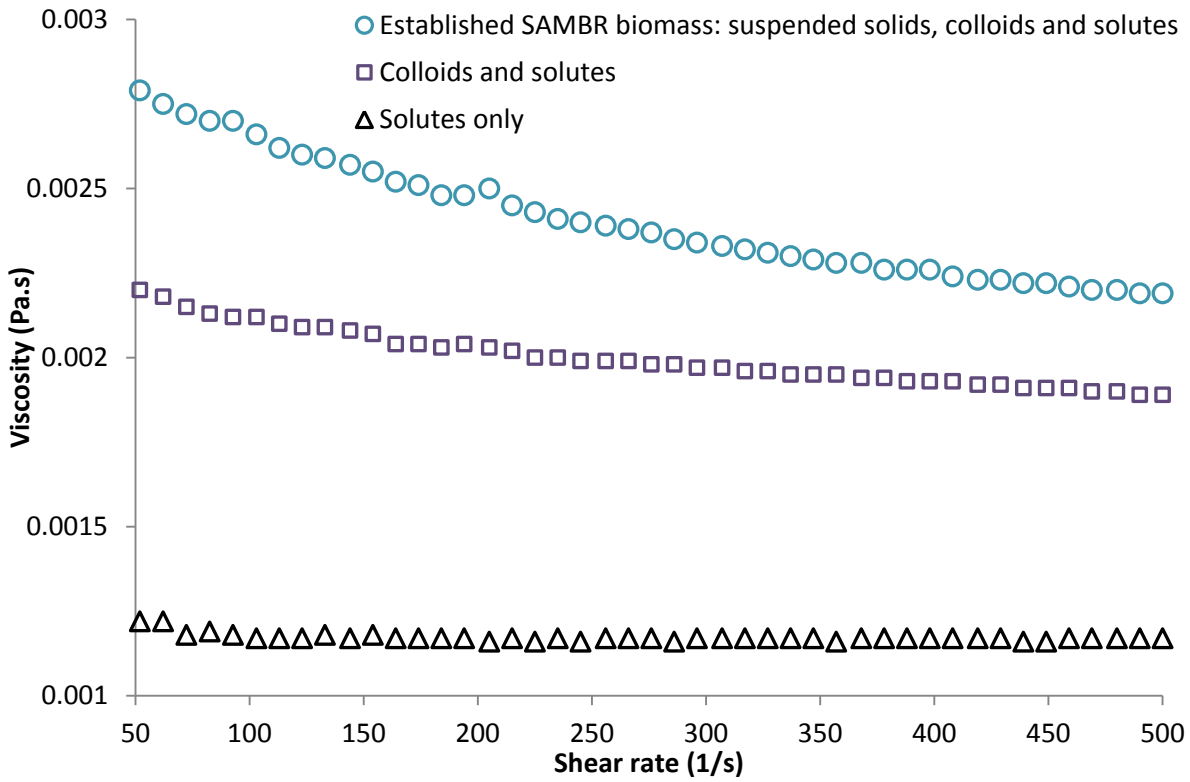


Figure 4-13 Viscosity variations for different sludge fractions for the established SAMBR biomass sample

This difference in the colloid fraction between the two samples is likely to be due to SMP production by the biomass. The SMPs are produced by the biomass when degrading complex substrates, and as the by-products of cell lysis, and are also produced when the cells are under stress (Aquino and

Stuckey, 2008; Barker and Stuckey, 1999). The stock biomass was fed only with easily degradable glucose and was not subject to intense agitation, the SMPs in this biomass sample would not be expected to be very high. Conversely, the biomass sample from the SAMBR would be expected to have quite high levels of SMPs due to the stress caused by the agitation of the gas scour, also the more complex nature of the feed would mean that SMP production is required to breakdown some of the larger substrates. These data seem to agree with the work by Bouhabila *et al.* (2001) on aerobic MBRs, and in their paper they also state that the colloid fraction of the reactor bulk phase had a large impact on reactor fouling.

Table 4-2 Comparing the contributing parts of the sample viscosity for the two biomass samples

	Stock solution	Established SAMBR sample
Suspended solids (mPa.s)	0.52	0.3
Colloids (mPa.s)	0.02	0.72
Solutes (mPa.s)	0.27	0.28
Water (mPa.s)	0.89	0.89
Total viscosity mPa.s	**1.7**	**2.19**

4.3.6 Small particle and solute rejection

4.3.6.1 Size exclusion chromatography

During SAMBR operation samples were taken from the reactor for size exclusion analysis. The samples were centrifuged at 13000 rpm for 2 minutes and the supernatant filtered through a 0.22µm syringe filter to remove particulates. The sample were analysed using an Aquagel-OH 30 column using refractive index (RI). In size exclusion chromatography the higher molecular weight (MW) solutes elute from the column first, while smaller molecules diffuse further into the column gel and hence are retained for longer time periods. The column was calibrated with PEG and PEO standards at 400, 4600 and 400,000 Da, and these standard samples are shown in both figures.

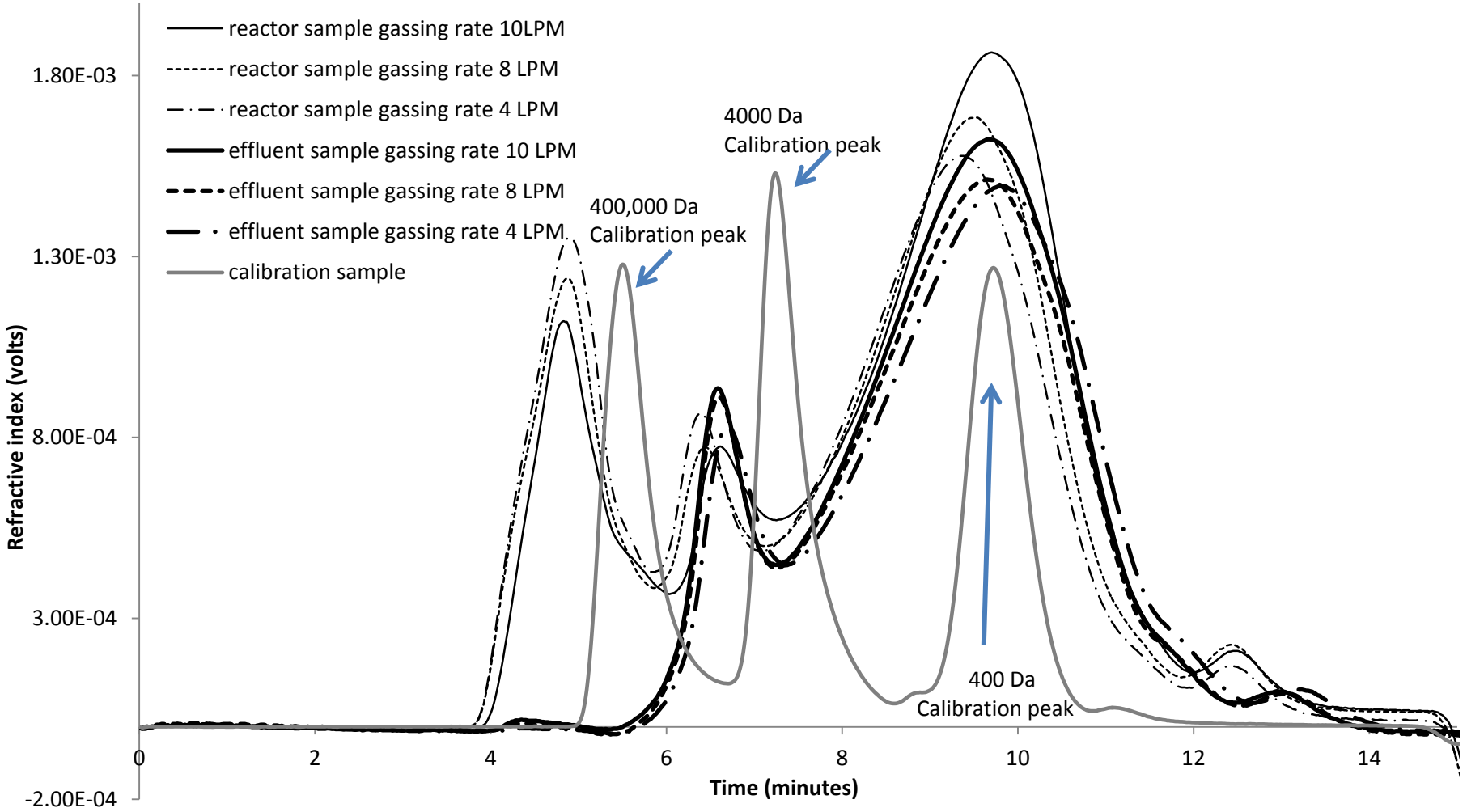


Figure 4-14 Size exclusion chromatograph for reactor samples (thin lines) and effluent samples(thick lines), before critical flux/gassing rate. The molecular weights of the calibrations peaks are labeled, and the differenence between the effluent and reactor chromatograms show the MWCO for the membrane.

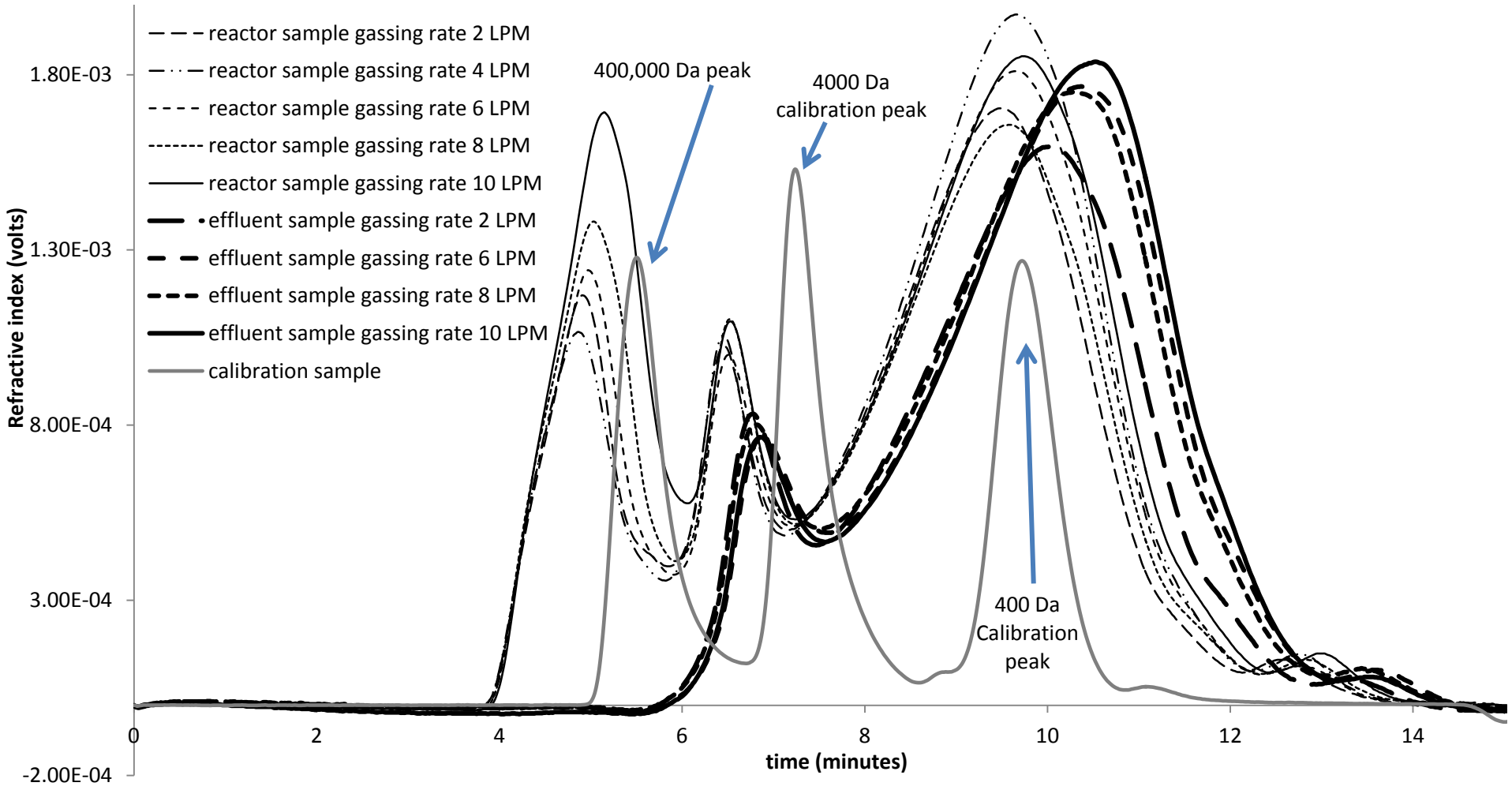


Figure 4-15 size exclusion chromatograph for reactor samples (thin lines) and effluent samples (thick lines) after operation beyond critical flux/gassing rate, the differenence between the effluent and reactor chromatograms showing the MWCO for the membrane has shifted compared to previous figure

Thus far it has been shown that there exists a critical flux and gassing rate, and that beyond these operational parameters significant amounts of irremovable fouling accumulates on the membrane. As such the results are grouped into two graphs: size exclusion before critical operation (Figure 4-14), and size exclusion after the SAMBR had been operated beyond its critical flux and gassing rates allowing for the build-up of surface and internal fouling, (Figure 4-15).Figure 4-14 shows that the molecules with a molecular weight greater than 400,000Da (the first peak in the calibration sample) are entirely retained by the reactor. This is fairly similar to the work carried out by Lyko *et al.* (2007) who found a MWCO of 170kDa in a similar aerobic MBR.

At lower molecular weights the chromatographs for the reactor and effluent sample are fairly similar, suggesting clear molecular weight cut-off, (MWCO) for the SAMBR under sub-critical operating conditions. For this data set there appears to be no clear trend in effluent or reactor samples relating to the operating gassing rate, so for small particles the removal appears to be independent of the gassing rate.

Figure 4-15 shows the size exclusion chromatographs after operation beyond the determined critical flux and gassing rate. In this case there is still a clear molecular weight cut-off at 400,000 Da as in Figure 4-14, however, for particles below this molecular weight some removal was observed. Particularly for particles between 400,000 and 4600 there was a significant difference in peak height suggesting that the molecular weight cut off was now in the region of 40,000. For the particles at the 400 Da peak, the effluent peaks appear to be shifted further right compared to the reactor sample, suggesting that the concentration of low molecular weight solutes is slightly higher in the effluent that in reactor. The reason for this apparent increase at very low molecular weights is unclear, although this peak is at a molecular weight lower than 100 Da (the minimum range for column), and therefore the data at this end of the scale may not be accurate enough to draw any conclusions.

For the data beyond critical operation (Figure 4-15) there are some trends that can be observed regarding operation at different gassing rates. For the bulk reactor samples there was an increase in the particle concentration above 400kDa as the gassing rate increases. This trend is most likely due to some settling of the larger particles occurring at the lower gassing rates. As the gassing rate is increased the larger particles are re-suspended into solution causing their apparent increase in concentration. For the smaller particles there is no observable trend within the reactor for different gassing rates.

When observing the effluent chromatograms in Figure 4-15 there is also a discernible trend for the low molecular weight solutes at different gassing rates. In this case the highest concentration was

found at the highest gassing rate (10 LPM), which decreases with decreasing gassing rate down to 2 LPM (the sample at 4 LPM was omitted due to sample spoiling). It is thought that the reason for this was due to the increased gassing rate removing more of the fouling layer that partially restricts the throughput of the low molecular weight particles. The shift in peak height between 4 LPM and 10LPM was actually within the margin of error, and therefore statistically insignificant, so no trend can be confirmed for this. However, the effluent chromatograph at 2LPM was significantly lower than all the other chromatographs. This suggests that the high TMP and fouling layer visible at this low gassing rate (see Figure 4-3 and Figure 4-8) can achieve a significant removal of low molecular weight (c. 400Da) particles.

4.3.6.2 Acetic acid rejection

The size exclusion columns used to collect the data in the previous section work best on size exclusion for non-charged particles. Within the SAMBR many charged particles and solutes are present, most notably the volatile fatty acids (VFAs). Acetic acid rejection across the membrane has been observed in the SAMBR by previous researchers (Akram, 2006; Martinez-Sosa *et al.*, 2011). To investigate this, the SAMBR was spiked with acetate to achieve a reactor concentration in the region of 500 mg/l, for the duration of this experiment the reactor was operated in batch mode with a 100% recycle ratio. The flux was held at 11 LMH throughout the experiment and the gassing rate held at 2 LPM. The reactor had been held under these conditions for a week previously under continuous operation with a HRT of 12 hours to allow the biofilm to fully develop.

VFA samples were collected every 15 minutes for the first hour, and every hour thereafter for 10 hours. To ensure the survival of the biomass the reactor was buffered with sodium bicarbonate; the pH of the reactor was also monitored throughout the experiment to ensure it remained within acceptable limits for anaerobic bacteria. Figure 4-16 shows the degradation of acetate in the reactor over time, which gives a specific degradation rate of 0.276 gCOD $gVSS^{-1}$ day^{-1}. There is also, for every sample, a notable difference between the reactor and effluent concentration of acetic acid. The average rejection for all the samples (excluding those at 0 and 0.25h, where the acetic acid was not fully mixed) is 3.35%, while this is a significant rejection it is much less that that previously achieved by Akram (2006).

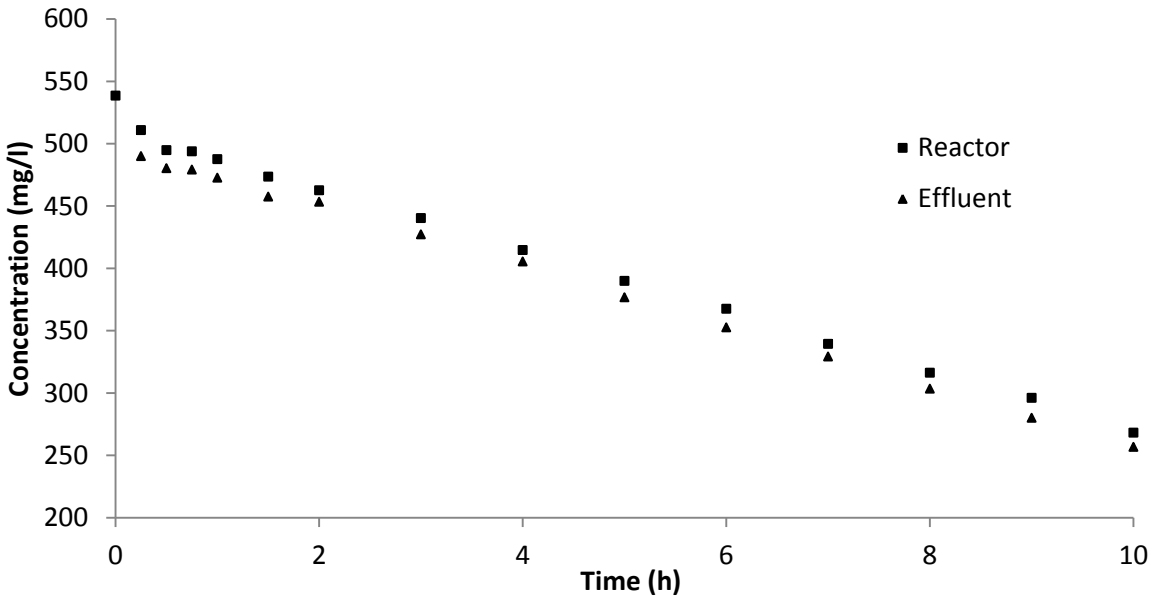


Figure 4-16 Acetic acid rejection in the SAMBR, comparing concentratin in the reactor to concentration in the effluent

It was suggested that the apparent rejection of acetic acid by the membrane and biofilm could simply be due to the anaerobes in the biofilm degrading the substrate. To investigate this, an approximate model for the acetic acid uptake in the biofilm was developed. The specific degradation rate of acetic acid in the SAMBR had already been determined to be 0.276 gCOD $gVSS^{-1}$ day^{-1}. It was also necessary to determine the mass of VSS on the biofilm- Park & Lee (2005) suggest a maximum density of 10g VSS m^{-2}, for a membrane attached biofilm. Since the membrane area is $0.1m^2$, this outputs a biofilm degradation rate of 0.276 g COD day^{-1}. To determine the contact time the biofilm thickness was estimated to be 1mm thick (Characklis *et al.*, 1990). This resulted in a contact time of 0.09 hours and hence a degradation of 0.00104 g COD or 1.09 mg acetic acid removal across the membrane.

The figure of 1mg/l degradation across the membrane is based on indirect assumptions and therefore, the actual degradation across the biofilm could be larger than this figure, however, it is unlikely to be as high as the measured values. The rejection measured in the experiment ranged from 9 to 15 mg acetate; this indicates that acetic acid degradation in the biofilm is not the only factor affecting VFA rejection. Two further theories for this increased rejection are; charge repulsion from the biofilm, and size exclusion. Size exclusion through the membrane seems an unlikely theory because this would mean restricting the pore size to a few tens of angstroms. Hence, a build-up of negatively charged solutes on the membrane surface is more likely to be able to explain acetic acid rejection.

4.3.6.3 Higher VFA rejections

To further investigate the rejection of these small solutes, an experiment into the rejection of higher VFAs was carried out. The above experiment was repeated with 2 higher molecular weight VFAs, isobutyric and isovaleric acids, because they were the most accurately detected on the Aminex HPLC column compared to other VFAs. The reactor was spiked simultaneously with acetic, isobutyric and isovaleric acids, so that the concentration was in the region of 500mg/l for each acid. The acetic acid result was included to compare with the previous result. The VFA concentrations were monitored every hour for 8 hours, the results from this experiment are shown in , Figure 4-18 and Figure 4-19.

In this case the average percentage rejection for acetic, isobutyric and isovaleric acids was 4.3%, 5.1% and 5.9%, respectively. As the most degradable, the acetic acid degraded the fastest; the isobutyric acid also showed significant degradation across the monitoring process. The isovaleric acid, after the initial mixing period, showed a reduction in concentration in the SAMBR of only 7mg/l over 7 hours of measurements.

This limited degradation of isovaleric acid in the SAMBR bulk is important, because in this case digestion in the biofilm cannot be the cause of the rejection which varies from 21 to 31 mg/l at each measurement. Since the biofilm is likely to contain a significant portion of amino acids it is likely that the surface of the biofilm will be negatively charged (Gerba, 1984). Because of this some charge repulsion between the biofilm and the VFAs may be partially responsible for the rejection. However, each VFA particle should have broadly the same -1 electrostatic charge, hence the difference in rejection across the membrane for the different sizes of the VFAs suggests that the size difference between the molecules plays a significant part in their rejection.

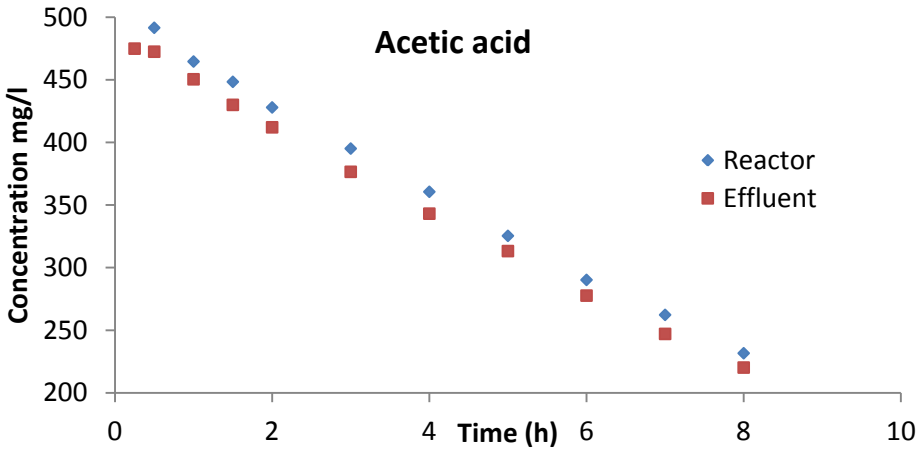


Figure 4-17 SAMBR rejection of acetic acid

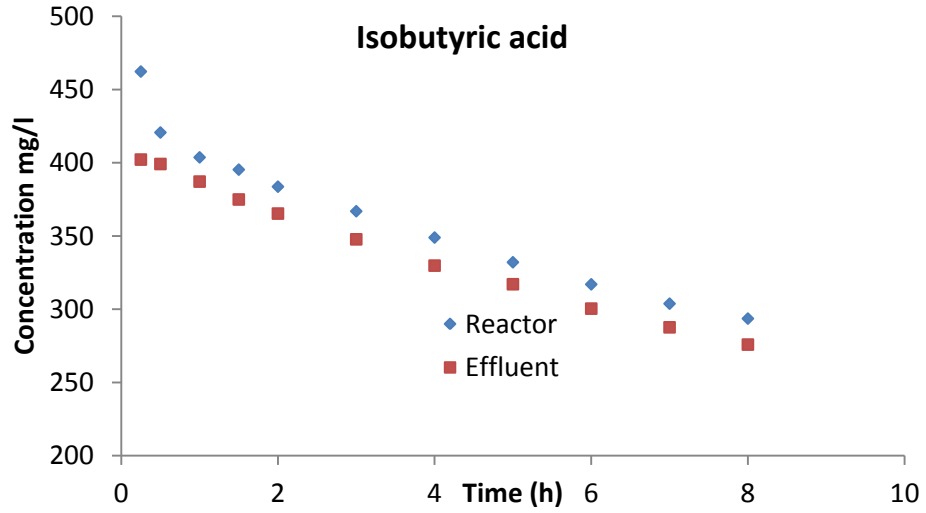


Figure 4-18 SAMBR rejection of isobutyric acid

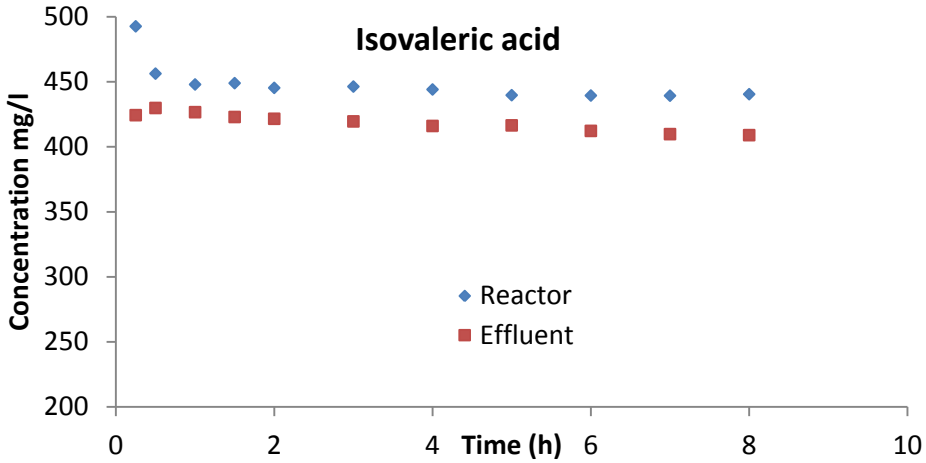


Figure 4-19 SAMBR rejection of isovaleric acid

During these experiments the reactor was operated beyond both the critical flux and critical gassing rate; as such the TMP in the reactor at the beginning of this experiment was quite high at 0.2 bar (see Figure 4-20).

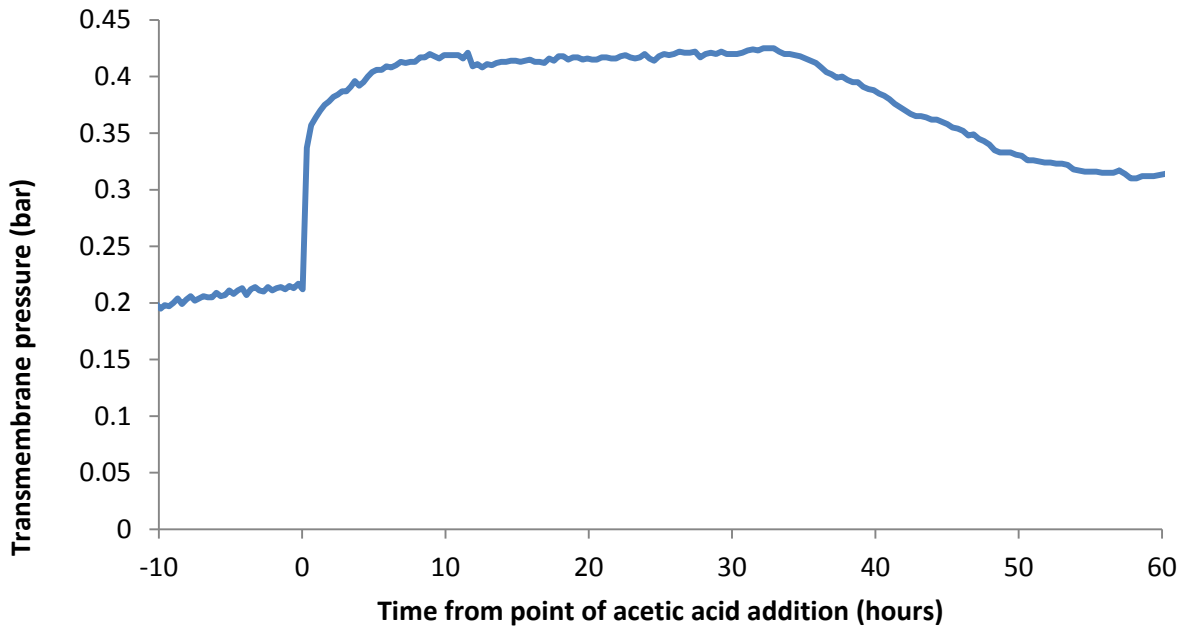


Figure 4-20 SAMBR TMP profile during acetic acid spike

On the point of VFA addition the TMP jumped to 0.4 bar, and remained at this value until the reactor was switched from batch flow back to continuous flow (at 30 hours), where the TMP started to drop again. There are a couple of possible reasons for this effect; Stoodley *at el.* (1997) demonstrated that under low pH a biofilm can become compacted by up to 69% of its original thickness. This compaction of the biofilm would likely increase the resistance of the biofilm, hence increasing the overall TMP. Another explanation for the rise in TMP could be due to the increase in charged colloids in the reactor bulk. By increasing the ionic strength of the solution the particles within the reactor are more likely to coagulate, and this can affect the rheology of the system making the reactor bulk more viscous and hence increasing the TMP.

The same VFA experiments were repeated in SAMBRs operated at higher gassing rates; in these cases no VFA removal was observed. This suggests that the VFA removal is linked to the extensive surface fouling only observed on the membrane at 2LPM (see pictures in Figure 4-8).

4.4 Summary

From the experiments discussed above the following conclusions can be drawn:

1) The critical flux for the SAMBR operating at a 6LPM gassing rate was 11.8 LMH; however, this figure is very much linked to the gassing rate, and also depends on the reactor not being operated beyond the critical values. Having operated the SAMBR beyond the critical flux and gassing rate, when the initial gassing and flux were resumed the reactor does not regain its initial critical flux value, instead the critical flux drops due to irreversible fouling on the surface of the membrane.
2) In addition to the critical flux, a critical gassing rate was determined for a flux of 7.2 LMH. The assumed definition for critical gassing rate was: 'there exists a critical gassing rate which when reached causes a steep rise in TMP'. By this definition the critical gassing rate under the reactor operating conditions was found to be 4 LPM, since operation below this caused a sharp rise in TMP. More interestingly, this critical gassing rate appeared to occur at the flow regime barrier between slug and bubble flow.
3) The membrane permeability of the SAMBR was also considered in this chapter. From the initial clean membrane the permeability is not reversible or recoverable due to the build-up of irremovable foulant on the membrane surface that cannot be removed by the gassing rate alone. However, once this fouling layer was fully developed the small increase in permeability at higher gassing rates was shown to be reversible.
4) The viscosity of the biomass was investigated due to the part it plays in the resistance across the membrane. It was found that once the biomass colony had been established within the SAMBR the build-up of colloids (most likely SMPs) had a significant effect on the overall viscosity of the biomass.
5) The size exclusion of the membrane was monitored over a period of time, and the MW cut-off for the membrane/biofilm was found to be in the region of 400 kDa, so long as the SAMBR was operated at fluxes lower than the critical flux (and gassing rates above the critical gassing rate). Once the SAMBR had been operated beyond the critical values, a sharp rise in TMP was observed, and the MW cut-off increased to be in the region of 40 kDa.
6) In order to monitor the effect of the membrane and biofilm on small charged solutes, VFA rejections across the membrane were monitored. VFA rejection was only observed at the lowest gassing rate of 2 LPM where low but significant removals of acetic, isobutyric and isovaleric acids were observed. It was suggested this was due to a combination of electrostatic charge in the biofilm, and a certain amount of size exclusion in the biofilm.

Chapter 5. Phage Removal in a SAMBR

5.1 Introduction

The removal of viruses in wastewater treatment is of growing importance due to the epidemiological nature of viral pathogens. Traditional methods of post treatment disinfection have focused on the removal of faecal coliforms, however, studies have shown that viruses are more resistant to disinfection agents compared to vegetative bacteria (Leong, 1983). The viral content of domestic sewage varies greatly from region to region depending on local socio-economic factors, immunisation programmes, and the time of year (U.S. EPA, 2006). In developed countries the concentration of viruses in raw sewage has been detected at concentrations as low as a few hundred viral units per litre, and up to over 100,000 viral units per litre.

The capacity of sewage treatment plants to remove viruses has been extensive reviewed by Leong (1983). The author reviewed the log removal of viruses through all the standard treatment units found in an average WWTP. The activated sludge unit (which is what the SAMBR would replace in the process flow model), had a median removal of 94%. While this is a significant removal figure, the total viral removal requirements across the wastewater system are much greater than this, and in most cases tertiary effluent processing is required. If the SAMBR could be demonstrated to show virus removals that are significantly higher than that of a standard activated sludge unit, this would serve to further promote the SAMBRs benefits for wider installations as a promising alternative for wastewater treatment in the future. Furthermore, when using the SAMBR to remove viruses the need for current viral removal techniques such as chlorination or ozonation, which are both expensive and hazardous, could be avoided.

Due to the hazards involved in using pathogenic viruses, bacteriophages are frequently used as viral indicators. Since phages are simply viruses that only infect bacteria they are a good model for pathogenic viruses due to their similarity in structure, size range and behaviour. Without tertiary treatment the potential for viral removal in a conventional STW is limited. Ueda *et al.* (2000) found a 1.31 log removal of a T4 phage across all units of a STW. The greatest individual unit was the activated sludge tanks which exhibited a 0.91 log removal for the T4 phage; this is a similar figure to that found by Leong (1983) for virus removal, thus further demonstrating the similarities between virus and phage data.

As reviewed in the literature review in Chapter 2, there have been some studies done on the removal of phages by aerobic MBRs. However, it is important to assess the impact of viruses on a SAMBR unit since the difference in microbial consortia means that the data for phage removal in an

aerobic MBR may not translate exactly to its anaerobic cousin. Hence, in this study the objectives were: to analyse the effectiveness of the SAMBR to remove viruses using bacteriophages as a model virus; and, to investigate the effect of different operational parameters, such as the gassing rate and the membrane history might have on virus removal.

5.2 Materials and methods

This study was performed on bench scale 3 litre SAMBR reactors detailed in Chapter 3. Throughout the experiments the COD removal, pH and gas composition were monitored. The COD removal remained steady and above 90% throughout, while the gas composition in the headspace of the reactor was 80% CH_4, and the pH was kept between 6.8 and 7.1 for stable anaerobic operation, and well above the pI (isoelectric point) for both phages to stop the phages coagulating. In all experiments the flux was kept constant between 6 and 7LMH.

For each experiment a 1ml sample of concentrated phage solution was injected into the reactor to give a concentrated viral spike in the reactor of approximately $1x10^7$ pfu/ml. However, the exact phage concentration in the reactor at any particular time was determined through direct sampling and analysis. Samples were collected inside the reactor from the bulk phase by connecting a 20ml syringe to the relevant sample port. Effluent samples were collected from a T valve situated just after the membrane pump.

The first samples were taken no earlier than 1 hour after injection to allow for complete mixing. Further samples were taken at either 2, 4 and 6 hours or 1, 2 and 3 hours after injection. The samples were treated and enumerated as described in Chapter 3. For each sample at least three plates were evaluated to give an average concentration, unless otherwise stated.

$$LRV = -\log\left(\frac{C_{eff}}{C_{rxr}}\right) \quad \text{5-1}$$

The log removal in the reactors was calculated using equation 5-1, where LRV is the log removal value, C_{eff} is the concentration of phage in the effluent in pfu/ml, and C_{rxr} is the concentration of phage in the reactor bulk.

When the gassing rate in the reactor was altered the SAMBR was left for 24 hours to reach a new equilibrium before any phage experiments were carried out (unless otherwise stated). To avoid any contamination between phages the experiments with the T4 phage were carried out in a separate reactor to the MS-2 experiments, and the different phage samples stored in separate fridges.

5.3 Results and Discussion

5.3.1 MS-2 interactions with anaerobic bacteria

It is important to understand the various interactions of the phage with anaerobic bacteria. It has been suggested that the coliphage may adsorb to the surface of other bacteria even if it is unable to use the bacteria to generate more phage. This would mean that any phage adsorbed to the surface of a bacterium would not be able to pass out through the reactor membrane, since the membrane pores are too small for the bacteria to pass through. In fact, some laboratory grown viruses have been observed to adsorb directly to aerobic activated sludge flocs (Farrah *et al.*, 1978). The effect of phage adsorption to biomass was not considered in any of the studies on phage removal listed in Table 2-12.

To investigate this, known concentrations of phage were injected into serum bottles containing different concentrations of biomass. The serum bottles were placed on a mixing tray at 60rpm to promote contact between the phage and the biomass; the serum bottles were then left for three hours to equilibrate, and the experiment was conducted at 30°C. If any significant amounts of phage adsorbed to the surface of the bacteria, the concentration in the serum bottles would appear to decrease with an increase in VSS. Initially the phage was injected into the serum bottles at a concentration in the region of 10^4 pfu/ml. A control serum bottle containing only a media solution was also tested. As shown in Figure 5-1, the concentration of phage in the serum bottles remained constant across all biomass concentrations. Each sample was analysed in duplicate and the individual data points are shown in Figure 5-1. This indicates that at this concentration of phage and biomass no adsorption to the bacterial surface was occurring.

It was possible that the concentration of phage was simply too high to detect any phage adsorption. To this end, the same experiment was then repeated with the injected phage concentration two logs lower at 10^2 pfu/ml. It can be seen in Figure 5-1, that once again the concentration remains constant across all biomass concentrations, including the control.

From these results it can be assumed that the MS-2 phage does not adsorb to the surface of the biomass at the concentrations being investigated. It is still possible that some adsorption may occur, however, for this study the concentration of phage in contact with the biomass was greater than 10^2pfu/ml, and hence for the purpose of this study the adsorption of phage to the surface of the biomass does not need to be considered.

The mechanism by which an MS-2 RNA infects an E. Coli bacteria is currently unknown (unlike the mechanism for the T4 phage which is well established); however the phage only infects E. Coli cells with an F-pilus, in addition to this the phage has an icosahedral shape (van Duin, 2006). These factors may help explain why this particular phage does not appear to adsorb strongly to the surface of the biomass. Additionally Davis *et al*. (2006) demonstrated that phages suspended in wastewater show a reduced propensity to adsorb to soil particles compared to those suspended in ground water, due to the ionic strength of the solutions; therefore in this case the ionic strength of the wastewater may be preventing adsorption.Gerba *et al.* (1984) also suggested that some viral adsorption to glass surfaces may occur, and since glass serum bottles were used in these experiments it is possible that some viral adsorption to the surface of the glass bottles may have happened. However, the phage concentration remained constant throughout the experiment, and therefore it is not likely that any significant adsorption to the glass bottles occurred.

This section demonstrates that the anaerobic biomass had no strong interactions with the MS-2 phage particles, and therefore when a sample is taken from the reactor biomass this can be directly enumerated as the concentration of phage in the reactor.

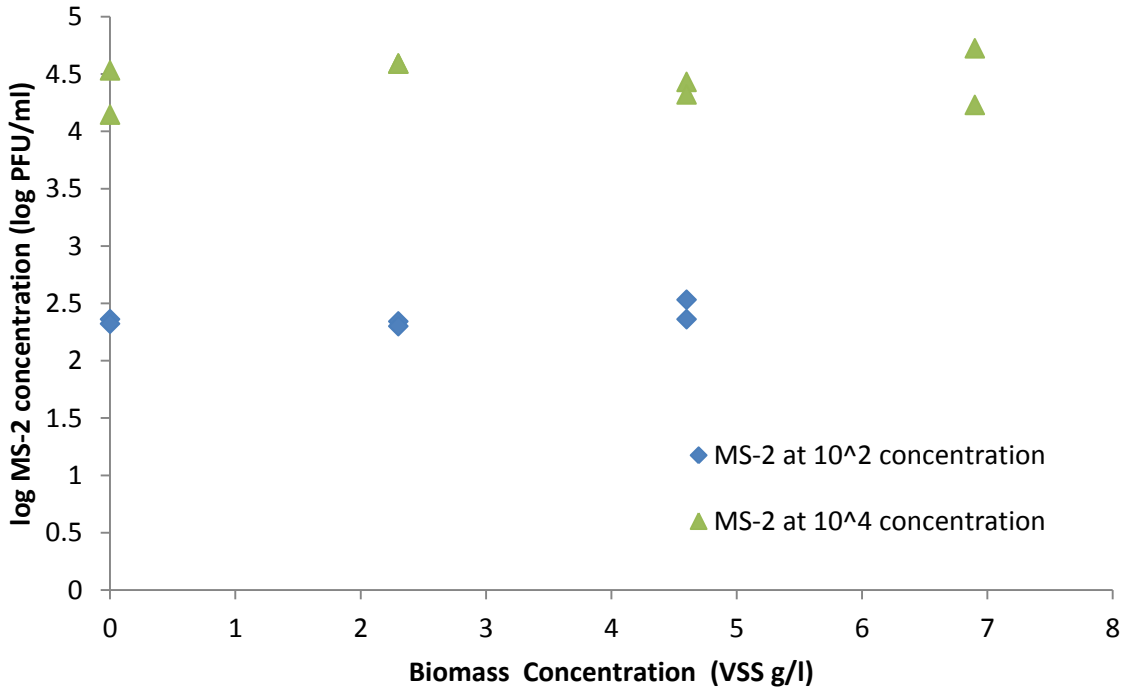


Figure 5-1 MS-2 concentration in biomass after 3 hours gentle mixing with various concentrations of anaerobic bacteria,demonstrating no adsorption of MS-2 to biomass

5.3.2 Phage removal in the SAMBR

In the previous chapter the removal of small particles less than 400 kDa was considered; in this section we look at the removal of one of the smallest phages MS-2. For comparison the MS-2 phage has a molecular weight between 3.5-3.8 million Daltons (Kuzmanovic *et al.*, 2003), and therefore is larger than the solutes considered in the previous chapter.

5.3.2.1 Gassing rate dependence

Before the reactors were filled with biomass and operated, the SAMBR was filled with DI water and left to run; this allowed for the testing of reactor control parameters. During this period a spike of MS-2 phage was added to the reactor to determine the removal properties of the clean membrane unit. This was repeated at various different gassing rates, however, as the operational flux was kept below the clean water flux for the membrane there was no detected pressure drop across the membrane. The phage removal in the clean reactor was found to be 0.7 LRV ± 0.4, unfortunately, at this stage of experimentation the phage enumeration technique was still being optimised, hence the wide margin of error in this result. The 0.7LRV for the clean system was slightly greater than the 0.4 LRV achieved by Shang *et al.* (2005) on similar membranes, but their result falls within the ± 0.4 log error margin determined for the SAMBR.

A SAMBR was then filled with biomass and operated under the standard conditions described in Chapter 3 for two months to establish an adapted biomass colony. The reactor showed steady performance with COD removals in excess of 90%, and an outlet gas stream at 80% CH_4. 72 hours prior to the start of the experiment the membrane was removed from the reactor and cleaned with a 1% oxalic acid and 0.5 % NaOCl solution, according to the protocol for Kubota membranes stated by Le-Clech *et al.* (2006). After this the clean membrane was re-submerged in the reactor and operated at the highest gassing rate, 10 LPM (In this case the phage removal was monitored over 3 days at 10LPM to ensure a stable LRV had been reached). Once the stable LRV at 10 LPM had been established, the gassing rate in the reactor was reduced and the reactor left for 24 hours to reach a new equilibrium, and the next LRV assessed.

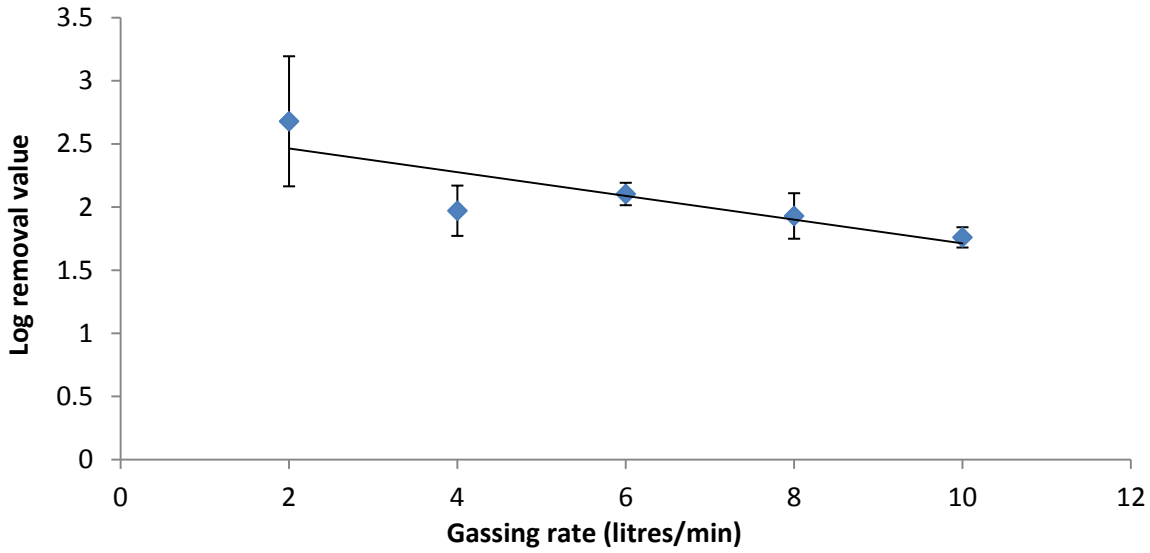


Figure 5-2 MS-2 Phage removal in the SAMBR at different gassing rates; error bars show 1 standard deviation for each measurement.

Figure 5-2 shows how the phage removal varies with the change in gassing rate, each data point is the average of 6 individual samples and the standard deviation calculated from this data is also shown, the raw data from this experiment can be found in appendix C. The TMP was negligible for gassing rates between 10 and 4 LPM, because the reactor was operated below the critical flux- the case at 2LPM will be considered later. However, in spite of the low TMP the LRV does increase with the decreasing gassing rate. This is most likely due to the increased deposition on the membrane surface, and while this deposition was not enough to affect the TMP, the increase in phage removal (with the exception of the 4LPM point) is evident. The log removal values broadly agree with the work by Shang *et al.* (2005) who found an average removal on 1.2 LRV in their aerobic MBRs.

At each gassing rate, after all the biomass and effluent phage samples were taken, the membrane was removed from the reactor for inspection; Figure 5-3 shows the photos of each membrane. In the pictures a gradual increase in the biofilm layer can be seen from the membrane at 10 LPM down to the membrane at 4 LPM. At 10 and 8 LPM there is essentially no visible deposition on the membrane surface, the markings on the membrane shown in the picture in Figure 5-3 come from the removal of the membrane from the reactor unit; in both these cases there was a large amount of foaming in the SAMBR which left deposits on the membrane surface as it was drawn out. When the gassing rate was dropped to 6 LPM and then 4 LPM it is possible to see the beginnings of a visible biofilm on the right hand side of membrane surface. The reason for this uneven build-up of the biofilm layer was

due to the channelling of the scouring gas; where the majority of the gas flow passes, in this case on the left hand side, there was much less of a visible biofilm.

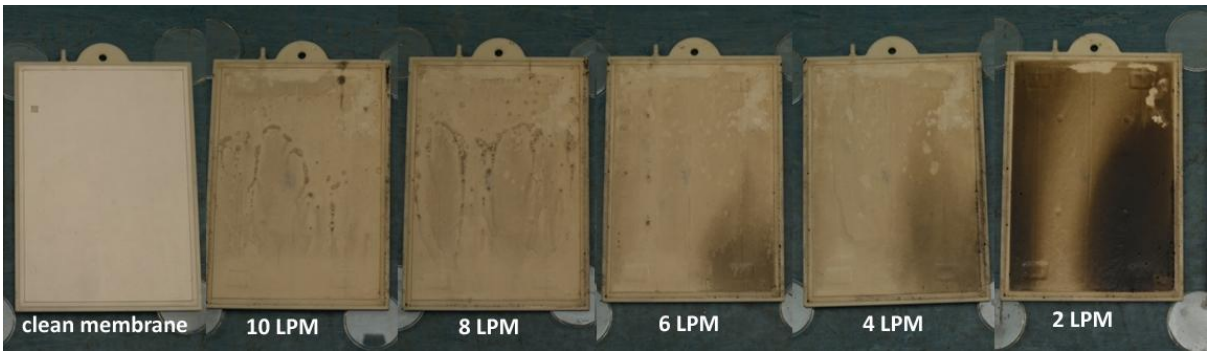


Figure 5-3 Photograph showing the membranes at each different gassing rate

In spite of the lack of a visible biofilm at the higher gassing rates, it was assumed that there must have been a thin layer on the surface that was causing the increase in MS-2 log removal from that of the clean membrane in a water filled reactor. Even at the highest gassing rate, the biofilm showed a 1.1 log removal increase from the clean membrane. It is assumed that the biofilm, through pore blocking or pore restriction is effectively reducing the size of the membrane pores, such that a significant amount of the MS-2 phage was retained within the reactor. It is also possible that charge played a part in the exclusion of the phage; the biofilm on the membrane is made up of bacteria and ECP, and this is likely to have a slight negative charge which would repel the negatively charged MS-2 particles (Gerba, 1984).

When the reactor was operated at 2 LPM a dramatic increase in fouling occurred which can be seen in the picture in Figure 5-3. With this data point, the LRV varied with the time each sample was taken, and hence the larger standard deviation error bars for that data point. As described in the Materials and Methods section, the reactor was operated at this gassing rate for 24 hours before the first sample was taken, however, for this state of operation it appeared that stable operation was not reached after 24 hours. In this case the TMP rose dramatically up to 0.65 bar for the first 12 hours of operation, but then settled down to 0.55 bar for the remainder of operation at 2LPM. The reactor was sampled again 48 hours after the change in gassing rate, and the LRV was found to have increased further to 3.2 (data not shown on graph). There was no further increase in TMP during this final 24 hours of operation, however the phage removal continued to increase. This increase in phage removal even after the TMP had stabilised indicates that further pore restriction was occurring.

5.3.2.2 *Operation at 2 LPM*

In order to fully investigate the effects of phage removal at low gassing rates the membrane was removed from the reactor and cleaned using the chemical cleaning method for Kubota membranes suggested by Le-Clech *et al.* (2006). The membrane was then re-submerged in the reactor and the unit operated at 2LPM until steady phage removal was achieved (Figure 5-4).

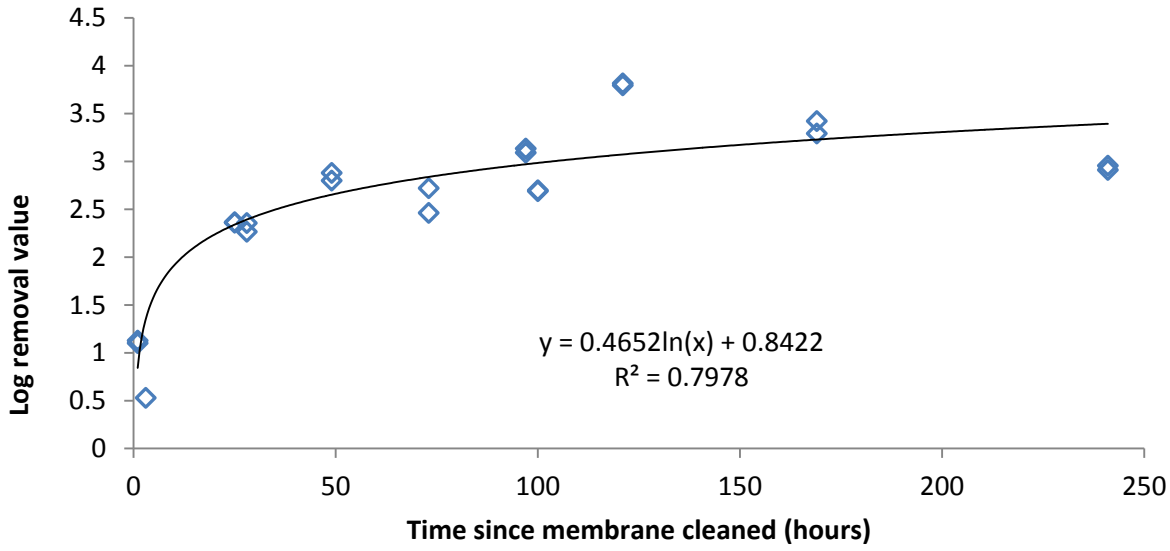


Figure 5-4 MS-2 phage removal in a reactor operated at 2 LPM starting from a clean membrane.

The phage removal increased over time, and a logarithmic relationship best fitted the data. The initial rejection data taken at 1 and 3 hours after the membrane was re-submerged into the reactor are quite low (<1log). At this time there would be very little build-up of biofilm, so most of the removal was down to that of the membrane alone; hence the LRV for the first 3 hours of operation was quite similar to that achieved in the initial SAMBR experiments without any biomass (see 5.3.2.1). After the first 24 hours the phage removal appears to gently increase at a much lower rate, tending towards a log removal slightly over 3. This suggests that the majority of the membrane fouling occurs quite quickly, within 24 hours, and after this time there is only a minor increase in pore restriction through membrane fouling.

5.3.2.3 *Membrane Hysteresis*

The previous results have dealt with MS-2 phage removal in a SAMBR starting with a clean membrane. In full scale operation the membrane is likely to be removed for cleaning over much longer intervals, typically 6-9 months for the Kubota membranes (Judd and Judd, 2006b). Therefore, it is important to consider the effect long term operation will have on phage removal within the SAMBR.

It was initially expected that after continued operation the SAMBR LRV would continue to rise slowly reaching an LRV of about 3, a similar LRV to that observed in Figure 5-4. It was also expected that the LRV would still be greater at a lower gassing rate similar to the data observed during initial operation in Figure 5-2.

The results for phage removal after extended operation are shown below in Figure 5-5. In fact, the SAMBR having been operated over an extended period of time shows the opposite trend to that observed in Figure 5-2. As the gassing rate increased, the phage removal appeared to increase also, each data point in this figure is the average of 6 samples and raw data is shown in appendix C.

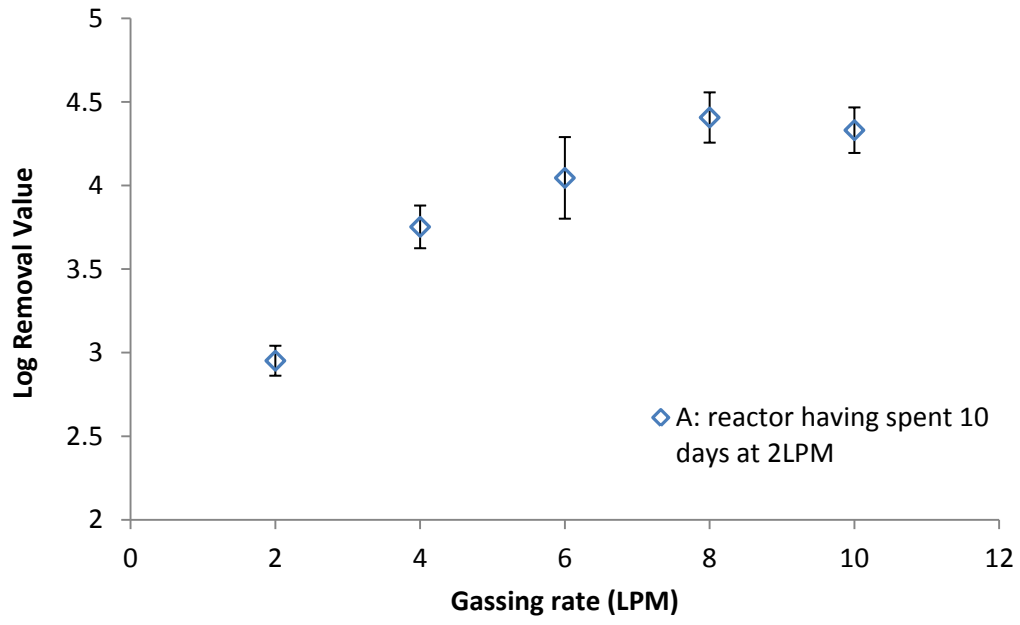


Figure 5-5 MS-2 Phage removal in the reactor after extended operation, LRV increases with gassing rate.

The above data in Figure 5-5 was collected from the same SAMBR run that the results in Figure 5-2 came from; between the two sets of data the reactor had been operated under standard conditions for a further 10 days at 2 LPM. In Figure 5-5 the phage log removal at 2 LPM was 2.95, which agrees with the data in section 5.3.2.2 regarding extended operation at a 2LPM scouring rate.

During this experiment photographs of the membrane were also taken to visually document the removal of the fouling layer as the gassing rate was increased. Figure 5-6 shows that once the gassing rate had been increased back to 10 LPM the visible fouling layer had almost completely been removed, and was at a similar extent to that observed in Figure 5-3. This suggests that something else besides surface fouling was controlling phage removal in the SAMBR.

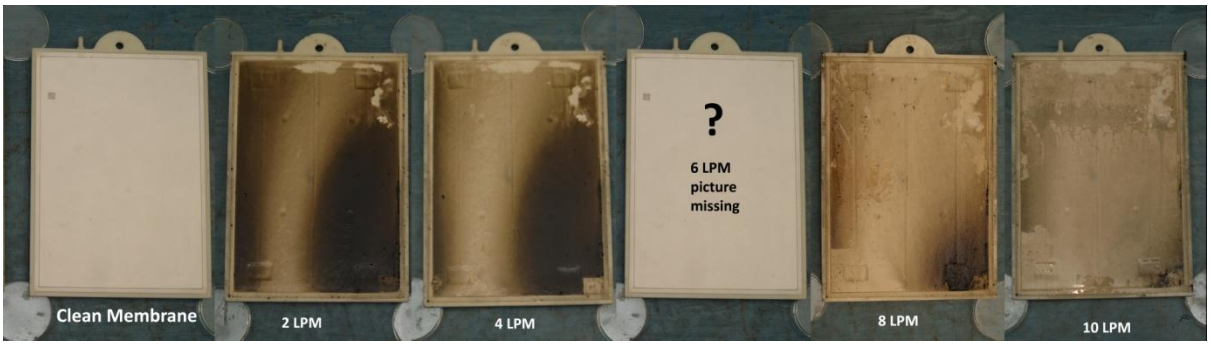


Figure 5-6 Membrane pictures as the gassing rate was increased.

Previous work in the literature that investigate virus removal in membrane reactors (summarised in Table 2-12) have not investigated the effect of gassing rate or any other mechanism that might bring the TMP down after fouling has occurred, and therefore this data has not been reported in the literature before. To confirm this result the experiment was repeated in another reactor; in this case the membrane had not been cleaned in over 6 months. During the final month of this period, the SAMBR was operated with a gassing rate of 2LPM for one month, so that the maximum possible amount of irreversible fouling had built up on the membrane before the experiment was carried out. This data are shown in Figure 5-7; the above results (data set A) are also included to provide a comparison. At 8 and 10 LPM gassing rates, the phage removal was roughly constant at 4.2 log removal, which suggests that the phage removal had reached a maximum, and that further increasing the gassing rate would not have any effect.

Figure 5-7 demonstrates that both reactors show the same trend of increased phage removal with increased gassing rate. The reactor operated for over 6 months (data sets B and C) shows a higher removal at each gassing rate compared to data set A, indicating that the longer the reactor is operated for, the higher the phage removal regardless of gassing rate. For data set B, in Figure 5-7, the gassing rate was increased in a stepwise fashion. To check for reversibility the phage removal was again monitored as the gassing rate was decreased in a stepwise fashion, and this is shown as data set C. With the exception of the 6LPM data point the data set C falls within 1 standard deviation of data set B, thus demonstrating good reversibility (the standard deviation of each data point is

represented by the error bars in the graph). This implies that, whatever phenomenon is causing phage removal, once it has occurred it is not affected by time.

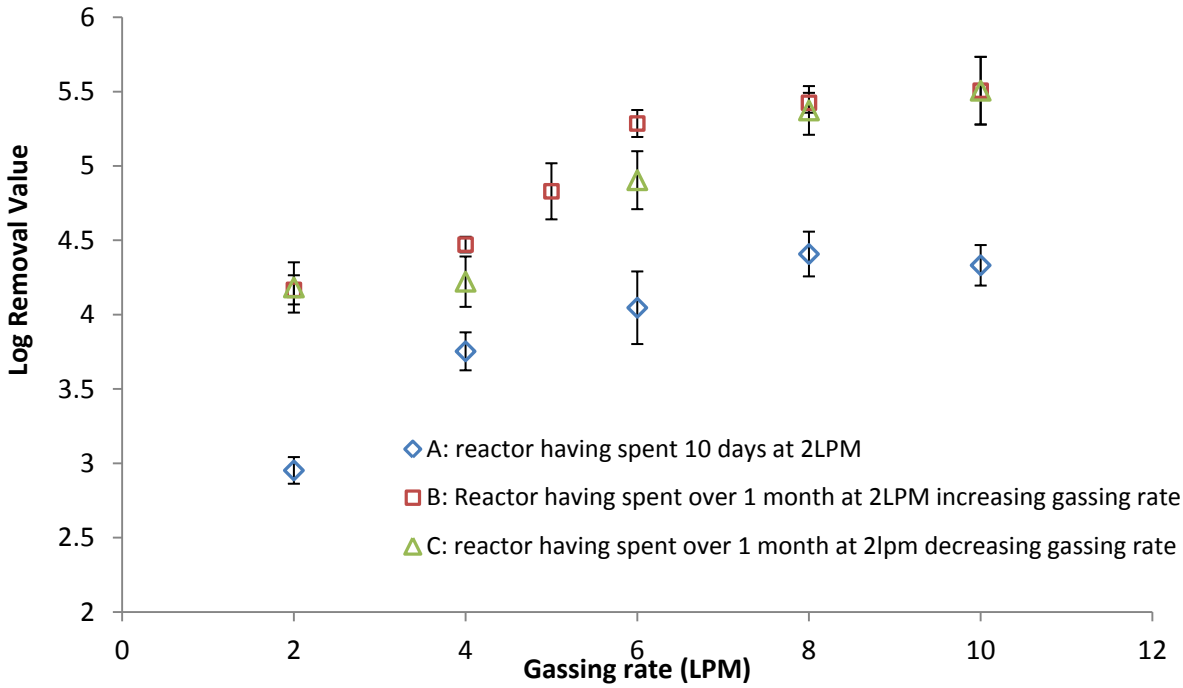


Figure 5-7 Phage removal in 2 reactors after extended operation at low gassing rates.

The removals observed here are significantly higher than those observed by Shang *et al.* (2005), who found a maximum log removal of 2.5. However, they only operated for 20 days, and therefore with extended operation they may have achieved a similar value. In fact the removal falls into the same range as that found by Chiemchaisri *et al.* (1992) for the Qβ phage. While the researchers used a different phage it is similar in size to the MS-2 phage, and therefore in good agreement with the data on extended operation.

The TMP was also recorded for the above data sets at each gassing rate. In section 5.3.2.2 it was demonstrated that there is no clear relationship between surface fouling and phage removal at low gassing rates. It is possible, however, that it is not the surface fouling but the internal 'irremovable' fouling that increases the phage removal due to significant pore blocking. The amount of fouling inside the membrane pores would be difficult to monitor, however, an increase in gassing rate would not remove the internal fouling since the gas bubbles only scour the surface of the

membrane. Once this internal fouling has occurred, the surface fouling would cease to have an effect on the phage removal, which is what is occurring in Figure 5-7 and Figure 5-8.

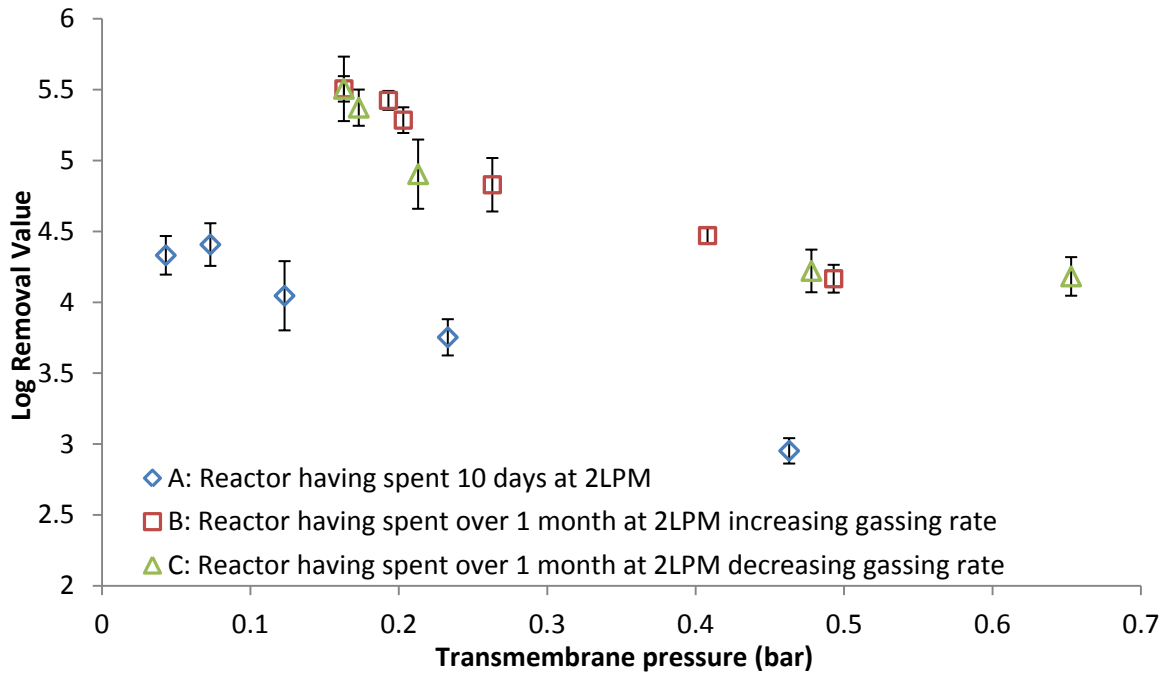


Figure 5-8 Phage removal in 2 reactors after extended operation at low gassing rates (dependence on TMP)

Figure 5-8 shows how the phage removal was affected by TMP. Similarly to the data in Figure 5-7 the reverse trend was observed to what was expected; as the TMP increases (with a decreasing gassing rate) the phage removal decreases.

This trend of increasing phage removal with a decrease in TMP has not been previously observed in other work on phage removal in MBRs. In other work the phage removal has been shown to increase with an increase in TMP (Shang *et al.*, 2005; Ueda *et al.*, 2000). In these other works, however, the TMP was only shown to increase over time as the fouling layer built up, and no attempt was made to decrease the TMP after the fouling layer became established.

Interestingly, in Figure 5-7 it can be seen that when the gassing rate was lowered back to 2 LPM in data set C the phage removal was essentially the same value as the initial removal rate at 2 LPM in data set B; however, in Figure 5-8 it can be seen that this occurred in spite of the fact that the TMP had significantly increased. Also, the discrepancies between the phage removal in data set A and that of B and C suggests that TMP alone is not a good indicator of phage removal.

Since the phage removal increases with both an increase in gassing rate, and a decrease in surface fouling and decrease in TMP, the explanation for the trend in phage removal in the reactors after

extended operation must be due to more than just the surface fouling layer. Cui *et al.* (2003) have shown that the membrane bioreactors have demonstrated an increased rejection at increased gassing rates for other compounds, it is suggested that this is down to reduced concentration polarisation at the membrane at higher gassing rates.

Once the internal fouling has occurred, and the fouling on the membrane surface ceases to affect the phage LRV, it is speculated that for the above results concentration polarisation has become the controlling parameter for phage removal. Concentration polarisation is defined as the build-up of charged particles and solutes on the surface of a membrane such that the concentration at the membrane surface is greater than in the bulk solution. So in this case the charged solutes are the phage particles, and the concentration of phage in the membrane boundary layer is higher than in the bulk solution. The larger this boundary layer, the higher the concentration of phage particles on the membrane surface, and therefore the phage concentration in the effluent would also be increased. To try and increase the phage rejection in this case the boundary layer would need to be reduced.

Gas scouring of the membrane surface had been shown to affect the concentration polarisation layer on the membrane wall (Cui *et al.*, 2003). A decrease in the phage surface concentration through an increase in gassing rate would therefore lead to the increase in phage removal as seen in Figure 5-7. Other methods for reducing this boundary layer (and thus increasing phage removal) could also include: operating at a lower flux or using a liquid crossflow to disrupt the boundary layer.

5.3.2.4 Phage degradation in the SAMBR

It has been shown that the SAMBR retains the vast majority of the phage particles inside the reactor; therefore, it is important to understand what will happen to the retained phages. To this end, the phage concentration in the reactor was monitored over a period of time. The LRV was measured at 4.5, and the gassing rate in the reactor was kept constant at 8 LPM. If the only factor affecting the phage concentration inside the reactor is the 0.001% of phages escaping through the membrane, then the number of phage particles inside the reactor could be modelled as a CSTR, whereby the rate of change of phage particles inside the reactor is directly proportional to the number of particles in the reactor as shown in equation 5-2.

$$\frac{dN_{MS2}}{dt} = -\frac{N_{MS2}R_{frac}F}{V} \qquad \textbf{5-2}$$

Where N_{MS2} represents the number of phages in the reactor, R_{frac} is the log removal value in fraction form, in this case the LRV was 4.5 so R_{frac} is $10^{-4.5}$, F is the flow rate out of the reactor in ml/day, V is the volume of the reactor in ml, and t is time in days. Equation 5-2 can be integrated and solved for t as shown in 5-3, so that the length of time taken for the concentration in the reactor to drop below a certain level can be computed using the parameters shown in Table 5-1.

$$t = \frac{V}{R_{frac}F}(lnN_0 - lnN_{MS2}) \qquad 5\text{-}3$$

Table 5-1 Parameters used to solve equation 5-3

Volume (ml)	3000
Flowrate (ml/day)	6000
R_{frac}	$10^{-4.5}$

Using the above equation it would take approximately 10 years for the concentration inside the reactor to drop by 1 log! However, as can be seen in

Figure 5-9, the phage concentration with the reactor dropped by 1 log over the space of 7 days, much faster that the CSTR model. In fact the phage concentration in the reactor appears to decrease in a linear-log fashion. Since this decrease in phage concentration is much faster than if it were only phage removal through the membrane that was causing the drop in concentration, it must be assumed that either the phages are being denatured by the anaerobic bacteria, or that the phages are being otherwise inactivated by the conditions within the reactor.

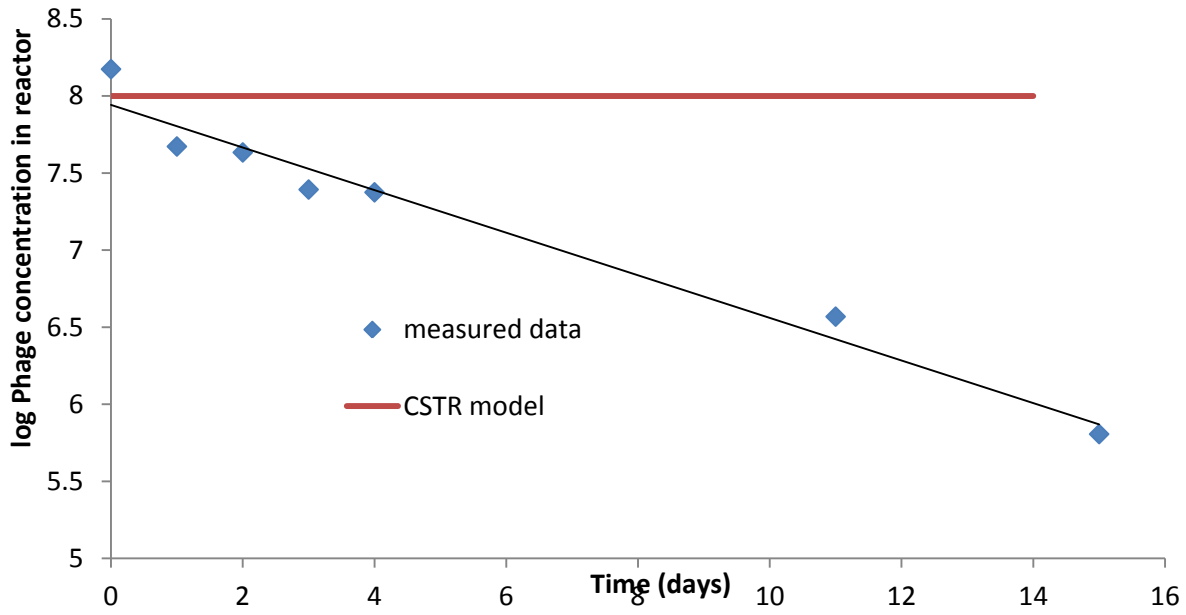


Figure 5-9 Phage concentration droppng in the SAMBR over time.

To ascertain the mechanism of phage reduction within the reactor some batch experiments were performed. Figure 5-10 shows the results of the batch experiment on the MS-2 phage. The bottles were set up as for a bio-methane potential (BMP) test, and different concentrations of phage were added. Unlike a standard BMP test, however, monitoring the methane output for these tests would not necessarily prove that the phage was being inactivated. By the nature of the phage harvesting process the phage suspension also contains significant amounts of meat extract and peptone, and these would be preferentially degraded to methane and skew the gas results. Instead of monitoring the methane output, a 1ml liquid sample from the bottles was removed and assessed for phage concentration. To determine whether the phage was being denatured by the biomass, or simply inactivated by the solute conditions, a control sample was also analysed. In this assay the suspended biomass was removed by centrifugation so that the serum bottle contained only phage and supernatant.

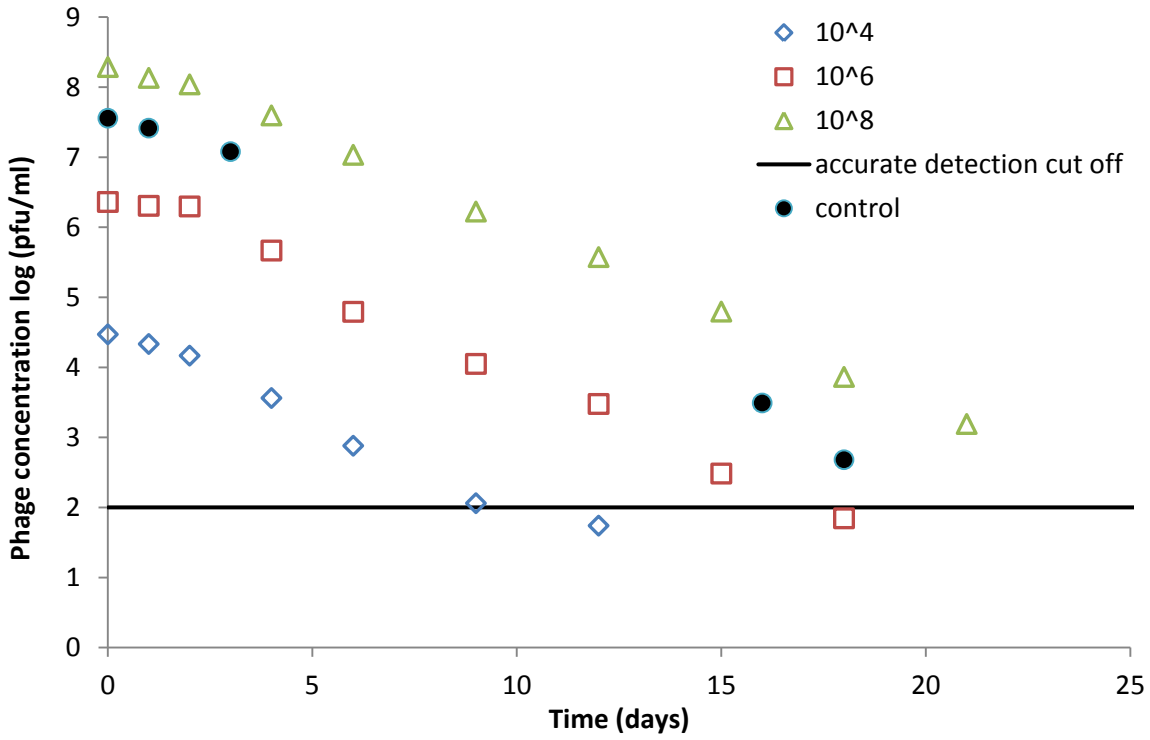


Figure 5-10 MS-2 phage concentration over time batch experiment.

The results in Figure 5-10 show that the phage concentration in the biomass decreased at a similar rate to that in the SAMBR (see

Figure 5-9), regardless of the initial concentration of the phage. In the batch experiment there appeared to be a lag phase of 3 days; initially it was thought that this lag-phase was due to the biomass, which had not previously been exposed to the phage, adapting to a new substrate. However, when considered alongside the control experiment, it can be seen that the phage suspended only in supernatant was removed at the same rate as the phage suspended in biomass. This indicates that it was the environmental conditions causing phage inactivation, rather than any denaturation/adsorption by the biomass.

5.3.3 T4 Phage Removal

Having considered the removal in the SAMBR for the very smallest phages, it was also important to consider what will happen to the larger viruses; for this the T4 phage was used as a model organism. The T4 phage has a longest dimension of 200nm making it one of the largest phages; with the membrane pores being 400nm the membrane alone would be expected to show significant T4 removal, but not complete removal. Therefore, to model larger viruses the T4 phage was selected,

and this has previously been used by other researchers due to its similarity to the SARS virus (Lv *et al.*, 2006).

Similar to the MS-2 experiments, the rejection efficacy of the clean membrane was assessed by placing the clean membrane in a reactor unit filled with water and monitoring the T4 throughput. The removal efficiency of the clean membrane was found to be 2.3 LRV ±0.2, and this was a slightly greater removal than Lv *et al.* (2006) who achieved a 1.7 LRV, and Ueda and Horan (2000) who found a 0-0.5 LRV for a T even phage through a 0.4µm membrane. The result from Ueda is surprisingly low considering the phages are about half the size of the pore, and hence some significant removal would be expected. However, since they used a T-even phage rather than T4 the difference may be due to the slight difference in the phage used. The result in this study was higher than expected; this is possibly due to the membrane not being entirely clean before the start of the experiment, or from some phage inactivation across the membrane.

The T4 experiments were done in a separate reactor to the one used for the MS-2 experiments. Initially a clean membrane was submerged in a reactor operating under the previously described standard conditions. The gassing rate was again varied and the log removal for each rate was monitored; the results are shown in Figure 5-11

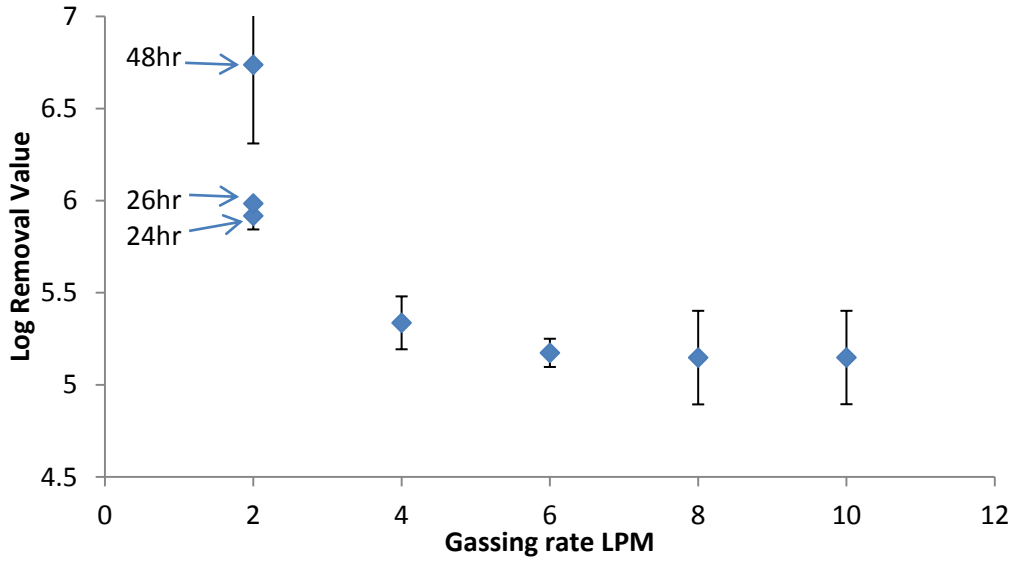


Figure 5-11 T4 phage removal in a SAMBR at different gassing rates, for the 2LPM data the time since the gassing rate was set is displayed next to the data point.

Under steady state operation the SAMBR shows a very good rejection of the large T4 phage. Even at the highest gassing rate the rejection remains above 5 log. Similar to the experiments for MS-2, the

T4 rejection appears to increase slightly with the gassing rate decreasing from 10 LPM to 4 LPM. However, since this is inside the margin of error for the experiments it is not statistically significant and therefore not possible to say that there is a definite trend. Additionally the data was analysed using an analysis of variance (ANOVA) F-test, with a null hypothesis of *"gassing rates from 10-4LPM have the same log removal value for T4"* and a significance value of 5%. The analysis returned a p value of 0.293 (full calculations are shown in appendix D), so there is a 29% chance of achieving a data set at least this extreme if the null hypothesis is true. This is greater than the 5% significance level; therefore the null hypothesis cannot be rejected, and any correlation between the gassing rate and the LRV is not statistically significant for gassing rates between 10 and 4 LPM.

As soon as the gassing rate was set at 2 LPM, the LRV increases in accordance with the TMP increase. The data points on Figure 5-11 at 2 LPM show the phage removal increasing at 24, 26 and 48 hours after the reactor was set to 2 LPM.

During further operation at 2 LPM the T4 removal continued to increase; however, the number of plaques appearing at the lowest dilution dropped below 10 (the minimum number for an accurate reading), and therefore it was not possible to accurately determine the LRV. Further to this more T4 were injected into the reactor at the highest practicable concentration; in this case the T4 concentration in the reactor surpassed 10^9pfu/ml. Since the concentration in the effluent remained below the lowest accurately determinable point (10^2pfu/ml), the T4 phage removal of the reactor was above 7 LRV.

These data is broadly in agreement with the rejections achieved by other researchers for T4 and T even phages. Ueda (2000) found removals for the T even phage to be between 2.3 and 5.9 LRV, and the log removals in the SAMBR were at the higher end of this spectrum. This is most likely due to the fact that Ueda and Horan only considered the first 12 hours of operation after a new membrane was used, and therefore it was possible that the biofilm on the membrane had not had a chance to fully develop causing their lower removals.

The work by Lv *et al.* (2006) and Zheng *et al.* (2005) both show complete removal of the T4 phage (in excess of 7 log); this is in agreement with the SAMBR data after operation below the critical gassing rate such that extensive fouling had occurred on the membrane surface.

5.3.4 Activated carbon effect on Phage removal

The effect of activated carbon has been widely studied for its adsorptive properties to remove toxins from waste streams. Several authors have reported an increase in SAMBR performance following the addition of activated carbon (Akram and Stuckey, 2008; Hu and Stuckey, 2007; Liu *et al.*, 2007;

Satyawali and Balakrishnan, 2009). This is thought to be due to small solutes such as SMPs and colloids being adsorbed onto the surface of the carbon so that they are retained within the reactor and can be degraded. Due to the prevalence of research into the effects of PAC and GAC in MBRs the effect of these particulates on virus removal should be considered.

Research has also been conducted on the effect of activated carbon on the viruses, again using phages as indicator organisms. Powell *et al.* (2000) have shown that there is a significant absorbance of MS-2 onto both PAC and GAC in column adsorption studies in a phosphate buffer solution (PBS) spiked with MS-2. However, there is no available data on the adsorption of phages onto PAC or GAC in a biomass environment.

In this section the effect of adding PAC and GAC to the SAMBR reactors is assessed in terms of its effects on MS-2 phage removal. Two separate reactors were spiked with phage and the log removals monitored once the initial removals were determined, 3g of PAC and GAC were added to each reactor to give a total concentration of 1g/l activated carbon, and the concentration of phage in the reactor and effluent further monitored to assess the effect of carbon addition on phage removal.

The GAC used was Norit PK 0.25-1 and the PAC was Norit SAE-2. In these experiments there were too many data points to repeat samples, and therefore the error bars on these graphs represent the standard error of ± 10 plaques on each plate.

5.3.4.1 GAC

Figure 5-12 shows how the log removal of MS-2 changed with the addition of the GAC. Initially the LRV of the reactor was fairly high at 3.5, but once the GAC was added to the reactor (just after 2 hours) the LRV started to drop. This was not the expected result; Figure 5-13 and Figure 5-14 show the concentration of the phage in the SAMBR bulk and in the effluent, respectively. While the MS-2 concentration in the SAMBR bulk remains fairly constant before and after GAC addition, the concentration in the effluent starts to increase after the GAC addition. It is assumed that the peak at 5 hours was an anomaly due to an experimental error.

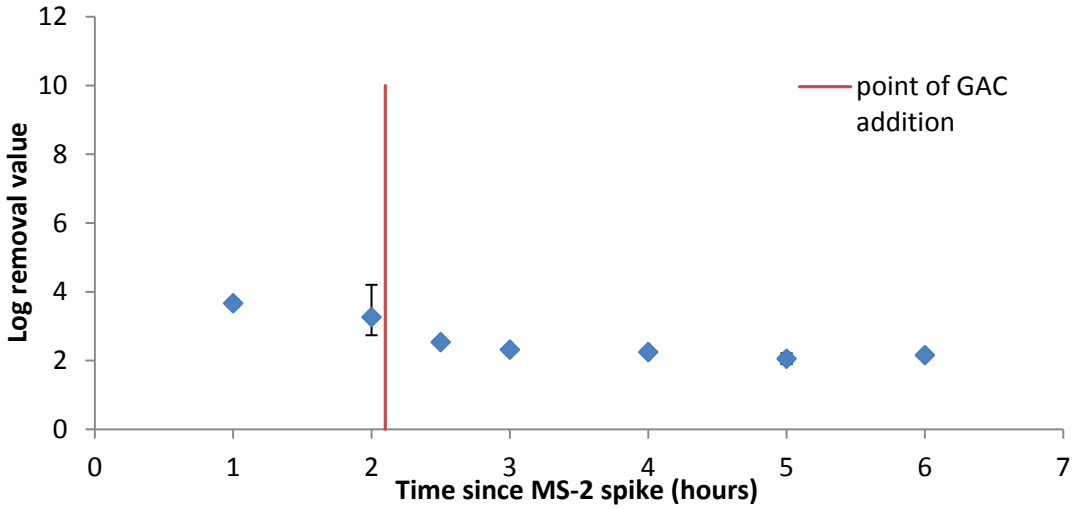


Figure 5-12 Phage LRV before and after GAC addition.

The lack of variation in the MS-2 concentration within the reactor demonstrates that adsorption is not occurring in the SAMBR. While the batch tests showed absorbance at this phage concentration in water, other components in the SAMBR such as VFAs, SMPs or other macromolecules have preferentially adsorbed to the surface of the GAC over the phage. The increase in phage in the effluent in the reactor after GAC addition indicates that by some mechanism the GAC is increasing phage throughput.

It is likely that the GAC is scouring the surface of the membrane as suggested by Hu and Stuckey (2007). This scouring reduces the fouling layer on the membrane surface that is partly responsible for the phage removal. This is important to note for industrial applications where GAC is added to MBRs to assist in the degradation or adsorption of other toxins, because a side effect of the GAC addition could cause an increased throughput in other unwanted particles such as viruses.

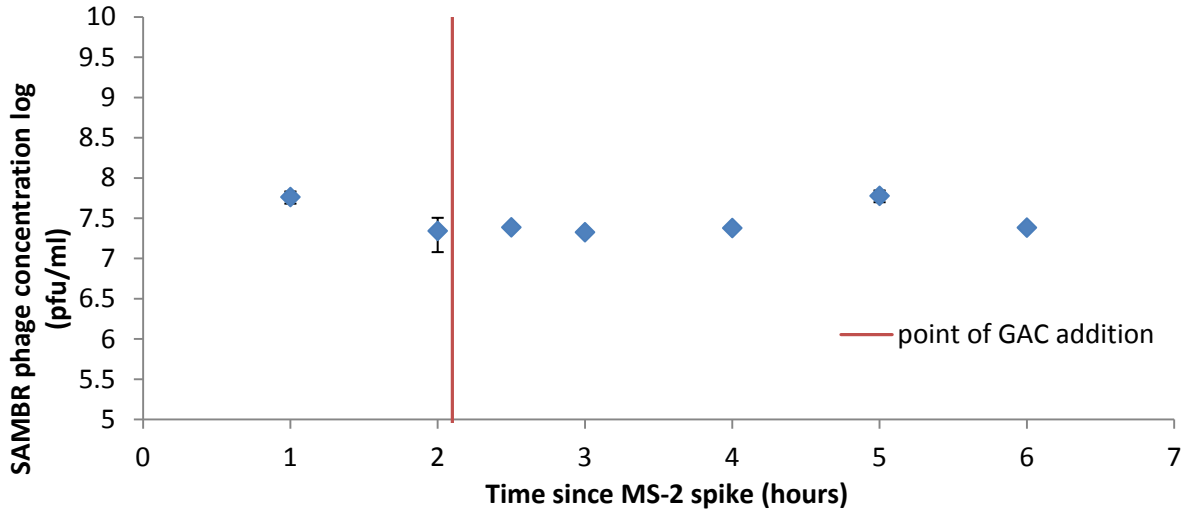


Figure 5-13 Phage concentration in the SAMBR bulk before and after GAC addition.

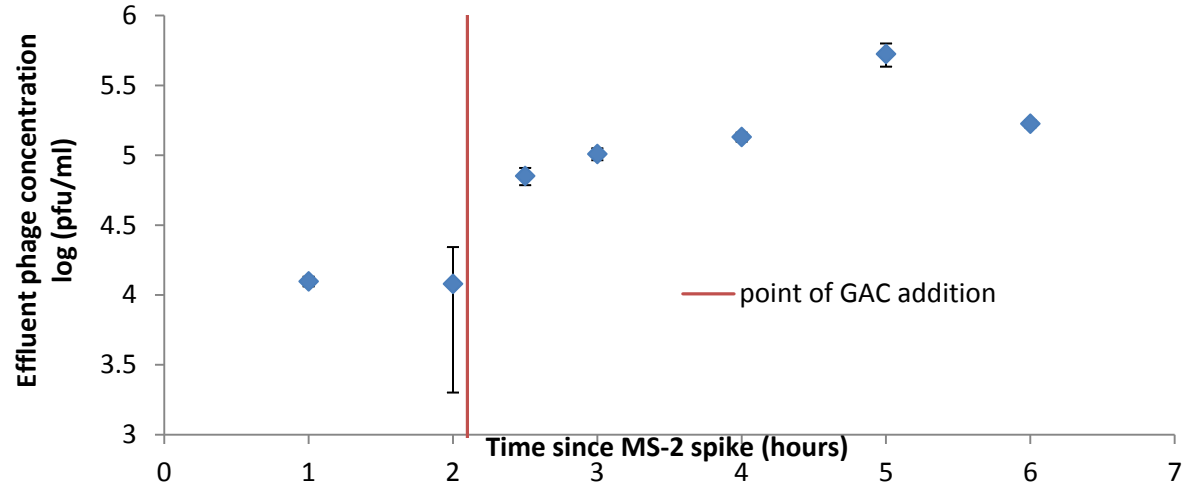


Figure 5-14 Phage concentration in the effluent before and after GAC addition.

5.3.4.2 PAC

Before the SAMBR was dosed with PAC, a batch test was run to assess the possibility of phage adsorption by the carbon. The PAC batch tests were run using a stock of biomass (previously uncontaminated by phage) as the suspended liquid matrix. The results (Table 5-2) show that very little phage adsorption occurs at PAC concentrations of 0.1 and 1 g/l. In fact, the log adsorption value for 0.1 and 1 g/l PAC is within the margin of error such that it is likely that there is no phage adsorption occurring at all.

Table 5-2 Phage adsorption by PAC in biomass batch results.

		PAC concentration g/l		
		0.1	1	10
		Log phage adsorption		
Initial phage concentration pfu/ml	**10^3**	-	0.05	0.93
	10^5	0.00	-0.01	1.18
	10^7	0.09	0.07	1.52

The results at 10g/l PAC show that there was approximately 1 log (90%) removal of phage in each batch sample due to adsorption (Table 5-2). Since such a high PAC concentration is required to provide any significant adsorption of phage, it is not likely that this has much useful application in the field of wastewater treatment. Adding this amount of PAC to remove viruses from within a biomass mixture would not be economically viable. A post treatment adsorption column would be more beneficial here, such as the one in the work carried out by Powell *et al.* (2000).

Other researchers have suggested the use of PAC in a SAMBR to improve performance, by lowering TMP, increasing COD removal and to provide a support for biomass growth (Hu and Stuckey, 2007). Therefore, it is still important to monitor the effect of PAC addition on phage removal, to see if it has any further effects such as the membrane scouring that occurred with GAC addition.

A spike of MS-2 phage was added to the reactors and the initial MS-2 removal determined; after 4 hours the PAC was added so that the concentration in the reactor was 1g/l and the effect of phage removal was monitored. Figure 5-15 shows some variation in the phage log removal before the PAC was added, which makes it difficult to determine an accurate initial LRV. However, after the PAC was added to the reactor the LRV does increase by 0.3 log. The reason for this increase is not clear, however looking at the concentration of phage in the reactor and in the effluent (Figure5-19 and Figure 5-18 respectively) it can be seen that the increase in LRV was due to an increase in phage concentration inside the reactor rather than a drop in the effluent concentration.

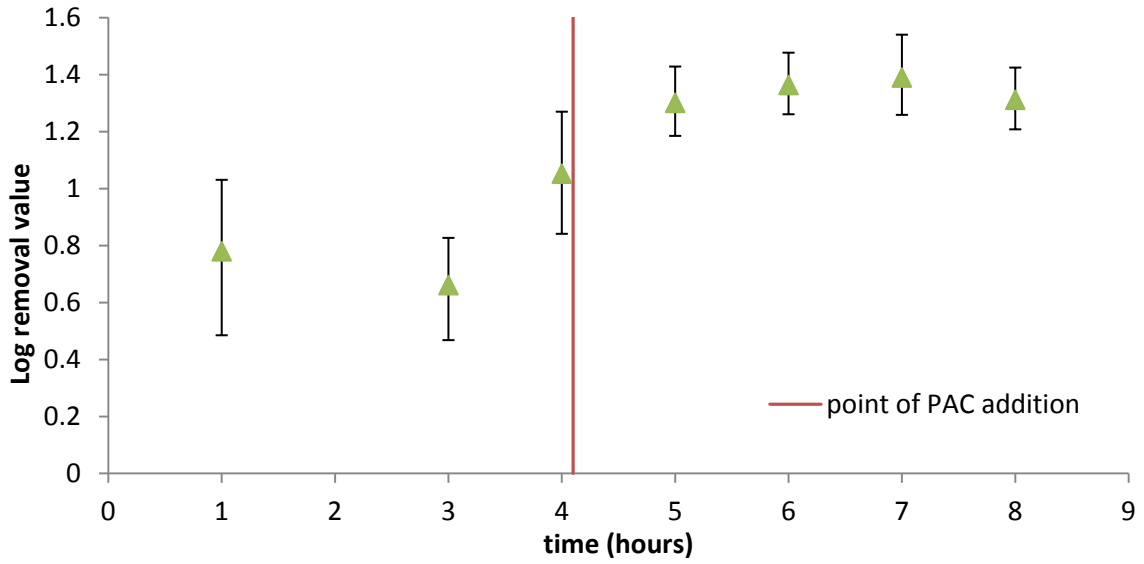


Figure 5-15 Log removal in the SAMBR before and after PAC addition.

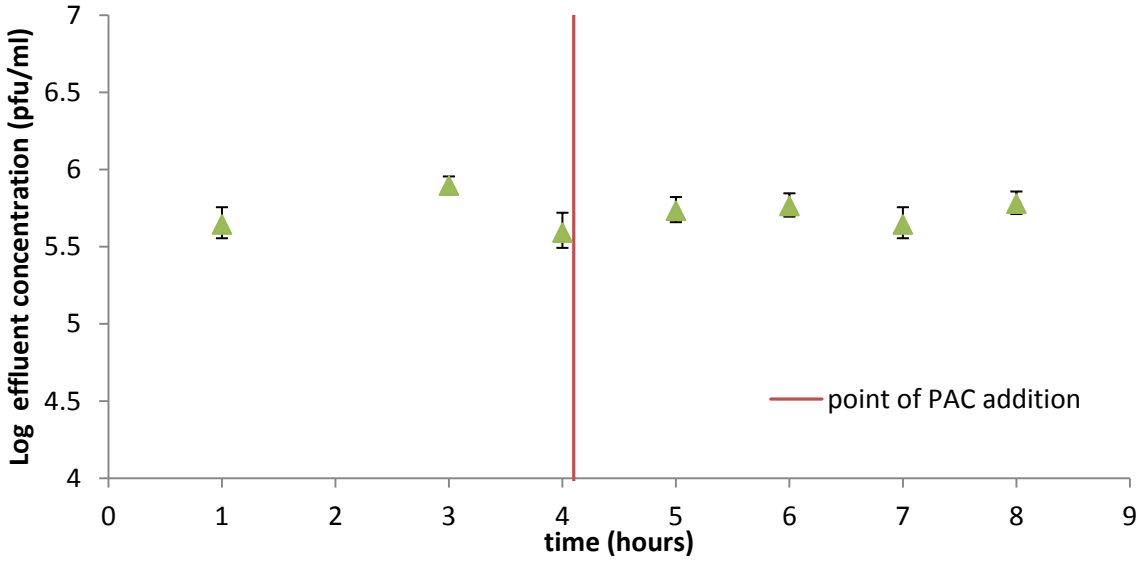


Figure 5-16 Phage concentration in the effluent before and after PAC addition.

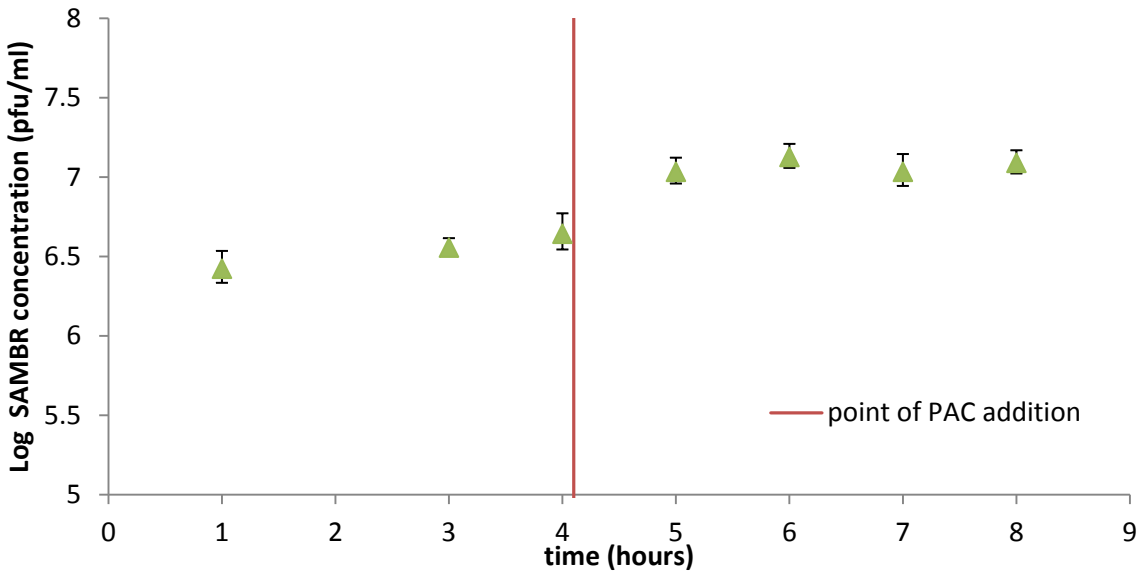


Figure 5-17 Phage concentration in the SAMBR before and after PAC addition.

Figure 5-16 shows that the concentration of phage in the reactor effluent remained fairly constant throughout the experiment at 5.7 ±0.1 log. The concentration in the SAMBR, however, appears to climb from 6.6 logs to 7.0 logs after PAC addition (shown in Figure 5-17). As expected from the batch results, a PAC concentration at 1g/l shows no tendency to adsorb the phage. Since no further phage was added to the SAMBR, the reason for the rise in phage concentration is thought to be due to propagation. The feed inlet to the reactor may contain some common E. coli which could be using the PAC as a platform to contact with the phage particles causing propagation.

5.4 Summary

The results have shown that:

Within the SAMBR reactors the MS-2 phage shows a significant log removal, however, the membrane alone only contributes 0.6 LRV to the overall removal. When under operation at gassing rates between 4-10 LPM, with an initially clean membrane, there is a positive correlation between decreasing the gassing rate and an increase in MS-2 removal. In this case the LRV varied from 1.75 up to 2.10.

Once the SAMBR was operated at low gassing rates of 2 LPM, significant fouling built up on the membrane surface causing a marked increase in the log removal of MS-2. In an experiment using a

clean membrane in a SAMBR operated at this gassing rate the LRV increased from 0.7 LRV to 2.5 LRV in the space of 2 days; the log removal continued to slowly increase after this.

While surface fouling on the membrane had some effect on phage removal, it was not the deciding factor. The membrane's individual history played an important part in determining the removal factor. The experiment seemed to indicate that the longer the membrane has spent at 2 LPM, the greater the log removals will be at all gassing rates. It has also been shown that once the fouling at 2 LPM has occurred, then increasing the gassing rate in the SAMBR serves to alleviate the effect of concentration polarisation; thus an increase in gassing rate results in an increase in LRV. In this case the LRV varies from 3 up to 5.5 LRV depending on the gassing rate. It has also been demonstrated that instead of building up in the SAMBR, the phages are inactivated by the conditions in the reactor.

Experiments with the much larger T4 phage show high log removals even during operation just after a clean membrane is inserted into the reactors. At 10 LPM an LRV of 5.1 was found, and this increased to 5.3LRV as the gassing rate was dropped to 4 LPM. Once the reactor was operated at 2 LPM again the removal dramatically increased as significant fouling occurred, and the LRV rose above detectable limits and can be approximated to complete removal.

Due to the prevalence of research on activated carbon in biotechnologies, the effect of both GAC and PAC on phage removal was studied. For GAC in the SAMBR, the log removal of the MS-2 phage actually decreased as the GAC particles appeared to scour the surface of the membrane unblocking some of the blocked pathways that prevented phage transmission. The addition of PAC to the SAMBR showed much less effect on phage removal. The PAC appears to act as a platform for phage generation, and hence the concentration of MS-2 in the SAMBR increased; however, this had little effect on the LRV. In both cases no evidence of MS-2 adsorption on the carbon surface appeared to occur at the commonly used concentrations of 1g/l. To achieve any notable phage adsorption the PAC concentration would need to be increased to around 10g/l, which would be impractical on an economic basis.

Chapter 6. Flowsheeting

6.1 Introduction

The water industry is the fourth most energy intensive sector in the UK; it uses approximately 2-3 % of net UK electricity and releases approximately two million tonnes of greenhouse gas emissions (carbon dioxide equivalent) every year (Environment Agency, 2012).These figures are expected to increase in the near future in order to meet the effluent standards set by the new European Water Framework Directive (Directive 2006/44/EC). In addition to the EU regulations, the UK government has also set targets on renewable energy production and carbon footprint. This presents a significant challenge to wastewater treatment companies, since the conventional activated sludge wastewater treatment process has limited scope for further optimisation in reducing power demand and CO_2 output. As such the traditional treatment flowsheets are unlikely to be able to meet both sets of regulations.

Anaerobic treatment of municipal wastewater has already been implemented in tropical countries, eg Brazil, where high-rate processes have been used to achieve chemical oxygen demand (COD) removals around 60-70%. These plants also have a smaller energy demand since there is no aeration requirement (Rogalla, 2007). The benefits of using anaerobic treatment have also been demonstrated during the treatment of low-strength industrial wastewater, with 23% electricity and 60% chemical expenditure savings (Lerner *et al.*, 2007).

Anaerobic sewage treatment of low strength (<700 mg/L COD) has been proved to be feasible at temperatures between 10-20°C. This has important benefits such as biogas production and reduced sludge production (Langenhoff and Stuckey, 2000; Lester *et al.*, 2009; Soares *et al.*, 2007). These advances have been possible due to new developments in bioreactor design, and enhanced knowledge on anaerobic microbial communities.

As discussed in the literature review the most comprehensive method for analysing different wastewater treatment flowsheets would be life cycle assessment (LCA). This ‘cradle to grave’ methodology would give an overview of the environmental impact of the wastewater treatment process. In this case however there is simply not enough information on the novel units to provide the data needed. Information on units such as the SAMBR, and ion exchangers for example has only been published up to the pilot scale, and therefore the full scale information needed to perform LCA

is lacking. Additionally LCA deals solely with the environmental impact of the process, it does not consider the cost or societal implications of the technology.

Because there has not previously been any attempt to compare anaerobic treatment units, in terms of a whole process view, it is necessary to take a simple flowsheeting approach to model the different units so that a comparison of the best treatment options can be developed.

The principal purpose of this chapter was to demonstrate how anaerobic treatment might fit in to modern wastewater treatment process design, if starting from a blank sheet of paper. To this end there were three aims in this chapter these were: To develop a basic model for various novel anaerobic treatment units; to design treatment flowsheets to demonstrate where the units would fit in to a wastewater treatment flowsheet; and to use a decision making analysis to compare the different flowsheets and come up with an optimal solution that takes into account all parameters. The flowsheets were modelled on a test case scenario based on a 10000 population equivalent plant in the UK.

6.2 Parameters used in flowsheet development

The basic parameters and constraints on which the model flowsheets were designed are shown in the tables below. The influent parameters (Table 6-1) are based on those of a treatment plant in Esholt, and the effluent constraints (Table 6-2) are those set by the EEC. The flowsheets were designed for a plant with a capacity of 10,000 population equivalent.

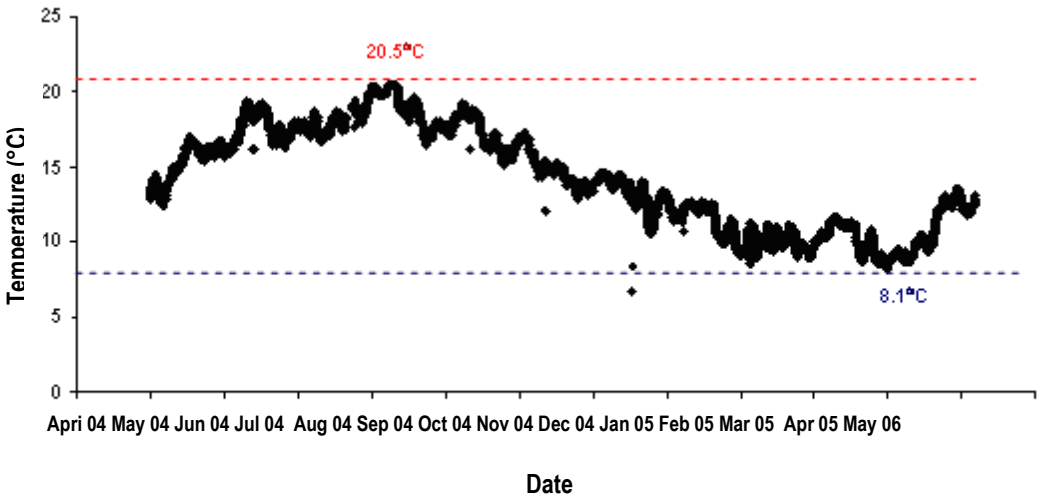


Figure 6-1 Temperature profile at Esholt over a 12 month period

Table 6-1 Influent characteristics from Esholt STW influent (average values measured over a period of 4 months)

Parameter	Unit	Average value
Suspended solids	mg/l	589.2
Total COD	mg/l	971.2
Soluble COD	mg/l	166.9
Total BOD	mg/l	313.2
Soluble BOD	mg/l	23.7
pH		7.1
Total ammonium	mg/l	28.8
Soluble nitrogen	mg/l	44
Nitrogen Kjeldahl	mg/l	54.8
Nitrite	mg/l	10.6
Nitrate	mg/l	0.1
Total phosphorus	mg/l	3.9
Total ortho-phosphate	mg/l	2.2
Total phosphate	mg/l	10.4
Acetic acid	mg/l	128
Propionic acid	µg/l	12
Butyric acid	mg/l	8.9
Iso-butyric acid	mg/l	5.6
Iso-valeric acid	mg/l	7.4
Pentanoic acid	µg/l	5.5
Temperature (see Error! Reference source not found.)	°C	8-20

Table 6-2 Effluent requirements- Adapted from the Freshwater Fish Directive (78/659/EEC; 2006/44/EC) Salmonid Waters

Parameter	Unit	Value
Suspended solids	mg/l	≤ 25
Total BOD	mg/l	± 5
pH		6-9
Total nitrogen	mg/l	<3
Total ammonium	mg/l	≤ 1
Nitrite	mg/l	0.01
Total phosphorus*	mg/l	1

6.3 Process units used in the model flowsheets

Since the purpose of this study was to re-flowsheet a 10,000 PE wastewater treatment plant to be based around anaerobic treatment. To take a holistic approach the entire process was re evaluated from pre-treatment to tertiary and post treatment.

6.3.1 Pre-treatment

6.3.1.1 Screens (6mm and 1mm)

Mechanically cleaned 6 mm coarse screens were taken into consideration for each one of the designed flowsheets. There is no precise definition of screenable material and hence no recognised method of measuring quantities of screenings (Tchobanoglous *et al.*, 2003). A recent study by Le Hyraic *et al*. (2009) on the characterisation of screenings from French WWTPs demonstrates that average production of screening is between 0.53 – 3.49 kg per capita per year (wet mass).

As an alternative to primary treatment, one of the designed flowsheets (#5) uses an additional fine screen with 1 mm openings before the wastewater enters the main anaerobic unit. For the fine screening, a rotary drum unit was selected. The specific removal efficiencies will depend upon the exact nature of the wastewater, however, it is assumed that COD, BOD and TSS removal will all be in the region of 30% (Tchobanoglous *et al.*, 2003). There is very little publically accessible information on fine screening efficacy, but there is anecdotal information from membrane bioreactor plants where pre-screening with 1-2 mm fine screens is mandatory. Most MBR plants operate with 6 mm coarse screening followed by grit removal (see below), and then 1-2 mm fine screens, the exact screening rating depends on the type of membrane. The most successful of these are rotary drum microscreens with cylindrical (“punched hole”) apertures, rather than slit or wedgewire screens which can lead to membrane channel clogging.

6.3.1.2 De-grit

Horizontal flow grit chambers are the simplest and most commonly installed. The typical grit content of wastewater is in the range of 0.004 to 0.037 $m^3/1000m^3$, with an average value of 0.02 $m^3/1000m^3$ (Tchobanoglous *et al.*, 2003). Assuming the grit chamber can remove 95% of the grit, the daily grit production on the model flow sheets is calculated to be 0.048 m^3/day or 125 kg/day (assuming sands, gravels and cinders of density 2600 kg/m^3).

6.3.1.3 *Primary settling tank*

Rectangular tanks were selected for this study since multiple units are required for a plant of the specified size and a collection of rectangular tanks requires a smaller footprint compared to multiple circular tanks.

The key design parameter for primary sedimentation tanks is the overflow rate at which effluent is drawn over the weirs. Typically this in the region of 30-50 $m^3/m^2.d$ for average flows; an overflow rate of 40 $m^3/m^2.d$ was selected for the unit model. A typical unit depth of 4 m was also selected. The remainder of the design was carried out using the protocol set out in Tchobanoglous *et al.* (2003). For the calculated retention time (2.4h) the removal efficiencies for BOD and TSS are 36 and 58% respectively as shown in Table 6-3. The COD removal rate of 50% was assumed using data from studies carried out by Tebbutt and Christoulas (1975).

Table 6-3 Primary settling tank performance (Tchobanoglous *et al.*, 2003; Tebbut and Christoulas, 1975)

Parameter	% removal
BOD	36
TSS	58
N	9
P	11
COD	50

6.3.2 Anaerobic Treatment

After pre and primary treatment has taken place the next step in the model flow sheet design is the main anaerobic unit. There are many important factors to take into consideration when designing anaerobic wastewater treatment units. Independent of which reactor type is selected, the reactor temperature was a large factor for concern. When considering anaerobic treatment at temperatures below 20°C, some additional parameters have to be considered. The solubility of methane in water increases as the temperature drops, and therefore a significant portion of the methane produced in the reactor may be lost in the liquid effluent. The lower temperature will also lead to an increase in CO_2 solubility, and hence a slight drop in the pH is expected. A reduced diffusivity of soluble matter is also expected at lower temperatures along with a decrease in microbial activity, and so the effectiveness of the anaerobic reactors may be adversely affected. However as stated in chapter 2

there is a growing body of research demonstrating the successful anaerobic treatment of domestic wastewater at psychrophilic temperatures.

6.3.2.1 Anaerobic baffled reactor

The ABR designed for the model flowsheets was designed to be used as a primary settling unit. A large amount of solids is predicted to be collected in the first and second compartments that demand the installation of a hopper (of similar design to that of a primary settling unit) so that sludge can be removed from the bottom of the reactor. The sludge in the bottom of the compartments breaks down in a similar manner to a sludge digester, such that the sludge leaving the reactor is stabilised, i.e. with low pathogens levels and reduced potential for odour formation.

There is no simple design protocol available for the design of ABR, because it is a relatively new unit. In addition, ABRs with the capacity to be used as a primary unit have not been demonstrated past pilot stage. However, there are data available in the literature, mostly from lab and pilot studies, that demonstrate the suitability of ABRs to degrade low strength wastewater. This data, summarised in the literature review was used to model this unit.

Due to the limitation on available designs, there is a degree of uncertainty in the ABR designed in the flowsheets, especially in the sludge outlet stream. Using the data from the ABR pilot plant at Ellesmere Port, the sludge outlet for the ABR was modelled on primary sedimentation followed by a digester. Further research is required to quantify the production and quality of sludge from an ABR when used as a primary unit.

For the model flowsheets an HRT of 8 hours was selected. At this retention time the COD, BOD and TSS removals were estimated to be 60%, 60% and 80% respectively, based on data from pilot plants in Ellesmere Port and Columbia, and also from the lab scale data discussed in chapter 2 (Barber and Stuckey, 1999; Clark *et al.*, 2000; Langenhoff and Stuckey, 2000; Orozco, 1997).

At an HRT of 8 hours the total volume of the ABR tank was 833m^3. The design of the tank was based on that of Boopathy and Tilche (1991), with the wastewater piped directly from the top of one upflow compartment to the bottom of the next to save space. An upflow velocity of 1 m/s was selected which is appropriate for a 4 compartment design (Clark *et al.*, 2000). The gas production was modelled at 0.175 m^3/kg $COD_{digested}$ based on standard gas production for anaerobic digestion and adjusted for operation at 14°C, including the increased methane solubility at low temperatures (Wen *et al.*, 1999). Sludge production, nitrogen and phosphorus removals were modelled using standard data for anaerobic units (Tchobanoglous *et al.*, 2003).

6.3.2.2 Submerged anaerobic membrane bioreactor

The SAMBR has not yet been demonstrated at full or pilot scale for the treatment of domestic wastewater. However there are many studies of lab scale unit treating domestic wastewater as well as some full scale unit treating high strength unit wastewater as discussed in chapter 2; results from these studies were used to more the SAMBR in the flowsheets.

The COD and BOD removals in the SAMBR for the model flowsheets is estimated to be 90%; based on work presented in Table 2-6 and others (Hu 2004; Hu and Stuckey, 2006; Akram, 2006). The high removals result from the use of the membrane which ensures all particles including the biomass are retained inside the reactor, and hence TSS removal is 100%. A hollow fibre membrane unit was selected since these require a smaller footprint compared to the flat sheet membranes. To obtain high COD/BOD removals an HRT of at least 6 hours is required, yielding a reactor volume of 620 m^3.

The gassing and cleaning requirements are based on data for aerobic MBRs. The membrane gassing requirement will be in the region of $0.3 m^3_{gas}/m^2_{membrane}$ (Judd, 2006). The membrane cleaning requirements are based on the recommendations for Zenon hollow fibre membranes as detailed by Le-Clech *et al.* (2006). It is expected that an intensive clean to remove inorganic fouling would be required roughly twice a year. It is also assumed that the membrane would require a weekly maintenance clean with a weak NaOCl solution; this is done by a 'cleaning in line' process (without removing the biomass) (Lim *et al.*, 2005). Gas production is modelled on the same data used for the UASB model (see UASB section), while sludge production, nitrogen and phosphorus removals are modelled using standard data for anaerobic units (Tchobanoglous *et al.*, 2003).

6.3.2.3 Up-flow sludge blanket (UASB)

The UASB design was based on the design process set out in Tchobanoglous *et al.* (2003), and the key parameter in UASB design is the upflow velocity. This ensures that the wastewater flows through the unit at an acceptable rate, but not so fast that the biomass is washed out. For the model flowsheets an upflow velocity of 1 m/s was selected; this is at the top end for domestic wastewater. A satisfactory COD removal for a UASB reactor at 14°C is in the region of 85%, and to achieve this, the HRT needs to be around 8 hours (Lettinga and Pol, 1991; Tchobanoglous *et al.*, 2003).

From the upflow velocity the required cross sectional area of the UASB can be calculated, (and consequently the height of the reactor). It is important to keep the diameter of the UASB reactor to a minimum, to avoid dead space or channelling within the reactor which impedes performance. To

this end the waste stream was split through 4 parallel UASB reactors to keep the height to diameter ratio for reach reactor below the recommended value of 1.4 (Tchobanoglous *et al.*, 2003).

The calculated gas production was based on data from Uemura and Harada (2000) and Lew *et al.* (2004), taking into account differences in methane generation for particulate and soluble COD fractions at low temperatures. Sludge production, nitrogen and phosphorus removals are modelled using standard data for anaerobic units (Tchobanoglous *et al.*, 2003).

6.3.2.4 Anaerobic unit comparison

Table 6-4 shows the key parameters and costs involved in the modelling of the main anaerobic units. Some parameters such as sludge accumulation and methane production are not listed here as the production depended greatly on the unit's position within the flowsheet. It is important to note that since the SAMBR and ABR units have not previously been designed at full scale for the parameters of this project, there is a degree of uncertainty in the figures; however the differences observed between the units demonstrate the strengths and weaknesses of each type of anaerobic reactor.

Table 6-4 Summary of key parameters and costs used in the design of the anaerobic flowsheets

Parameter	units	**SAMBR**	**ABR**	**UASB**
COD removal	%	90	60	85
BOD removal	%	90	60	70
Suspended solids removal	%	100	80	75
MLSS requirement	g/l	5.5	5	23
Nitrate/nitrite removal	%	96	100	96
Phosphorus removal	%	25	20	25
Retention time	h	6	8	8
Reactor volume	m3	620	958	829
Foot print	m2	248	417	103
Capital cost	£	£ 1,864,610	£ 1,396,662	£ 157,310
Operational cost	£/yr	£ 87,185	£ 1,451	£ 11,482

In terms of cost the SAMBR is the most expensive unit both in terms of capital and operational expenditure. The capital cost is due to the requirement of building a more complex unit; the SAMBR will require both membrane units and gas compressors which are not requirements of the other two units. The operational expenditure for the SAMBR is 60 times greater than for the ABR and 7.6 times greater than for the UASB. The reason for this is due to the cost of replacing and cleaning the membranes The frequency assumptions for this were taken from 'The MBR Book' (Judd 2006) however the data presented from the installation at Ken's foods suggests that this may be a

conservative estimate (Christian *et al.*, 2011). The SAMBR also has the highest COD, BOD and suspended solids removal, this is due to the membrane in the reactor which prevents particulate matter passing into the effluent stream. Another benefit of the membrane system is that there cannot be any sludge washout a lower retention time of 6 hours is possible compared to the 8 hours required by the abr and UASB reactors.

The ABR is the simplest of the three unit mechanically requiring only influent pumps, the rest is based on a gravity feed; because of this the operational cost for the ABR is considerably lower compared to the UASB and ABR units. The design process for the ABR however means that to keep the upflow velocity within an acceptable bound the unit cannot be very high and therefore the ABR is required to have a larger foot print that the other units (4 times greater than the UASB and 1.7 times greater that the SAMBR) this can be important if there are space restrictions on the build site. The ABR also has the lowest predicted rate of COD and BOD removal this is mostly due to the unit being used in place of a primary unit and therefore it will take greater loads of BOD and COD compared to the SAMB and UASB.

The UASB reactor required the highest degree of suspended solids in its design, to ensure adequate contact between bacteria and substrate, because of this it is predicted to have the lowest suspended solid removal at 75% due to washout.

6.3.3 Post treatment

The post treatment and sludge treatment model development in this study was carried out by Sebastian Zacharias at Cranfield University. Different process units were considered depending on the effluent parameters from the anaerobic units. Where the BOD/COD in the effluent was significantly high, biological post treatment was employed which was comprised of an activated sludge unit or a trickling filter. In other cases a novel combination of GAC adsorption in conjunction with cation and anion ion exchange resins was modelled. The final option for post treatment was a coagulant dosing option which was also considered. The sludge treatment was modelled as either one of the two common methods of thickening followed by dewatering, or by further anaerobic digestion followed by dewatering.

6.4 Flowsheeting

Having assessed all the data from the unit operations considered above, five potential flowsheets were selected and subsequently modelled to demonstrate how such a plant based on anaerobic treatment might perform in terms of effluent quality, energy cost and capital and running costs

(Table 6-5). These flowsheets were short-listed from a large number of possibilities that can be achieved from combining the process unit processes. The selection of the final five flow sheets was based on literature information, personal experience and structured discussions during interim project meetings. The assumptions and reasoning behind the key decisions were:

- The effluent from the SAMBR would not allow biological post-treatment due to the COD being so low (<55mg/l), and therefore the membrane units were always followed by chemical treatment.
- The UASB reactor alone does not remove sufficient organic matter, and therefore both biological and chemical post treatments were considered.
- The main purpose of the primary treatment was to reduce the solids content, and therefore a flowsheet replacing primary treatment with fine screens was considered.
- The ABR unit can be designed as an alternative to a conventional primary settler, therefore both types of primary treatment were compared in the flowsheets

An activated sludge plant was also modelled on the same parameters to allow for a full comparison with conventional treatment processes.

Table 6-5 Overview of potential model flowsheets.

Flowsheet number	Pre-treatment	Anaerobic reactor	Post-treatment	Sludge treatment
1	Screens 6mm + de-grit + primary settling	SAMBR	Coagulation dosing, clarification + GAC + cation IEX	Anaerobic digestion + dewatering
2	Screens 6mm + de-grit + primary settling	UASB	Biological treatment (TF or ASP) + coagulation dosing + clarification	Anaerobic digestion + dewatering
3	Screens 6mm + de-grit	ABR + SAMBR	GAC + cation and anion IEX	Thickening + dewatering
4	Screens 6mm + de-grit	ABR + UASB	Biological treatment (TF or ASP) + cation	Thickening + dewatering
5	Screens 6mm + de-grit + screens 1 mm	SAMBR	GAC + cation and anion IEX	Thickening + dewatering
6	Screens 6mm + de-grit + primary settling	ASP + secondary clarifier	Sand filtering	Anaerobic digestion + dewatering

The following section shows the process flow diagram for each of the modelled flowsheets along with a table summarising the main features of each flowsheet.

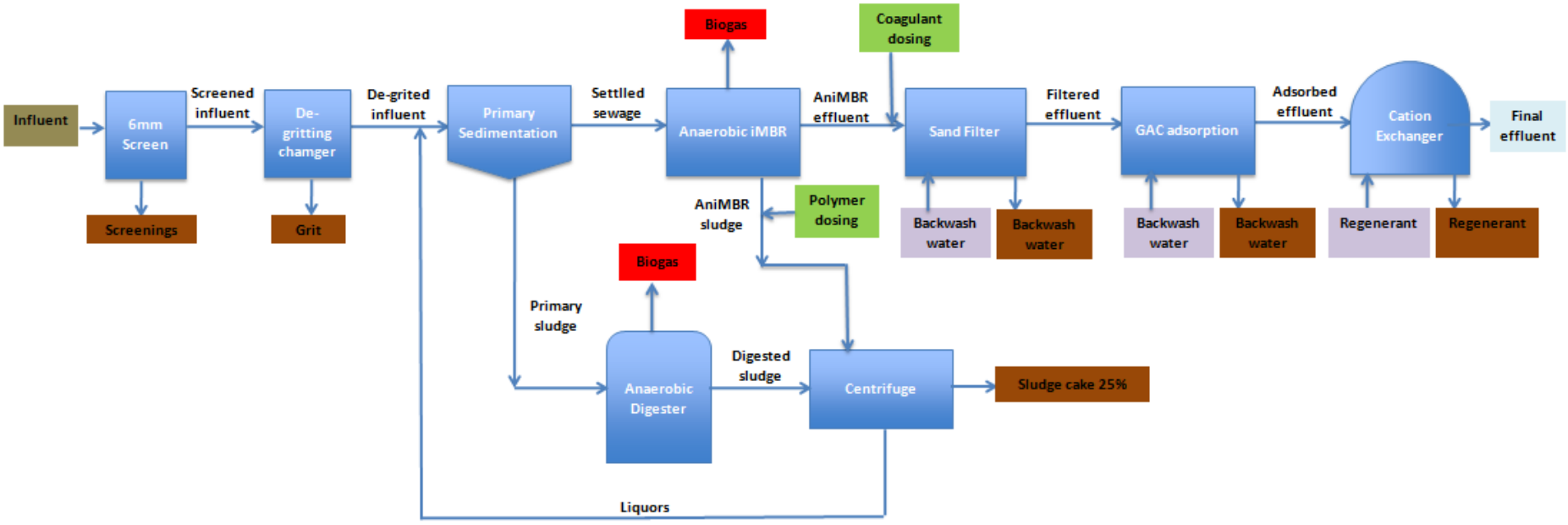


Figure 6-2 PFD Flowsheet 1

Table 6-6 Key outcomes flowsheet 1

Parameter	In	Out	Removal / balance
Flow (m^3/day)	2500	2479	
TCOD / TBOD / SS	971/313/589	24/5/0	98/99/100 %
TN / TP (mg/l)	65.5/10.4	1/0	98/97 %
Energy (kW/m^3)	0.052	0.313	-0.262
Capital costs (£)	£4,513,602		
Operational costs (£/year)	£125,570*		
Sludge production (kg/day)	48		

Plant footprint (m^2)	419
Resource recovery	digester CH_4 + aqueous CH_4 + nutrients + effluent
Plant reliability	AnMBR not well tested at full-scale
Maintenance	Medium

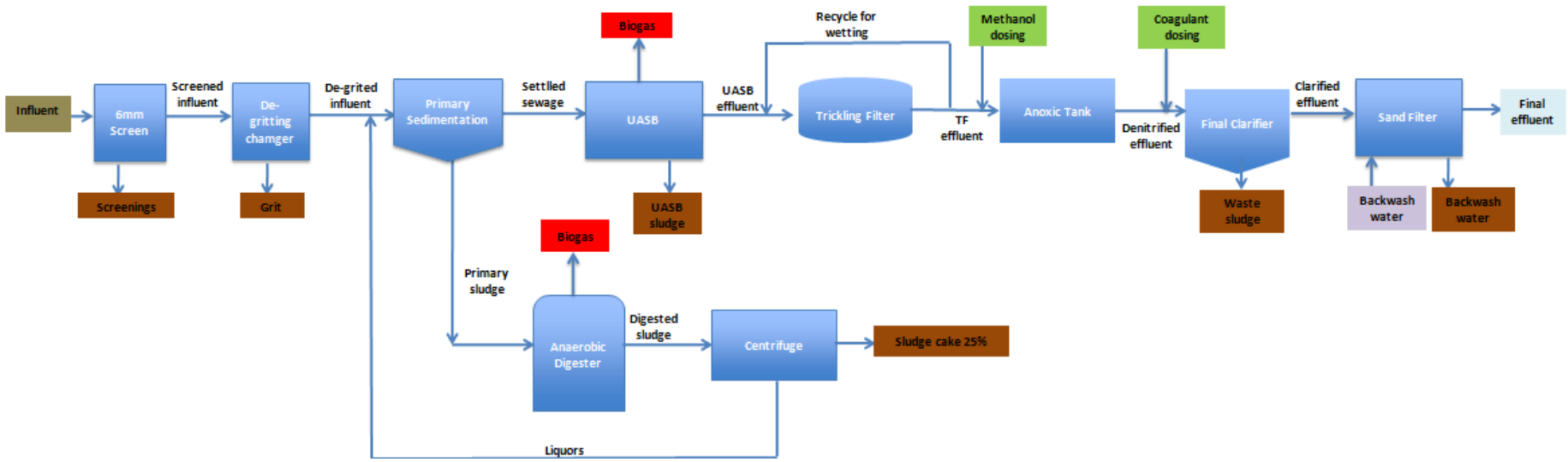


Figure 6-3 PFD Flowsheet 2

Table 6-7 Key outcomes flowsheet 2

Parameter	In	Out	Removal /
Flow (m^3/day)	2500	2471	
TCOD / TBOD / SS (mg/l)	971/313/589	81/2/22	92/99/96 %
TN / TP (mg/l)	65.5/10.4	10/0.7	85/93 %
Energy (kW/m^3)	0.018	0.401	-0.383
Capital costs (£)	£5,299,313		
Operational costs (£/year)	£82,306		
Sludge production (tons DS/year)	96		

Plant footprint (m^2)	641
Resource recovery	digester CH_4 + aqueous CH_4
Plant reliability	>700 full-scale UASB worldwide, but not tested at
Maintenance	Low

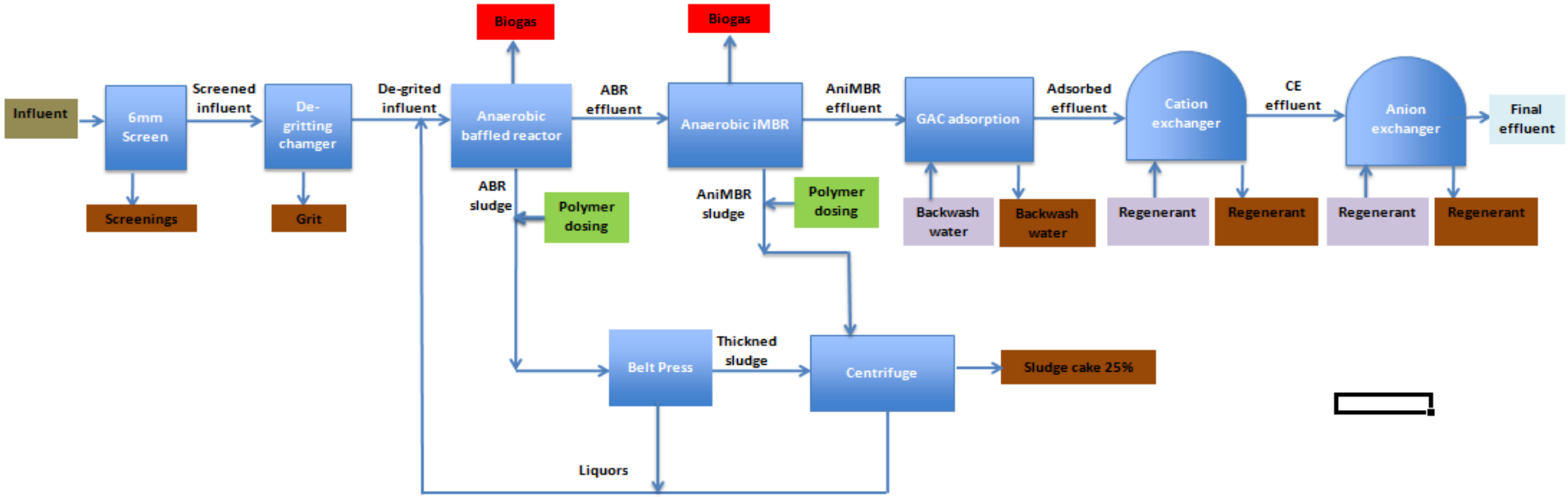


Figure 6-4 PFD Flowsheet 3

Table 6-8 Key outcomes flowsheet 3

Parameter	In	Out	Removal / balance
Flow (m^3/day)	2500	2479	
TCOD / TBOD / SS (mg/l)	971/313/589	41/6.3/0	96/99/100 %
TN / TP (mg/l)	65.5/10.4	2/0	98/97 %
Energy (kW/m^3)	0.013	0.347	-0.334
Capital costs (£)	£5,200,494		
Operational costs (£/year)	£126,416*		
Sludge production (kg/day)	74		

Plant footprint (m^2)	701
Resource recovery	digester CH_4 + aqueous CH_4 + nutrients + effluent
Plant reliability	ABR + AnMBR not well tested at full-scale
Maintenance	Medium

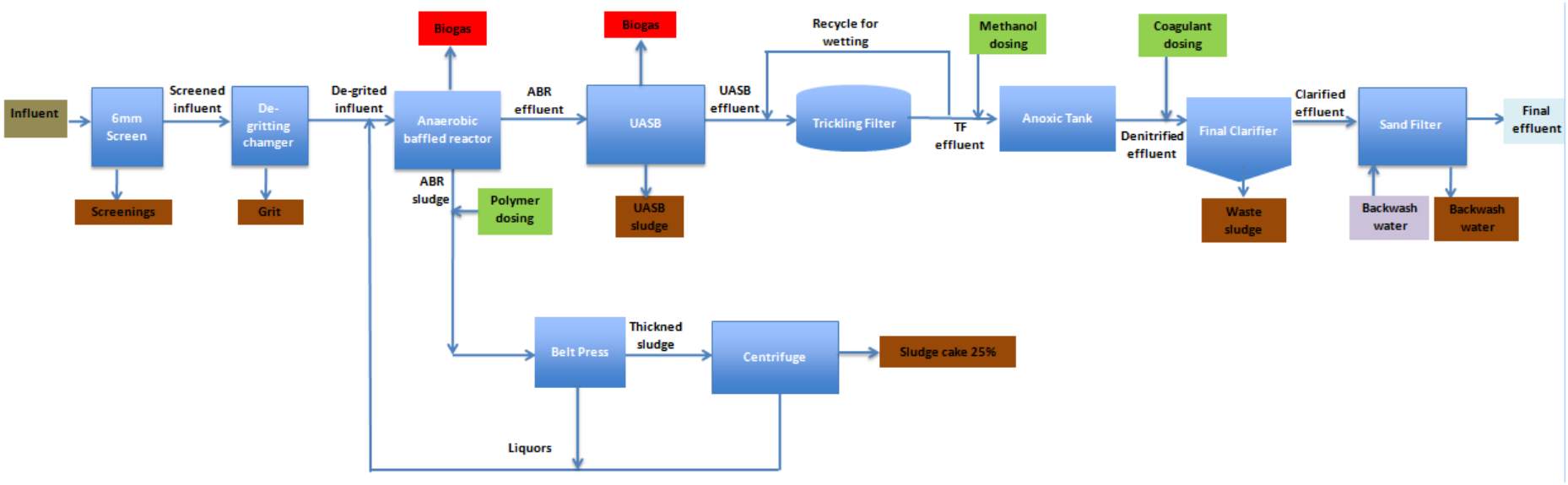


Figure 6-5 PFD Flowsheet 4

Table 6-9 Key outcomes flowsheet 4

Parameter	In	Out	Removal /
Flow (m^3/day)	2500	2470	
TCOD / TBOD / SS (mg/l)	971/313/589	81.3/2/11	92/99/98 %
TN / TP (mg/l)	65.5/10.4	10/0.7	85/93 %
Energy (kW/m^3)	0.013	0.370	-0.357
Capital costs (£)	£5,652.021		
Operational costs (£/year)	£80,079		
Sludge production (kg/day)	96		

Plant footprint (m^2)	847
Resource recovery	digester CH_4 + aqueous CH_4
Plant reliability	ABR not widely tested at full-scale. >700 full-scale UASB worldwide, but not well tested at low temperatures
Maintenance	Medium

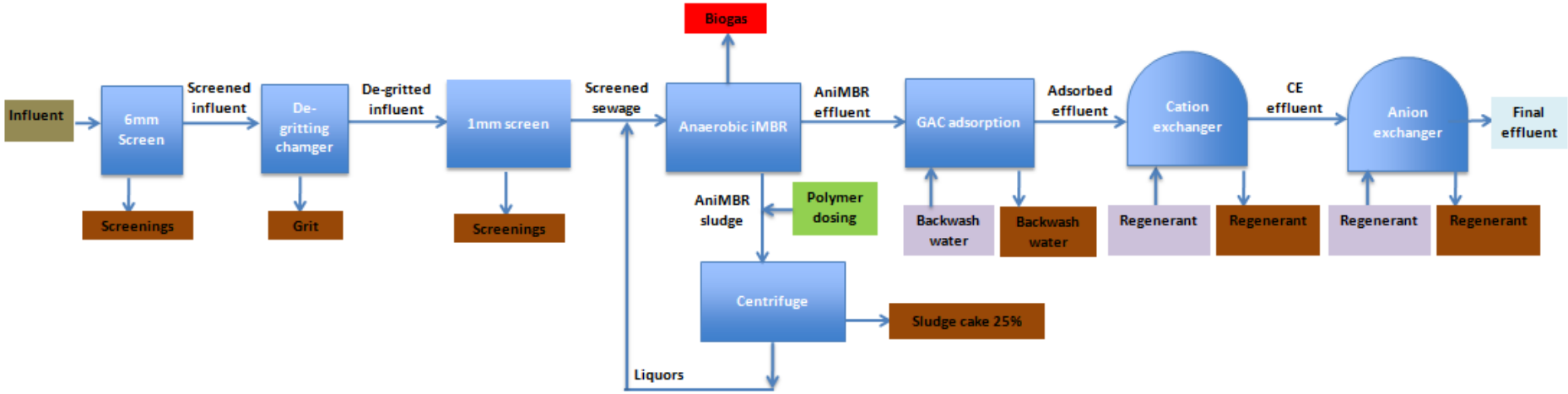


Figure 6-6 PFD Flowsheet 5

Table 6-10 Key outcomes flowsheet 5

	In	Out	Removal /
Flow (m^3/day)	2500	2479	
TCOD / TBOD / SS (mg/l)	971/313/589	52/11/0	96/97/100 %
TN / TP (mg/l)	65.5/10.4	3/1	95/95 %
Energy (kW/m^3)	0.008	0.309	-0.301
Capital costs (£)		£3,287,597	
Operational costs (£/year)		£119,031*	
Sludge production (kg/day)		61	

Plant footprint (m^2)	239
Resource recovery	digester CH_4 + aqueous CH_4 + nutrients + effluent
Plant reliability	AnMBR + anion exchanger not well tested at full-scale
Maintenance	Medium

**excludes IEX regenerant costs*

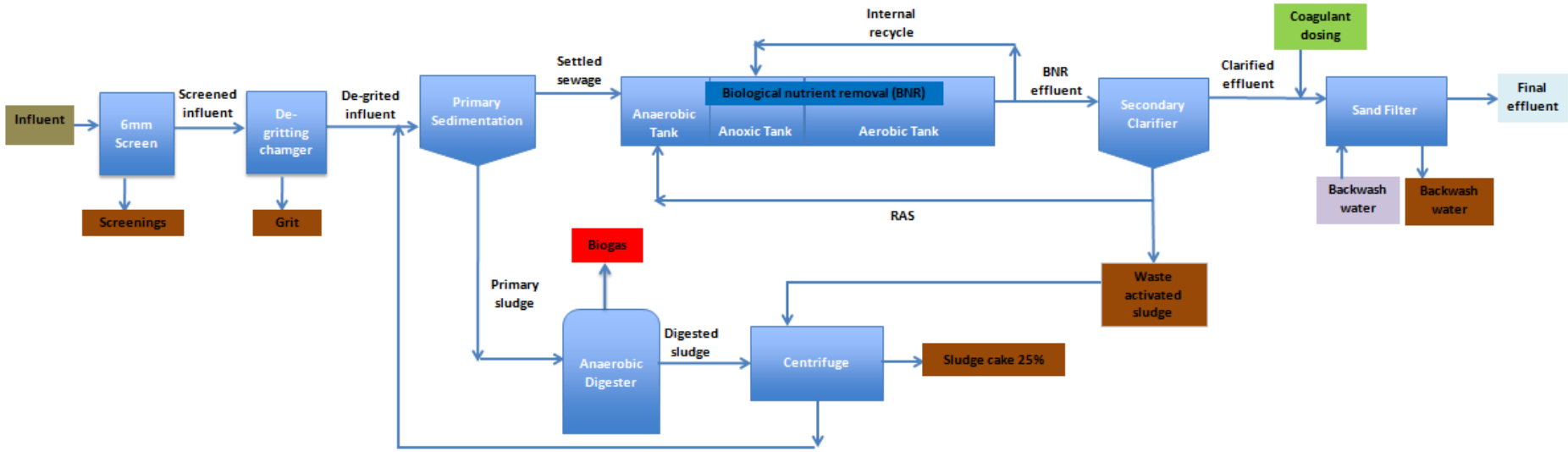


Figure 6-7 PFD Flowsheet 6

Table 6-11 Key outcomes flowsheet 6

	In	Out
Flow (m^3/day)	2500	2365
TCOD / TBOD / SS (mg/l)	971/313/589	34.0/7.9/40.2
TN / TP (mg/l)	65.5/10.4	0.2/1.0
Energy (kW/m^3)	0.01	0.98
CAPEX (£)	£5,304,819	
OPEX (£/year)	£106,498	
Sludge production (kg/day)	96	

Plant footprint (m^2)	641
Resource recovery	digester CH_4
Plant reliability	Very well known processes

Table 6-12 main results for each flowsheet

Flowsheet	1	2	3	4	5	6
Effluent COD (mg/l)	24	81	41	81	52	34
Effluent BOD (mg/l)	5	2	6.3	2	11	8
Effluent Suspended solids (mg/l)	0	22	0	11	0	40
Effluent Total Nitrogen (mg/l)	1	10	2	10	3	0.2
Sludge production (kg/day)	48	96	74	96	61	96
Capital Cost	£ 4,500,000	£ 5,300,000	£5,200,000	£ 5,700,000	£3,300,000	£5,300,000
Operational cost (/year)	£ 125,000	£82,000	£ 126,000	£80,000	£ 119,000	£ 106,000
Plant footprint (m^2)	419	641	701	847	239	641

6.5 Kepner Tregoe flowsheet comparison

Each of the flowsheets have their own benefits and drawbacks, for example it can be seen in Table 6-12 that flow sheets 1, 3 and 5, (the flowsheets containing a SAMBR,) have the cleanest effluent in terms of the zero suspended solids. These flowsheets, however, are also the most expensive to operate on a yearly basis due to the limited life time of the membrane, and this means a replacement cost has to be factored into the model. To find the optimum flowsheet for this particular circumstance a decision making approach is required. As mentioned previously a life cycle assessment approach would provide an indication of the environmental of each plant, however the sheer volume of data required to undertake this analysis would make it impractical for this assessment additionally for some of the more novel units the data simply is not available. Since this is the first attempt to analyses the role of anaerobic wastewater treatment in a holistic flowsheet based approach a simple Kepner Tragoe decision making tool was employed.

The Kepner Tregoe (KT) decision making process is a structured methodology for gathering information and prioritising and evaluating it. This process is capable of differentiating possible solutions on the basis of their overall suitability to a broad objective. An important aspect of KT decision making is the assessment and prioritising of risk. Thus, the purpose is not to find a perfect solution but rather the best possible choice; it is a means of decision making that attempts to limit the impact of the 'deciders' biases. It is most useful, in cases such as this, where there are many potential solutions and multiple parameters to consider.

In attempting to determine the optimum flowsheet model, the KT process required the following steps:

1. A list of objectives (e.g. COD removal, capital expenditure, plant footprint) was created, a table showing all the parameter is shown in Table 6-13.
2. Every member of the project team (7 individuals) awarded each of these objectives a number (from 1-10) based on how important they considered each objective to be. For example, if the team member considered COD removal to be an important objective they would give it a 9 or 10. The average of every team member's numbers designation is assigned to each parameter as its individual weighting.
3. For each flowsheet a ranking from 1-5 is awarded for every objective, on how successfully they achieve the objective. For example, in the energy balance a total requirement greater than or equal to 1kW/m^3 achieved a score of 1, a requirement between 0.99 and 0.7 kW/m^3

achieved a score of 2, and so on up to energy requirement less than 0.19kW/m^3 which scored 5. A full breakdown of the scores for each parameter is shown in appendix E

4. The overall KT score for each flowsheet is calculated by the sum of all the average weightings multiplied by their corresponding ranking number.

Table 6-13 list of parameter used in the KT analysis

Objectives
Energy balance
BOD removal
COD removal
Solids removal
Total nitrogen removal
Total P removal
CAPEX
OPEX
Sludge production
Plant footprint
Resource recovery
Plant reliability
Maintenance
Plant flexibility

The flow sheet with the highest overall KT score was put forward as the optimal flowsheet for this project. The KT scores for each flow sheet are summarised in Table 6-14, and a full breakdown of the weightings and rankings for each flowsheet is detailed in Appendix D.

6.5.1 Comparison with standard aerobic flowsheet

In conjunction with the model anaerobic flow sheets, an aerobic flowsheet was also designed based on the same influent/effluent constraints. This was also assessed under the Kepner Tregoe process, and Table 6-14 shows that of all the simulated flowsheets the aerobic flowsheet came out with the lowest score. This was due primarily to the fact that the conventional activated sludge plant scores poorly in the areas of total plant footprint, energy usage and sludge production.

According to the assumptions made, the operational cost of the conventional plant is lower than that modelled in flowsheets 1 and 5, whilst both the capital cost and footprint are substantially higher. In terms of product water quality the SAMBR produces a clarified effluent but may not

achieve the same degree of COD or nutrient removal achieved by the biological nutrient removal (BNR) plant.

The conventional BNR plant is subject to the risk of failure during shock loads of nutrients, or when the plant receives a toxic shock. If the shock is sufficient to disrupt the biomass, the effluent would be adversely affected such that the EEC effluent restrictions would not be met. Whilst this is also possible with the SAMBR flowsheets, the biomass is entirely retained by the membrane; in addition, the GAC and ion exchange resin added to the reactor will limit the extent of the toxic shock being released into the final effluent stream.

6.6 Flowsheeting outcomes

Table 6-14 Final Kepner Tregoe scores for each model flowsheet listed in order of final score.

Flowsheet number an principal design units	KT score
Flowsheet 5: 1mm screen + SAMBR + chemical treatment	438
Flowsheet 1: Primary settling tank + SAMBR + chemical treatment	436
Flowsheet 2: Primary settling tank + UASB + biological treatment	416
Flowsheet 3: ABR + SAMBR + chemical treatment	411
Flowsheet 4: ABR + UASB + biological treatment	381
Flowsheet 6: Conventional aerobic flowsheet	371

According to this KT process, Flowsheets 5 and 1 (Figure 6-2 and Figure 6-6) standout as the best possible flowsheets of the 6 modelled. There are two key differences between these two flowsheets; firstly flowsheet 1 employs a standard primary settler to remove solids while flow sheet 5 relies solely on a fine 1mm screens to protect the SAMBR from clogging by suspended solids. Secondly, while both flowsheets employ chemical post treatment, flowsheet 1 uses coagulation dosing and clarification; while flowsheet 5 requires an anion exchange (both flowsheets use GAC and cation exchange).

For the development of the model it was assumed that the SAMBR process can be sustained with little or low input to maintain membrane permeation. In reality this is likely to be dependent on the suspended solids loading, therefore flowsheet 5 presents the highest risk of failure if a shock load of suspended solids enters the process stream because this may cause the SAMBR to fail.

Of the remaining anaerobic flowsheets, numbers 3 and 4 scored lowest; this is largely due to the inclusion of the anaerobic baffled reactor in place of the primary settler and sludge digester. The ABR appeared to be promising in the initial research and design phase; however, in the KT

assessment it did not score well. Due to the particular shape requirements of the ABR design, the unit must be very shallow to allow the waste stream to pass through each of the baffles with an acceptable HRT. Because of the shallow design the ABR requires a larger footprint compared to the other units considered for primary treatment. The large size of the ABR unit will also increase the capital expenditure required to build the plant. Both these factors (cost and footprint) decrease the KT score of the unit, and since the effluent stream from the ABR is not clean enough to avoid further biological treatment steps, the rest of the flowsheet has a similar design to the flowsheet with a less novel initial approach.

The UASB based flow sheets do not score as highly as the SAMBR flowsheets. There are two main reasons for this; firstly, to keep to the design constraints the flow had to be split across 4 parallel units, thereby increasing process cost. Secondly, the effluent from the UASB is not as clean as that from the SAMBR, and therefore more intensive post treatment is required.

Based on the KT decision process, flowsheets 1 and 5 are suggested as the best possible flow sheets for future development in anaerobic wastewater treatment. In reality flowsheet 5 may not be as effective as the model suggests, due to the potential for blocking in the 1mm screen. Also, the SAMBR may require higher sludge wastage than modelled, to keep membrane fouling within acceptable limits. Since the SAMBR has not been well tested beyond lab scale, it is not possible to say for certain whether the flowsheets would be practicable without further pilot scale data.

6.7 Experimental data on a 15°C SAMBR

In this study the SAMBR was identified as the key anaerobic unit for the future of anaerobic technology, however, questions remain as to its operational efficiency at low temperatures. As such one of the lab scale SAMBRs used for the results in Chapters 4 and 5 was operated at 15°C for 45 days to assess the reactor performance at this low temperature. The COD profile for this reactor is shown in Figure 6-8, and this shows that the SAMBR shows good performance throughout operation. Unfortunately, due to degradation of the feedstock the influent COD was lower than desired, and thus the SAMBR was operating at very low influent CODs (between 350 and 150 mg COD/l).

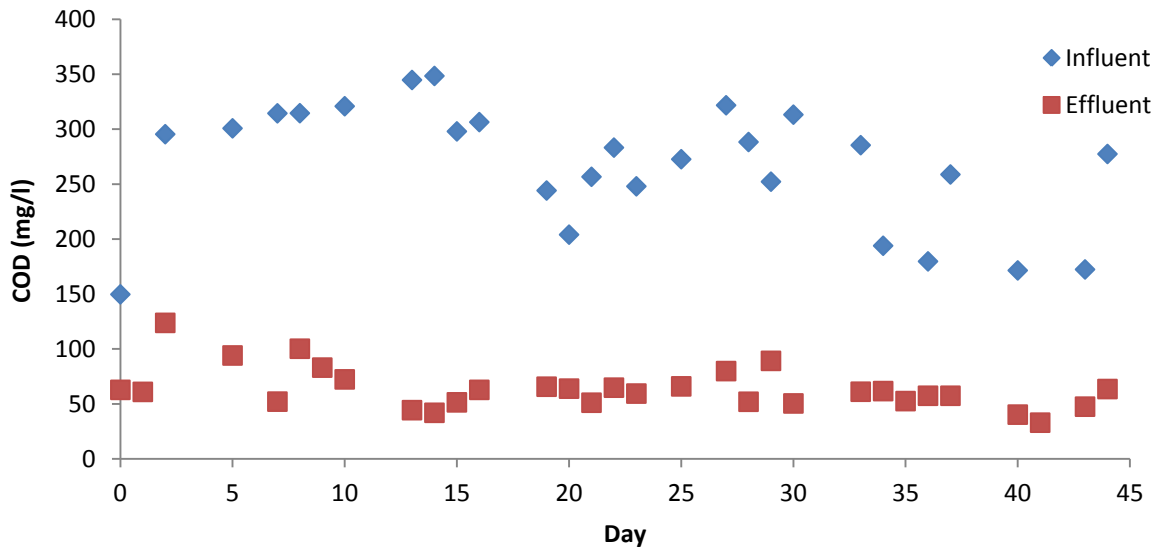


Figure 6-8 COD profile for SAMBR operated at 15°C

The COD removal efficiency of the SAMBR (Figure 6-9) shows that in spite of the low COD influent significant COD removal was achieved; the average COD removal was found to be 76%. As expected, this is lower than the COD reading obtained for standard mesophilic operation where the COD removal remained above 90%. The 76% removal figure is lower than that predicted for the model flowsheet, however, since the influent COD here is so low, it is thought that with the higher influent COD in the flowsheets (700 mgCOD/l), this is not an unreasonable model. Additionally in this experiment the biomass was acclimated for 45 days only. McKeown *et al.*(2009) successfully operated an EGSB style reactor at 4-15 °C with COD removal efficiencies in excess of 85%; however the authors operated the reactor for 1243 days, which allows the biomass much longer time to adapt. Throughout operation the gas in the headspace of the reactor remained between 65-75% CH_4, thus showing that in spite of the lower temperatures the methane fraction in the gas remained at a good level for gas recovery.

For this reactor the biomass was seeded from mesophilic anaerobic sludge, and therefore while the SAMBRs show good performance in the psychrophilic range, it can be assumed that the sludge retains its mesophilic characteristics (Lettinga *et al.*, 1999; Elmitwalli *et al.*, 1999; McKeown *et al.*, 2009). A truly psychrophilic sludge may also increase the COD removal, and therefore it is suggested that research into bioaugmentation of the anaerobic biomass is carried out in future.

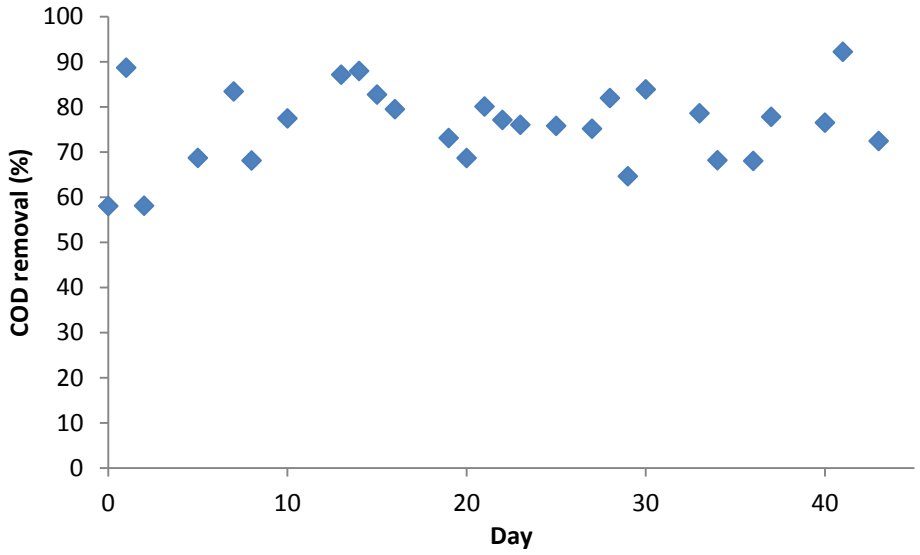


Figure 6-9 Percentage COD removal in the SAMBR

The VSS was first measured on day 14 of operation and showed a steady increase of 0.027gVSS $l^{-1}d^{-1}$. This translates to a yield of 0.063gVSS/gCOD which is in the range proposed by Pavlostathis *et al.* (1991) for long chain fatty acids. During operation no sludge was wasted apart from the samples removed for pH monitoring (this is assumed to be negligible). This slow rate of growth is typical of anaerobic bacteria and is one of the main benefits of the technology.

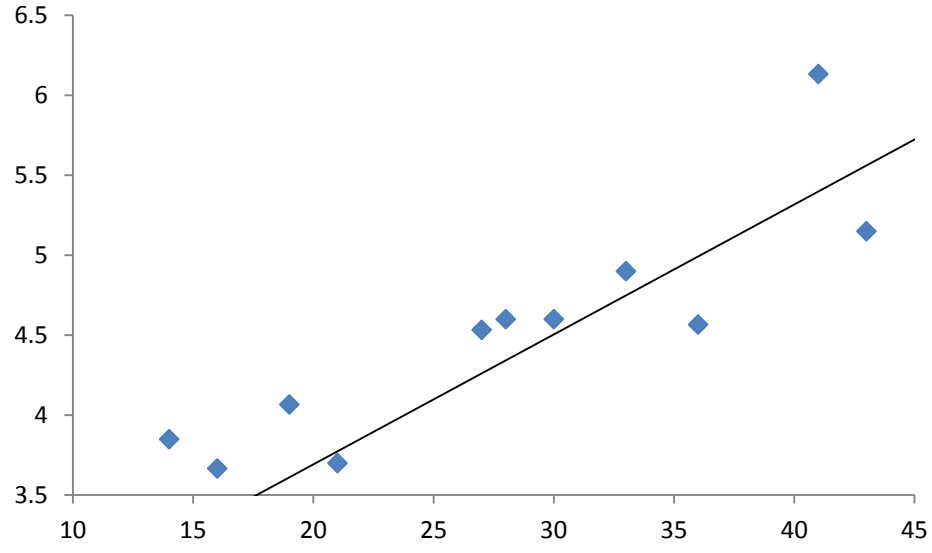


Figure 6-10 VSS increase from days 14 to 45.

6.8 Summary

This chapter presents an overview of how a wastewater treatment flowsheet, designed on the principals of anaerobic digestion, might look if designed from scratch. This is the first time that anaerobic treatment has been modelled using a holistic flowsheeting approach to compare different novel units. As such the preliminary models present an overview of the future for anaerobic wastewater treatment in the UK, however more extensive data is required for future studies to present a more rigorous analysis such as LCA. The following conclusions can be drawn from the compared flowsheets.

It can be concluded that anaerobic wastewater treatment is a practical and promising alternative to conventional activated sludge plants in the UK. More research is required into the effectiveness of some of the units at low temperatures, in particular the SAMBR. Also the effect increased suspended solids loading rates would have on the reactors would need to be considered.

Of all the units the modelled SAMBR shows the most promise for the future of anaerobic treatment due to its ability to produce a clarified effluent, free of solids. The SAMBR is also more protected from washout problems, compared to anaerobic units such as the UASB because of its ability to retain 100% of the biomass due to the membrane.

Using the Kepner Tregoe analysis the most promising flowsheets for domestic wastewater treatment are using either a primary settler or 1mm screens in conjunction with an SAMBR and chemical post treatment. This option does have a higher operational cost compared to the conventional activated sludge plant; however, it performs better in terms of footprint and resistance to toxic shock.

In a psychrophilic situation the lab scale SAMBR demonstrated good COD removal at 76%. This shows promise for full scale operation, and in addition low anaerobic growth rates were observed in the reactor.

Chapter 7. Conclusions and Future Work

This chapter analyses to what extent the work completed in Chapters 4, 5 and 6 meets the objectives set out at the end of Chapter 2, and makes recommendations for future work to be carried out.

7.1 Fouling and small particle throughput in the SAMBR

Investigate the parameters that affect the membrane resistance, including critical flux, permeability gas scour and biomass rheometry.

The critical flux for the SAMBR operating under standard conditions at a 6LPM gassing rate was found to be 11.8 LMH. After irremovable fouling had been allowed to build up on the membrane surface the critical flux dropped to 7.2 LMH for a SAMBR operating under the same conditions, thus demonstrating a degree of hysteresis in the membrane operation.

Permeability of the membrane was also found to be non-reversible. An increase in gas scour reduced the removable cake layer, and the drop in permeability was largely attributed to irremovable fouling, and therefore the only way to regain initial clean water permeabilities was to chemically clean the membrane.

The viscosity of the anaerobic biomass was found to be between 1.9 and 2.5 times higher than water, and this had a large effect on the resistance across the membrane. The biomass that had been operated in the SAMBR for an extended period of time showed a much higher viscosity (0.0022Pa.s), and this was largely attributed to the colloid fraction that had built up in the reactor. As stated by Pevere *et al.* (2006), the biomass was found to have non Newtonian shear thinning properties, indicating that an increase in gas scour would cause a drop in the viscosity of the biomass.

Investigate the possibility of a critical gas scouring rate.

A critical gas scouring rate was determined for the SAMBRs for the hypothesis that: *'there exists a critical gassing rate which when reached from higher rates causes a steep rise in TMP'*. By this definition the critical gassing rate for the SAMBR set up used in this thesis was found to be 4 LPM, since operation at lower gassing rates caused a sharp rise in TMP. Similarly to the critical flux, after operation beyond the critical value had occurred, an irremovable fouling layer occurred such that as the gassing rate was increased the TMP did not drop to its original low value. In addition, at the

lowest gas sparging rates the flow regime appeared more like a bubble flow pattern, which could be responsible for the gain in TMP.

Investigate small particle rejection in the SAMBR and the effect fouling has on this.

When operated below the critical parameters (i.e. at low TMPs) the molecular weight cut-off for the membrane/biofilm in the SAMBR was found to be in the region of 400,000 Da. After operation beyond critical flux or gassing rate, the MWCO dropped to approximately 40,000 Da, due to the build-up of irremovable fouling on the membrane surface.

In order to monitor the effect of the membrane and biofilm on the smallest particles, VFA rejections across the membrane were monitored. VFA rejection was only observed at the lowest gassing rate of 2 LPM where low but significant removals of acetic, isobutyric and isovaleric acids were observed. It is suggested that this was due to a combination of electrostatic charge in the biofilm, and a certain amount of size exclusion caused by the biofilm.

7.2 Phage removal in the SAMBR

Monitor the log removal of bacteriophages MS-2 and T4 within a SAMBR

The membrane alone showed poor removal for MS-2 and T4 phages at 0.7 LRV ± 0.4 and 2.3 LRV ±0.2, respectively. When the SAMBR was operating with biomass the log removals for both phages increased; the small MS-2 phage showed removals from 1.75-2.10 LRV while under operation at low TMP. When the TMP was increased, due to irremovable fouling, the phage removals increased significantly to between 3.0 and 5.5 LRV depending on the gassing rate. For the larger T4 phage in the SAMBR the minimum rejection was 5.1 LRV, and this increased until complete removal was achieved as the fouling layer built up.

Even at the highest removal rates there was very little phage accumulation in the SAMBRs. This is because the conditions in the reactor caused significant amounts of phage inactivation. The conditions in the reactor caused a 99% phage inactivation over the space of two weeks. Batch tests determined that degradation had a log-linear relationship, independent of the initial phage concentration. Once the phages have been inactivated, they may be degraded by the biomass, however, degradation by the biomass does not play a significant part in the removal of active phages from the SAMBR.

Analyse the impact of the gas scouring rate on phage removal

Before operation beyond the critical parameters (gassing rate and flux) there was a weak correlation between the log removal of the phage and the gas scouring rate, so that when the gas scour was decreased the phage removal increased. This was the case for both MS-2 and T4 phages.

After the SAMBR had been operated beyond the critical gassing rate and critical flux; and the irremovable fouling layer had been allowed to build-up, the relationship between gassing rate and phage removal changed. The fouling layer caused complete removal of the T4 phage, and the MS-2 phage removal was governed instead by concentration polarisation. As the gas scour increased the concentration polarisation layer reduced and hence phage removal increased. There is, however, a maximum gassing rate beyond which further increases did not affect phage rejection. In this case the maximum phage rejection was achieved at 8 LPM gassing rate, and beyond this there was no apparent increase in phage removal.

Assess the impact adding activated carbon to the SAMBR has on phage removal

The MS-2 phage shows poor adsorption to the surface of the activated carbon. As suggested by other researchers, this is most likely due to the active sites on the internal pores of the carbon being too small to be accessed by the phage particles.

On the addition of GAC to the SAMBR, the log removal decreased. This is because the carbon granules scour the surface of the membrane removing some of the fouling layer that causes phage rejection. The addition of PAC resulted in a slight increase in concentration in the SAMBR, and this was most likely due to the PAC providing a platform for phage propagation. The concentration in the effluent, however, remained unchanged. Thus while activated carbon has been recorded to have many benefits regarding SAMBR operation, it is of no benefit to phage or virus removal, indeed the addition of GAC has a detrimental effect.

7.3 Flowsheet modelling

Investigate the potential anaerobic treatment options that could be utilized in the UK today.

Five shortlisted flowsheets for anaerobic wastewater treatment were modelled to demonstrate the potential for wastewater treatment in the UK. To meet the EEC directive for Salmoniod waters (Table 6-2) all the flowsheets required some form of post treatment. The major anaerobic units investigated were: Anaerobic immersed membrane bioreactors (AniMBRs aka SAMBRs), upflow anaerobic sludge blanket reactors (UASB) and the anaerobic baffled reactor (ABR).

Model potential flowsheets with a focus on anaerobic digestion to contrast with a conventional aerobic treatment option.

The modelled anaerobic flowsheets were compared with a model of a conventional aerobic flow sheet. Using a decision making Kepner Tregoe matrix, all of the flowsheets scored higher than the conventional aerobic flowsheet, thereby demonstrating the viability of anaerobic wastewater treatment for use in the UK.

Use the decision making process to recommend an optimal anaerobic flowsheet for waste water treatment.

From the Kepner Tregoe matrix, two flowsheets scored significantly higher than the rest. These were the flowsheets that contained a SAMBR reactor as the main method of anaerobic treatment. Of all the units the modelled, the SAMBR shows the most promise for the future of anaerobic treatment due to its ability to produce a clarified effluent, free of solids. The SAMBR is also slightly more protected from toxic shock due to its ability to retain all the biomass inside the membrane, while other units could suffer from washout.

7.4 Future work

Inevitably, alongside meeting the objectives these results highlight many areas where further research is required. In this section recommendations are made for future studies in this area.

1. The results in Chapter 4 indicate that the critical gassing rate in the SAMBR may be partly determined by the flow regime of the gas sparging. Further research could investigate the relationship between the flow regime of the gassing stream and the steep jump in TMP observed at the critical gassing rate. It would be interesting to discover if the critical gassing rate remains the same for all (sub critical) fluxes, or if there is a relationship between the two. Understanding the trade off between gassing rate and flux would allow for better optimised reactor design.

2. The results on the biomass viscosity seem to indicate a dependence on the colloid fraction. Determining the relationship between colloid fractions and viscosity would facilitate greater understanding of the resistance across the membrane. Furthermore, this study into colloid mitigation through coagulation and sedimentation would be beneficial to effective SAMBR operation.

3. Irremovable fouling has a large part to play both in terms of phage and small particle rejection, and in terms of the critical flux and gassing rate. A greater understanding of the mechanisms involved in irremovable fouling and how to mitigate it would be beneficial.

4. The results for VFA rejection suggest that there may be some charge repulsion on the biofilm. Further study in this area could involve analysis of the throughput of both positive and negative particles of the same molecular weight, thus determining if charge repulsion on the membrane surface has an effect on effluent characteristics.

5. Similarly to the above, it is postulated that the charge on the biofilm may be responsible for some of the phage removal. Therefore, the above investigation could also be combined with a study into the effect of biofilm charge on phage removal. Additionally some SEM pictures of the membrane would provide further evidence of the types of fouling occurring.

6. It is thought that concentration polarisation has an important effect on the log phage removal after significant fouling has occurred. Attempting to measure the extent of concentration polarisation on the membrane at different gas scouring rates would help to prove or disprove this hypothesis.

7. The effect of gas scouring on phage removal has been extensively studied; however, there are many other factors that are likely to have a significant effect on the phage throughput. Evaluating the effect of parameters such as ionic strength of the feed, and the suspended solids in the SAMBR would allow for a greater understanding of the parameters that need to be controlled to achieve the required virus removals.

8. While the SAMBR proved to be the most promising anaerobic unit in the flow sheet modelling process, there is very little full scale operational data for this unit. Demonstrating the SAMBRs operability at low temperatures with domestic wastewater is the next key step before wider implementation of this unit can occur; this has been achieved at lab scale, but not in any units large than $1m^3$ (Herrera-Robledo *et al.*, 2011; Martinez-Sosa *et al.*, 2011).

9. The experimental data at the end of Chapter 6 suggests that the SAMBR operated in the psychrophilic range shows good performance, however, there is room for improvement. An

investigation into the possibility of bioaugmenting the biomass with a strictly psychrophilic Archaea could lead to improved COD removal in the SAMBR.

10. The flowsheets modelled in Chapter 6 are quite inflexible in their design, and the model does not allow for many parameter variations. A more dynamic model for the selected flowsheets could allow for more adaptability in the model so that the scope for its application is wider.

11. The most favourable flowsheet in the decision making process involved using a set of 1mm fine screens rather than a primary settler. This flowsheet would not be practical for high solids feeds, and therefore a pilot study should be able to assess the extent to which the 1mm screens can protect the SAMBR from excessive solids build up.

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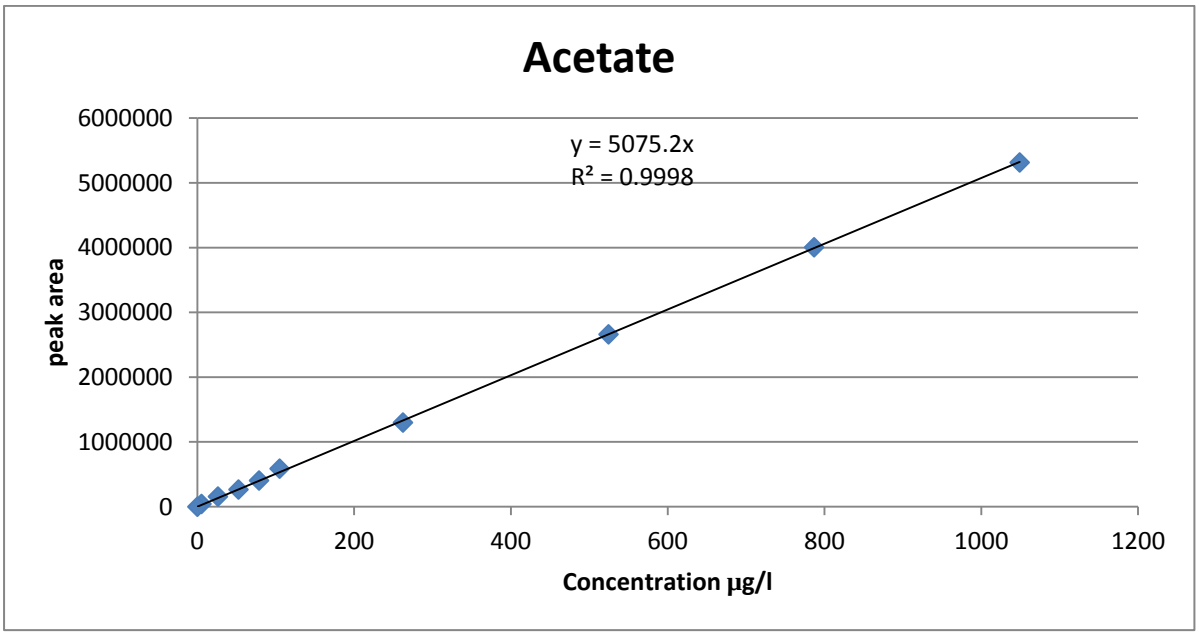
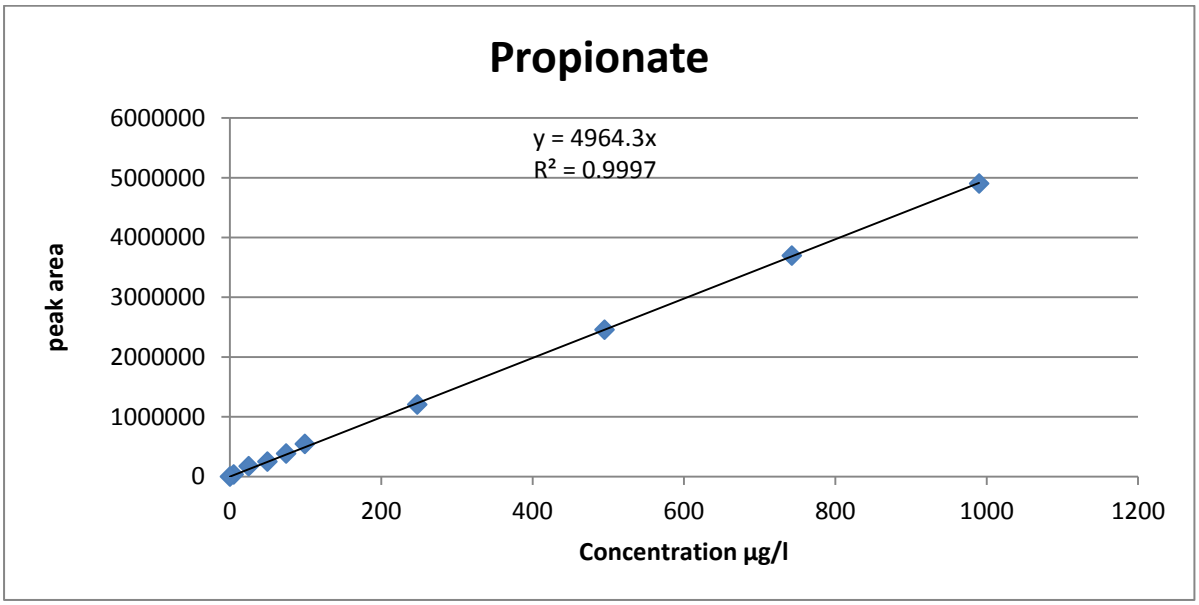
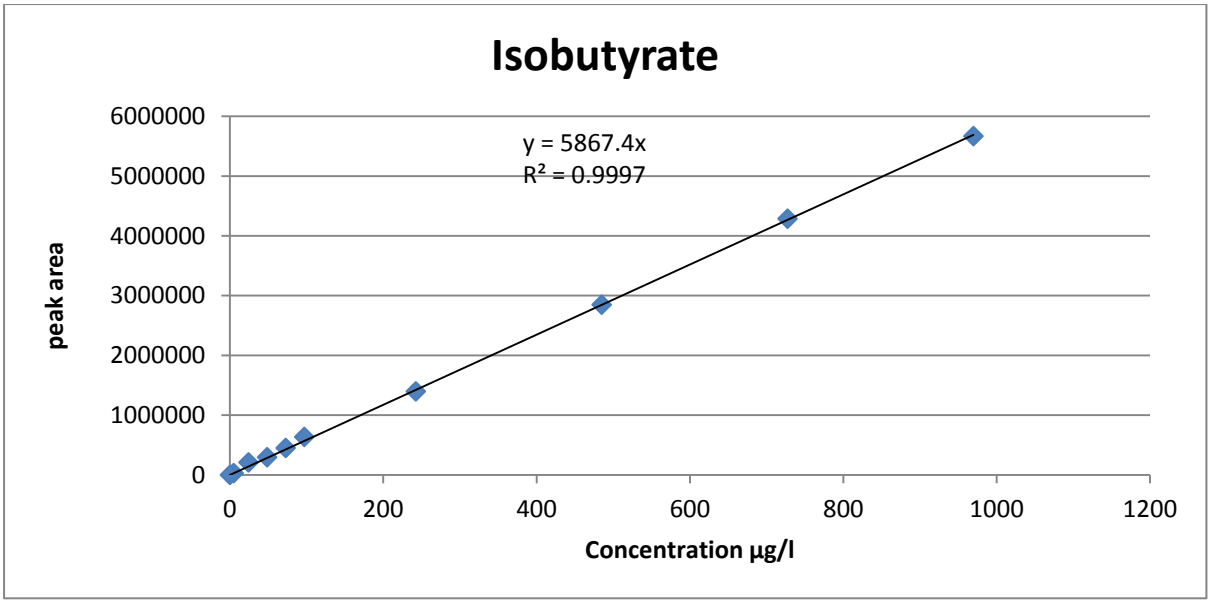
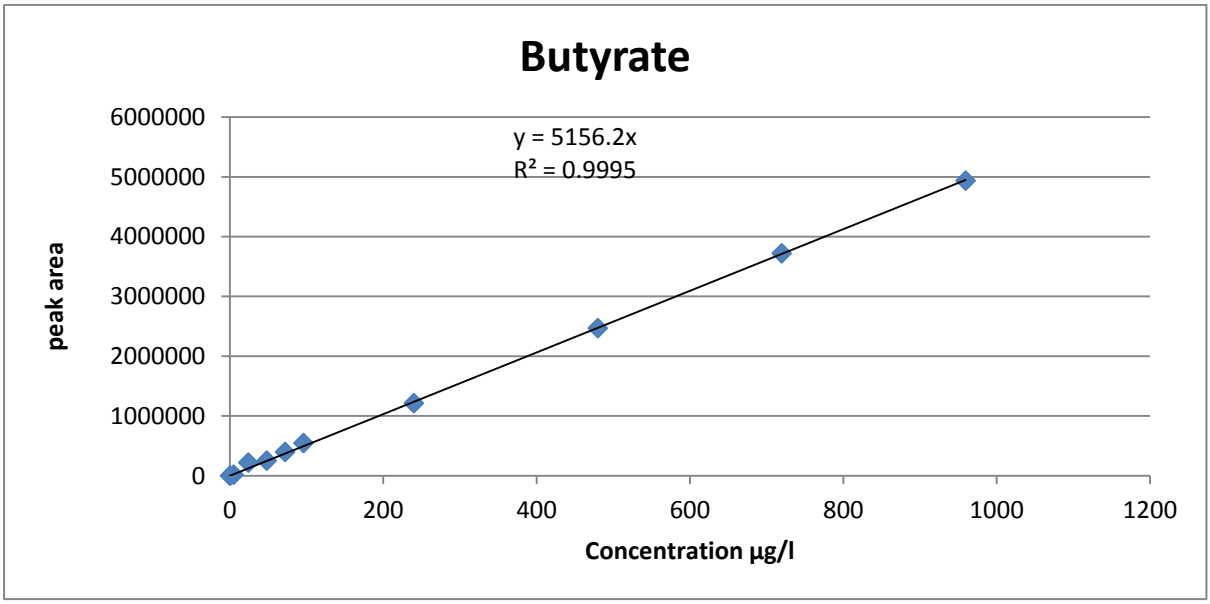
Chapter 9. Appendix A: calibration plots

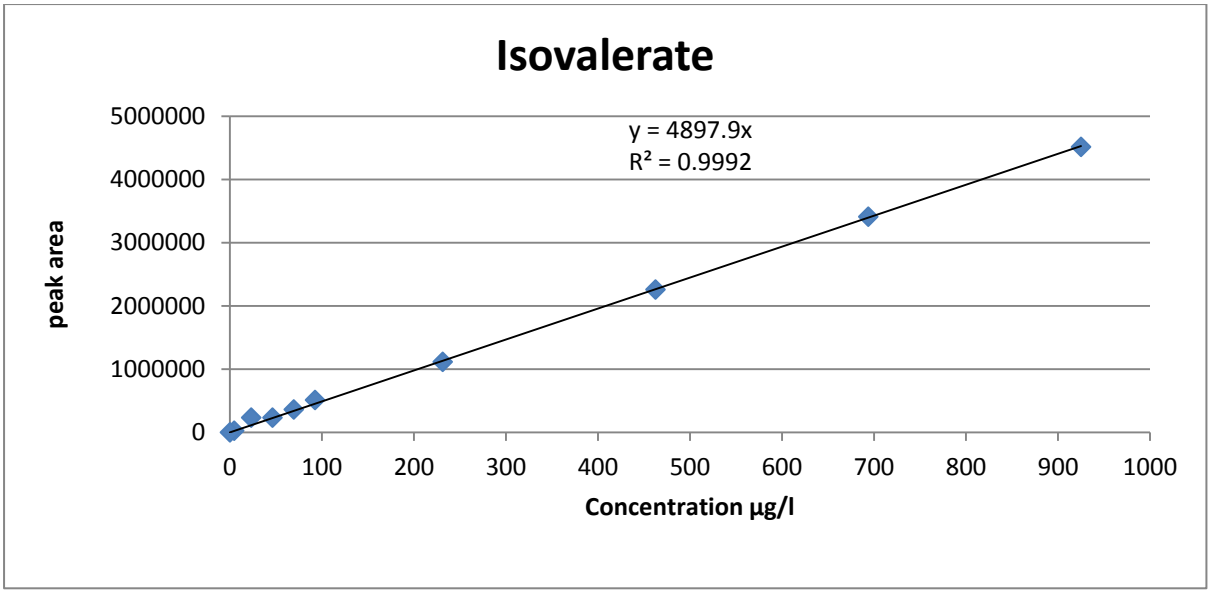
Vfa calibration data, samples assessed at concentrations of 5, 25, 50, 75, 100, 250, 500, 750 and 1000 µl/l, and converted to µg/l using densities shown in Table 9-1.






Isobutyrate
y = 5867.4x
R² = 0.9997
peak area
6000000
5000000
4000000
3000000
2000000
1000000
0
0
200
400
600
800
1000
1200
Concentration µg/l


Butyrate
y = 5156.2x
R² = 0.9995
peak area
6000000
5000000
4000000
3000000
2000000
1000000
0
0
200
400
600
800
1000
1200
Concentration µg/l



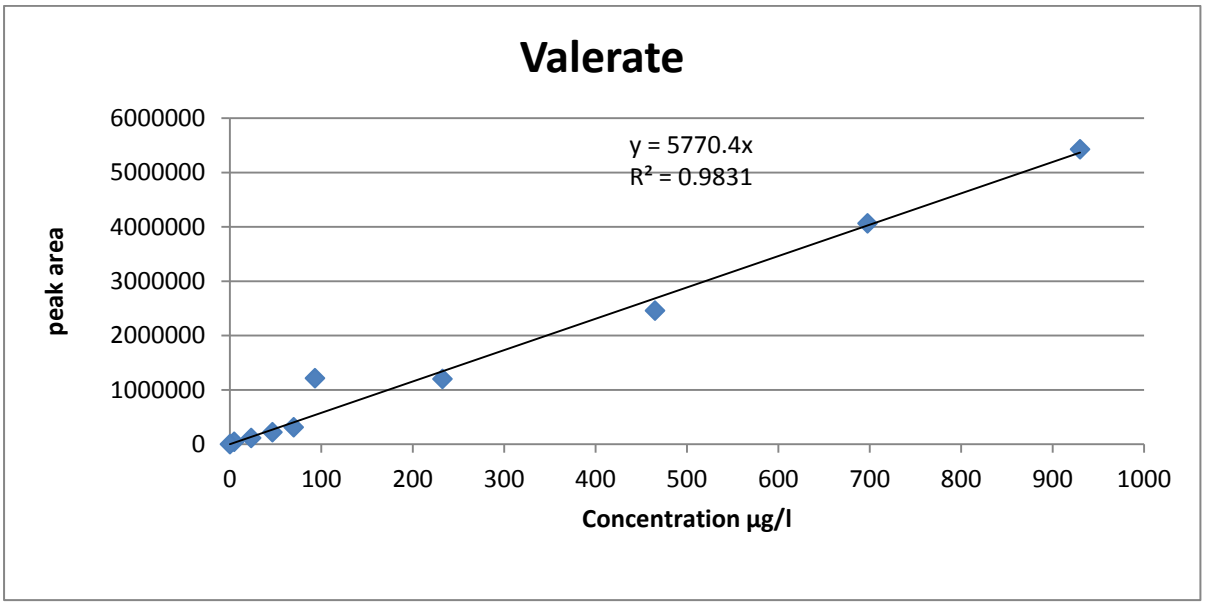


Table 9-1 vfa densities (Sigma-Aldrich, 2011)

VFA	Density g/ml
acetate	1.049
propionate	0.99
isobutyrate	0.9697
butyrate	0.9595
isovalerate	0.925
valerate	0.93

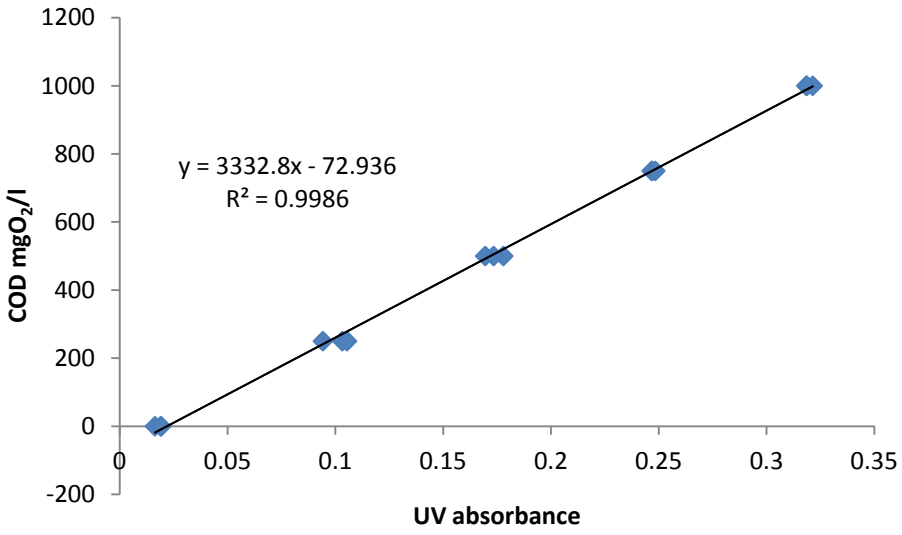


Figure 9-1 Sample COD calibration graph

Chapter 10. Appendix B: SAMBR Operational Data

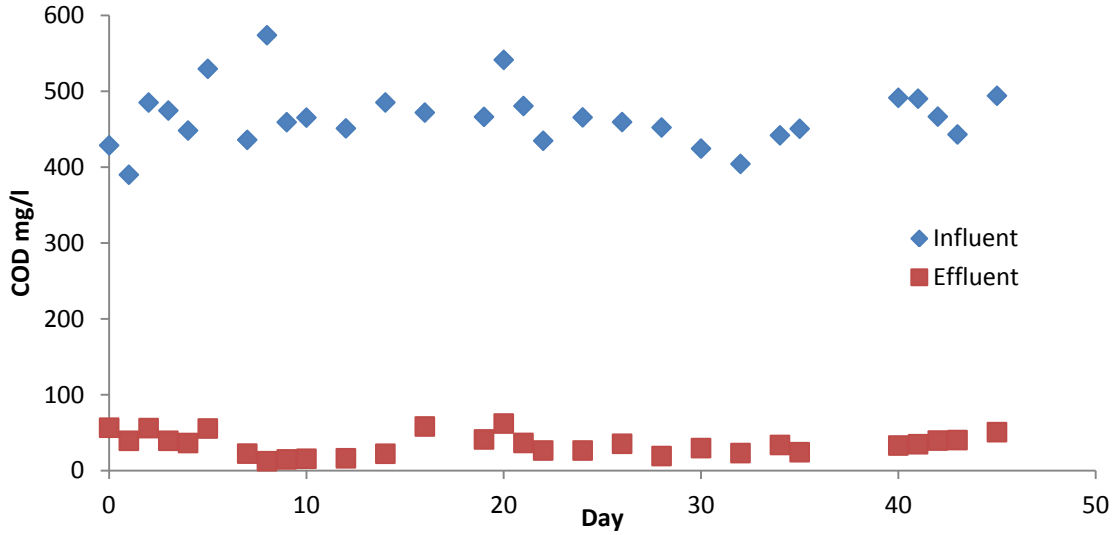


Figure 10-1 COD influent and effluent measurements for SAMBR operation during with the experiments listed in

Table 10-1 were carried out, (each data point is an average of three measured values).

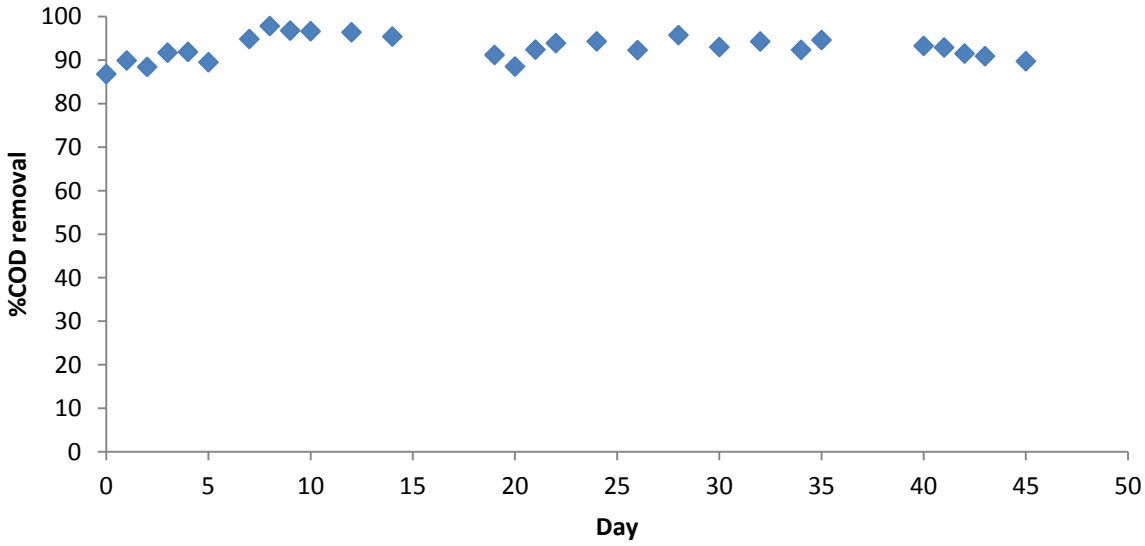


Figure 10-2 COD removal effiency for SAMBR measurements for SAMBR operation during with the experiments listed in Table 10-1 were carried out.

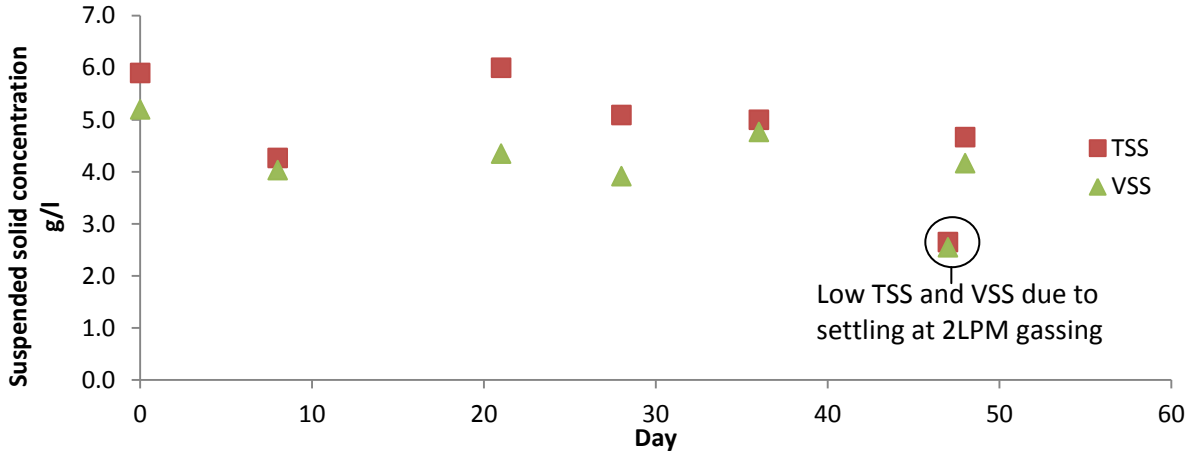


Figure 10-3 total suspended solids and volatile suspended solids measurements during operation

Table 10-1 Days on which experiments in chapter 4 and 5 were carried out in relation to the collected COD and suspended solid data shown above

Day	Gassing rate	Experiments from Chapter 5	Experiments from Chapter 4		
0	10	**clean membrane inserted into SAMBR**			
1	10				
2	10				
3	10	MS-2 phage removal in reactor (Figure 5-2)	critical gassing rate data (Figure 4-3)	Membrane permeability monitored (Figure 4-7)	Size exclusion data collected (Figure 4-14)
4	8				
5	6				
6	4				
7	4				
8	2				
9	2				
10	2				
11	2				
12	2				
13	2				
14	2				
15	2		VFA removal 1 (Figure 4-16)		
16	2		VFA removal 2 (, Figure 4-18, Figure 4-19)		
17	2				
18	2				
19	2	extended operation MS-2 removal (Figure 5-5)	Critical gassing rate reincreasing gassing rate (Figure 4-5)	Membrane permeability monitored (Figure 4-7)	Size exclsion data collected (Figure 4-15)
20	4				
21	6				
22	8				
23	4				
24	10				
25	8	phage concentration			

26	8	in reactor over time (Figure 5-9)			
27	8				
28	8				
29	8				
30	8				
31	8				
32	8				
33	8				
34	8				
35	8				
36	8				
37	8				
38	8				
39	2	Phage removal at 2LPM over time (Figure 5-4)	permeability drop at 2LPM (Figure 4-10)	membrane cleaned and reinserted into reactor	
40	2				
41	2				
42	2				
43	2				
44	2				
45	2				
46	2				
47	2				
48	2				
49	6				
50	6				
51	6		critical flux after extended operation experiment (Figure 4-2)		

Chapter 11. Appendix C: Phage sampling raw data

Table 11-1 raw data collected for the removal of MS-2 in a SAMBR (Figure 5-2)

experiment date	14-Jan							15-Jan							16-Jan		
day	3							4							5		
gasssing rate lpm	10	10	10	10	10	10		8	8	8	8	8	8		6	6	6
ml	5	5	5	5	5	5		5	5	5	5	5	5		5	5	5
time (sec)	26	26	26	26	26	26		26	26	26	26	26	26		26	26	26
Flux lmh	6.9	6.9	6.9	6.9	6.9	6.9		6.9	6.9	6.9	6.9	6.9	6.9		6.9	6.9	6.9
pressure monitor reading	1	1	1	1	1	1		0.99	0.99	0.99	0.99	0.99	0.99		0.98	0.98	0.98
TMP	0.013	0.013	0.013	0.013	0.013	0.013		0.023	0.023	0.023	0.023	0.023	0.023		0.033	0.033	0.033
sample time since spike (h)	1	1	2	2	3	3		1	1	2	2	3	3		1	1	2
SAMBR bulk																	
dilution	4	4	5	4	5	4		4	4	5	4	5	4		4	4	5
plaques	90		17	172	12	97		109		20	201	41	181		86		19
pfu	9000000	0	1.7E+07	1.7E+07	1.2E+07	9700000		1.1E+07	0	2E+07	2E+07	4.1E+07	1.8E+07		8600000	0	1.9E+07
effluent																	
dilution	2	3	2	3	2	3		2	3	2	3	2	3		2	3	2
plaques	158		297	35	203	17		99		183	25	376	43		74		130
pfu	158000	0	297000	350000	203000	170000		99000	0	183000	250000	376000	430000		74000	0	130000
LRV	**1.75559**	-	**1.75769**	**1.69146**	**1.77169**	**1.75632**		**2.04179**	-	**2.03858**	**1.90526**	**2.0376**	**1.62421**		**2.06527**	-	**2.16481**

Table 11-1 continued.

experiment date	16-Jan				18-Jan							19-Jan					
day	5				7							8					
gasssing rate lpm	6	6	6		4	4	4	4	4	4		2	2	2	2	2	2
ml	5	5	5		5	5	5	5	5	5		5	5	5	5	5	5
time (sec)	26	26	26		26	26	26	26	26	26		26	26	26	26	26	26
Flux lmh	6.9	6.9	6.9		6.9	6.9	6.9	6.9	6.9	6.9		6.9	6.9	6.9	6.9	6.9	6.9
pressure monitor reading	0.98	0.98	0.98		0.98	0.98	0.98	0.98	0.98	0.98		0.55	0.55	0.55	0.55	0.55	0.55
TMP	0.033	0.033	0.033		0.033	0.033	0.033	0.033	0.033	0.033		0.463	0.463	0.463	0.463	0.463	0.463
sample time since spike (h)	2	3	3		1	1	2	2	3	3		1	1	2	2	3	3
SAMBR bulk																	
dilution	4	5	4		4	5	4		4	5		4	5	4	5	4	5
plaques	206	19	208		194	13	226		171	12		201	19	154	26	215	19
pfu	2.1E+07	1.9E+07	2.1E+07		1.9E+07	1.3E+07	2.3E+07	0	1.7E+07	1.2E+07		2E+07	1.9E+07	1.5E+07	2.6E+07	2.2E+07	1.9E+07
effluent																	
dilution	2	2	3		2	3	2		2	3		1	1	2	2	2	2
plaques	130	202	16		120	15	173		219	24		370	372	163	163	12	12
pfu	130000	202000	160000		120000	150000	173000		219000	240000		37000	37200	163000	163000	12000	12000
LRV	**2.19992**	**1.9734**	**2.11394**		**2.20862**	**1.93785**	**2.11606**		**1.89255**	**1.69897**		**2.73499**	**2.70821**	**1.97533**	**2.20279**	**3.25326**	**3.19957**

Table 11-2 raw data collected for the removal of MS-2 in the SAMBR after operation at 2LPM (for Figure 5-5)

experiment date	30 January 2011							31 January 2012							01 February 2012		
Day	19							20							21		
gasssing rate lpm	2	2	2	2	2	2		4	4	4	4	4	4		6	6	6
ml	5	5	5	5	5	5		5	5	5	5	5	5		5	5	5
time (sec)	29	29	29	29	29	29		26	26	26	26	26	26		25.5	25.5	25.5
Flux lmh	6.2	6.2	6.2	6.2	6.2	6.2		6.9	6.9	6.9	6.9	6.9	6.9		7.1	7.1	7.1
pressure monitor reading	0.55	0.55	0.55	0.55	0.55	0.55		0.78	0.78	0.78	0.78	0.78	0.78		0.89	0.89	0.89
TMP	0.463	0.463	0.463	0.463	0.463	0.463		0.233	0.233	0.233	0.233	0.233	0.233		0.123	0.123	0.123
sample time since spike (h)	1	1	2	2	3	3		1	1	2	2	3	3		1	1	2
SAMBR bulk																	
dilution	5	4	5	4	5	4		4	5	4	5	4	5		4	5	4
plaques	16	190	20	262	15	165		225	26	282	22.5	201	24		182	45	130
pfu	1.6E+07	1.9E+07	2E+07	2.6E+07	1.5E+07	1.7E+07		2.3E+07	2.6E+07	2.8E+07	2.3E+07	2E+07	2.4E+07		1.8E+07	4.5E+07	1.3E+07
effluent																	
dilution	2	2	2	2	2	1		1	1	1	1	1	1		1	1	1
plaques	18	18	22	22	21	232		62	48	53	44	27	29		32	37	6
pfu	18000	18000	22000	22000	21000	23200		6200	4800	5300	4400	2700	2900		3200	3700	600
LRV	**2.94885**	**3.02348**	**2.95861**	**3.07588**	**2.85387**	**2.852**		**3.55979**	**3.73373**	**3.72597**	**3.70873**	**3.87183**	**3.91781**		**3.75492**	**4.08501**	**4.33579**

Table 11-2 continued.

experiment date	01 February 2012				02 February 2012							04 February 2012					
Day	21				22							24					
gasssing rate lpm	6	6	6		8	8	8	8	8	8		10	10	10	10	10	10
ml	5	5	5		5	5	5	5	5	5		5	5	5	5	5	5
time (sec)	25.5	25.5	25.5		25.5	25.5	25.5	25.5	25.5	25.5		25.5	25.5	25.5	25.5	25.5	25.5
Flux lmh	7.1	7.1	7.1		7.1	7.1	7.1	7.1	7.1	7.1		7.1	7.1	7.1	7.1	7.1	7.1
pressure monitor reading	0.89	0.89	0.89		0.94	0.94	0.94	0.94	0.94	0.94		0.97	0.97	0.97	0.97	0.97	0.97
TMP	0.123	0.123	0.123		0.073	0.073	0.073	0.073	0.073	0.073		0.043	0.043	0.043	0.043	0.043	0.043
sample time since spike (h)	2	3	3		1	1	2	2	3	3		1	1	2	2	3	3
SAMBR bulk																	
dilution	5	4	5		5	5	5	5	5	5		5	5	5	5	5	5
plaques	14	102	14		72	73	73	84	53	74		178	193	115	141	123	161
pfu	1.4E+07	1E+07	1.4E+07		7.2E+07	7.3E+07	7.3E+07	8.4E+07	5.3E+07	7.4E+07		1.8E+08	1.9E+08	1.2E+08	1.4E+08	1.2E+08	1.6E+08
effluent																	
dilution	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1
plaques	8	18	11		20	18	44	43	23	29		96	102	56	35	71	83
pfu	800	1800	1100		2000	1800	4400	4300	2300	2900		9600	10200	5600	3500	7100	8300
LRV	**4.24304**	**3.75333**	**4.10474**		**4.5563**	**4.60805**	**4.21987**	**4.29081**	**4.36255**	**4.40683**		**4.26815**	**4.27696**	**4.31251**	**4.60515**	**4.23865**	**4.28775**

Table 11-3 Raw data for the removal of MS-2 in a SAMBR after extended operation at 2LPM (for Figure 5-7 and Figure 5-8)

experiment date	12-Dec							13-Dec					14-Dec					
gasssing rate lpm	2	2	2	2	2	2		4	4	4	4		5	5	5	5	5	5
ml	5	5	5	5	5	5		5	5	5	5		5	5	5	5	5	5
time (sec)	28	28	28	28	28	28		28	28	28	28		28	28	28	28	28	28
Flux lmh	6.4	6.4	6.4	6.4	6.4	6.4		6.4	6.4	6.4	6.4		6.4	6.4	6.4	6.4	6.4	6.4
pressure monitor reading	0.52	0.52	0.52	0.52	0.52	0.52		0.59	0.59	0.62	0.62		0.75	0.75	0.75	0.75	0.75	0.75
TMP	0.493	0.493	0.493	0.493	0.493	0.493		0.423	0.423	0.393	0.393		0.263	0.263	0.263	0.263	0.263	0.263
sample time since spike (h)	1	1	2	2	3	3		1	1	2	2		1	1	2	2	3	3
SAMBR bulk																		
dilution	5	5	5	5	5	5		5	5	5	5		6	6	6	6	6	6
plaques	160	166	91	104	91	91		234	269	214	272		11	16	20	26	11	15
pfu	1.6E+08	1.7E+08	9.1E+07	1E+08	9.1E+07	9.1E+07		2.3E+08	2.7E+08	2.1E+08	2.7E+08		1.1E+08	1.6E+08	2E+08	2.6E+08	1.1E+08	1.5E+08
effluent																		
dilution	1	1	1	1	1	1		1	1	1	1		1	1	1	1	1	1
plaques	63	71	65	56	56	83		67	103	74	94		22		55	29	12	16
pfu	6300	7100	6500	5600	5600	8300		6700	10300	7400	9400		2200	0	5500	2900	1200	1600
LRV	**4.40478**	**4.36885**	**4.14613**	**4.26885**	**4.21085**	**4.03996**		**4.54314**	**4.41692**	**4.46118**	**4.46144**		**4.69897**	-	**4.56067**	**4.95258**	**4.96221**	**4.97197**

Table 11-3 continued.

experiment date							16-Dec							17-Dec					
gasssing rate lpm	6	6	6	6	6		8	8	8	8	8	8		10	10	10	10	10	10
ml	5	5	5	5	5		5	5	5	5	5	5		5	5	5	5	5	5
time (sec)	28	28	28	28	28		28	28	28	28	28	28		27	27	27	27	27	27
Flux lmh	6.4	6.4	6.4	6.4	6.4		6.4	6.4	6.4	6.4	6.4	6.4		6.7	6.7	6.7	6.7	6.7	6.7
pressure monitor reading	0.81	0.81	0.81	0.81	0.81		0.82	0.82	0.82	0.82	0.82	0.82		0.85	0.85	0.85	0.85	0.85	0.85
TMP	0.203	0.203	0.203	0.203	0.203		0.193	0.193	0.193	0.193	0.193	0.193		0.163	0.163	0.163	0.163	0.163	0.163
sample time since spike (h)	1	2	2	3	3		1	1	2	2	3	3		1	1	2	2	3	3
SAMBR bulk																			
dilution	5	5	5	5	5		6	6	6	6	6	6		6	6	6	6	6	6
plaques	242	253	295	278	286		40	35	37		49	44		41	42	28	41	29	31
pfu	2.4E+08	2.5E+08	3E+08	2.8E+08	2.9E+08		4E+08	3.5E+08	3.7E+08	0	4.9E+08	4.4E+08		4.1E+08	4.2E+08	2.8E+08	4.1E+08	2.9E+08	3.1E+08
effluent																			
dilution	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1
plaques	18	15	13	13	14		13	17	14	10	17	16		12	6	14	21	8	
pfu	1800	1500	1300	1300	1400		1300	1700	1400	1000	1700	1600		1200	600	1400	2100	800	
LRV	**5.12854**	**5.22703**	**5.35588**	**5.3301**	**5.31024**		**5.48812**	**5.31362**	**5.42207**		**5.45975**	**5.43933**		**5.53**	**5.85**	**5.30**	**5.29**	**5.56**	

Table 11-3 continued.

experiment date	18-Dec							19-Dec							20-Dec		
gasssing rate lpm	8	8	8	8	8	8		6	6	6	6	6	6		4	4	4
ml	5	5	5	5	5	5		5	5	5	5	5	5		5	5	5
time (sec)	27	27	27	27	27	27		27	27	27	27	27	27		27	27	27
Flux lmh	6.7	6.7	6.7	6.7	6.7	6.7		6.7	6.7	6.7	6.7	6.7	6.7		6.7	6.7	6.7
pressure monitor reading	0.84	0.84	0.84	0.84	0.84	0.84		0.8	0.8	0.8	0.8	0.8	0.8		0.535	0.535	0.535
TMP	0.173	0.173	0.173	0.173	0.173	0.173		0.213	0.213	0.213	0.213	0.213	0.213		0.478	0.478	0.478
sample time since spike (h)	1	1	2	2	3	3		1	1	2	2	3	3		1	1	2
SAMBR bulk																	
dilution	6	6	6	6	6	6		6	6	6	6	6	6		6	6	6
plaques	42	44	21	24	43	35		61	26	15	19	15	16		22	23	12
pfu	4.2E+08	4.4E+08	2.1E+08	2.4E+08	4.3E+08	3.5E+08		6.1E+08	2.6E+08	1.5E+08	1.9E+08	1.5E+08	1.6E+08		2.2E+08	2.3E+08	1.2E+08
effluent																	
dilution	1	1	1	1	1	0		1	1	1	1	1	1		1	1	1
plaques	15	17	16	14	12	118		53	46	15	14	21	38		154	123	145
pfu	1500	1700	1600	1400	1200	1180		5300	4600	1500	1400	2100	3800		15400	12300	14500
LRV	**5.45**	**5.41**	**5.12**	**5.23**	**5.55**	**5.47**		**5.06**	**4.75**	**5.00**	**5.13**	**4.85**	**4.62**		**4.15**	**4.27**	**3.92**

Table 11-3 continued.

experiment date	20-Dec				21-Dec					
gasssing rate lpm	4	4	4		2	2	2	2	2	2
ml	5	5	5		5	5	5	5	5	5
time (sec)	27	27	27		33	33	33	33	33	33
Flux lmh	6.7	6.7	6.7		5.5	5.5	5.5	5.5	5.5	5.5
pressure monitor reading	0.535	0.535	0.535		0.36	0.36	0.36	0.36	0.36	0.36
TMP	0.478	0.478	0.478		0.653	0.653	0.653	0.653	0.653	0.653
sample time since spike (h)	2	3	3		1	1	2	2	3	3
SAMBR bulk										
dilution	6	6	6		6	6	6	6	6	6
plaques	19	25	26		15	47	20	21	20	14
pfu	1.9E+08	2.5E+08	2.6E+08		1.5E+08	4.7E+08	2E+08	2.1E+08	2E+08	1.4E+08
effluent										
dilution	1	1	1		1	1	1	1	1	1
plaques	108	106	112		155	154	127	150	125	117
pfu	10800	10600	11200		15500	15400	12700	15000	12500	11700
LRV	**4.25**	**4.37**	**4.37**		**3.99**	**4.48**	**4.20**	**4.15**	**4.20**	**4.08**

Table 11-4 Raw data collected for the removal of bacteriophage T4 in a SAMBR (for Figure 5-11)

experiment date	13/02/2011							14-Feb							15-Feb		
gasssing rate lpm	10	10	10	10	10	10		8	8	8	8	8	8		6	6	6
ml	5		5		5			5		5		5			5		5
t	25.5		25.5		25.5			25.5		25.5		25.5			25.5		25.5
Flux lmh	7.1		7.1		7.1			7.1		7.1		7.1			7.1		7.1
pressure monitor	0.995	0.995	0.995	0.995	0.995	0.995		0.995	0.995	0.995	0.995	0.995	0.995		0.995	0.995	0.995
TMP	0.018	0.018	0.018	0.018	0.018	0.018		0.018	0.018	0.018	0.018	0.018	0.018		0.018	0.018	0.018
time (h)	1	2	2	3	3	4		1	1	2	2	3	3		1	1	2
biomass																	
dilution	5	6	5	5	5	5		5	6	5	6	5	6		5	5	5
plaques	294	10	77	43	44	32		69.5	6	66	15	42.5	6		74	87	78
pfu	2.9E+08	1E+08	7.7E+07	4.3E+07	4.4E+07	3.2E+07		7E+07	6E+07	6.6E+07	1.5E+08	4.3E+07	6E+07		7.4E+07	8.7E+07	7.8E+07
effluent																	
dilution	1	0	1	0	0	0		0	1	0	1	0	0		0	0	0
plaques	16	35	12	30	26	33		41	11.5	38	5	39	39		55	51	63
pfu	1600	350	1200	300	260	330		410	1150	380	500	390	390		550	510	630
LRV	5.26423	5.45593	4.80731	5.15635	5.22848	4.98664		5.2292	4.71745	5.23976	5.47712	5.03732	5.18709		5.12887	5.23195	5.09275

Table 11-4 continued.

experiment date	15-Feb				16-Feb							17-Feb					18-Feb					
gasssing rate lpm	6	6	6		4	4	4	4	4	4		2	2	2	2		2	2	2	2	2	2
ml		5			5		5		5			5		5			5		5			
t		25.5			26		25.5		26			25.5		26			25.5		26			
Flux lmh		7.1			6.9		7.1		6.9			7.1		6.9			7.1		6.9			
pressure monitor	1	1	1		0.995	0.995	0.995	0.995	0.995	0.995		0.99	0.99	0.975	0.975		?					
TMP	0.02	0.02	0.02		0.018	0.018	0.018	0.018	0.018	0.018		0.023	0.023	0.038	0.038							
time (h)	2	3	3		1	1	2	2	3	3		2	2	4	4		1	1	2	2	3	3
biomass																						
dilution	5	5	5		5	5	5	6	5	5		5	5	5	5		5	5	5	5	5	5
plaques	81	87	88		183	152	120	19	123	124		112	88	95	98		131	143	125	130	104	96
pfu	8E+07	9E+07	9E+07		2E+08	2E+08	1E+08	2E+08	1E+08	1E+08		1.1E+08	8.8E+07	9.5E+07	9.8E+07		1.3E+08	1.4E+08	1.3E+08	1.3E+08	1E+08	9.6E+07
effluent																						
dilution	0	0	0		0	0	0	0	0	0		0	0	0	0		0	0	0	0	0	0
plaques	55	44	66		59	48	81	78	67	77		12	12	10	10		1	2	1	0	8	3
pfu	550	440	660		590	480	810	780	670	770		120	120	100	100		10	20	10	0	80	30
LRV	5.17	5.3	5.12		5.492	5.501	5.171	5.387	5.264	5.207		5.97004	5.8653	5.97772	5.99123		7.11727	6.85431	7.09691		6.11394	6.50515

Chapter 12. Appendix D: T4 ANOVA F-test calculations

null hypothesis (H0) --> **all gassing rates from 10-4 have the same log removal**

Gassing rate	number of samples	LRV					
10	6	5.2642	5.4559	4.8073	5.1563	5.2285	4.9866
8	6	5.2292	4.7175	5.2398	5.4771	5.0373	5.1871
6	6	5.1289	5.2319	5.0928	5.1681	5.2961	5.1249
4	6	5.4916	5.5006	5.1707	5.3867	5.2638	5.2069

Mean	**Overall mean**		between group sum of squares (Sb)
5.1498	**5.2021**		0.0164
5.148			0.0176
5.1738			0.0048
5.3367			0.1088
		total	**0.1475**

Between group degrees of freedom (fb)		**between-group mean square value (MSb)**		**"within-group" sum of squares (Sw)**	**within group degress of freedom (fw)**	**within-group mean square value (MSw)**
3		0.0492		0.8594	23	0.0374

F ratio	corresponding p value		Fcrit for p(0.05)
1.316	0.293		3.028

Centered group data squared					
0.0039	0.0644	0.1558	0.0021	0.0007	0.0464
0.0007	0.2349	0.0014	0.0756	0.0271	0.0002
0.0054	0.0009	0.012	0.0012	0.0088	0.006
0.0838	0.0891	0.001	0.0341	0.0038	2E-05

Chapter 13. Appendix E: Kepner Tregoe Detailed Results

Table 13-1 Individual and average weightings for the set objectives.

		Individual weightings*							
		A	**B**	**C**	**D**	**E**	**F**	**G**	**Average±Stdev**
Objective	**Unit**								
Energy balance	kW/m^3	8	10	9	6	9	10	8	8.6 ± 1.5
Efficiency:									
BOD removal	%	10	9	10	10	7	9	9	9.1 ± 1.2
COD removal	%	10	9	9	10	7	9	9	9.0 ± 1.1
Solids removal	%	10	8	9	10	7	8	9	8.7 ± 1.2
Total nitrogen removal	%	10	8	8	10	7	8	9	8.6 ± 1.2
Total P removal	%	10	8	8	10	7	8	9	8.6 ± 1.2
Other parameters:									
CAPEX	£	7	6	6	10	6	6	5	6.6 ± 1.6
OPEX	£	8	9	8	10	6	9	8	8.3 ± 1.4
Sludge production	kg/day	5	6	5	3	4	6	4	4.7 ± 1.2
Plant footprint	m^2	5	4	3	3	5	4	3	3.9 ± 0.9
Allows resource recovery**	%	6	4	6	3	6	4	8	5.3 ± 1.3
Plant reliability		8	7	10	8	8	7	10	8.3 ± 1.1
Maintenance		9	7	7	8	8	7	7	7.6 ± 0.8
Plant flexibility		9	6	8	7	8	6	7	7.3 ± 1.2

*Weightings - reflect the importance of the objective: 1-2 (very low); 3-4 (low); 5-6 (medium); 7-8 (high) and 9-10 (very high).

**(e.g.: struvite, water re-use, etc)

Table 13-2 Objective ranking

		Ranking				
Objective	**Unit**	**1**	**2**	**3**	**4**	**5**
Energy balance	kW/m^3	>1.00	0.99-0.70	0.69-0.40	0.39-0.20	<0.19
Efficiency:						
BOD removal		<49%	50-69%	70-84%	85-94%	>95%
COD removal	%	<49%	50-69%	70-84%	85-94%	>95%
Solids removal	%	<49%	50-69%	70-84%	85-94%	>95%
Total nitrogen removal	%	<49%	50-69%	70-84%	85-94%	>95%
Total P removal	%	<49%	50-69%	70-84%	85-94%	>95%
	%	<49%	50-69%	70-84%	85-94%	>95%
Other parameters:						
CAPEX	M£	>£6M	£5.9-5.5M	£5.4-4.5M	£4.4-3M	<£2.9-1M
OPEX	k£/year	>£200K	£200k-£150k	£149k-£100k	£99k-£50k	<£49k
Sludge production	kg/day	80-60	60-40	40-20	20-5	>5
Plant footprint	m^2	>1000	999-800	799-600	599-300	>300
Allows resource recovery *		No	1	1+2-	1+2+3-	1+2+3+4
Reference installations		Lab-scale	Small pilot-scale	Large pilot-scale	Few full-scale	Widely implemented
Maintenance		Very high	High	Medium	Low	Very low
Plant flexibility		None	Allows 1 process variation	Allows 2 process variations	Allows 3 process variations	Allows 4 process variations

Table 13-3 Comparison of flowsheets using the KT methodology

Flowsheet 1: Primary settling tank + anMBR + chemical treatment

Objective	Unit	Weighting *W*	In	Out	Balance/ removal	Ranking *R*	*W* x *R*
Flow	m^3/day		2500.0	2478.6			
Energy balance	kW/m^3	8.6	0.052	0.313	-0.262	4	34
Efficiency:							
BOD		9.1	313.2	4.7	98.5	5	46
COD	%	9	971.3	23.7	97.6	5	45
Solids	%	8.7	589.2	0.0	100.0	5	44
Total nitrogen	%	8.6	65.5	1.1	98.3	5	43
Total P	%	8.6	10.4	0.3	97.1	5	43
Other parameters:							
CAPEX	£	6.6			£4,513,602	3	20
OPEX	£/year	8.3			£125,570	3	25
Sludge production	kg/day	4.7			48	2	9
Plant footprint	m^2	3.9			419	4	16
Allows resource recovery		5.3		1+2+3+4		5	27
Plant reliability		8.3		anMBR not well tested		4	33
Maintenance		7.6		medium		3	23
Plant flexibility		7.3		Allows 3 process variations		4	29
						Total	**436**

Flowsheet 2: Primary settling tank + UASB + biological treatment

Objective	Unit	Weighting *W*	In	Out	Balance/ removal	Ranking *R*	*W* x *R*
Flow	m^3/day		2500.0	2469.6			
Energy balance	kW/m^3	8.6	0.018	0.401	-0.383	4	34
Efficiency:							
BOD		9.1	313.2	2.0	99.4	5	46
COD	%	9	971.3	81.3	91.6	4	36
Solids	%	8.7	589.2	22.1	96.2	5	44
Total nitrogen	%	8.6	65.5	10.0	84.7	5	43
Total P	%	8.6	10.4	0.7	93.3	4	34
Other parameters:							
CAPEX	£	6.6			£5,299,313	3	20
OPEX	£/year	8.3			£82,306	4	33
Sludge production	kg/day	4.7			96	1	5

Plant footprint	m^2	3.9		641	3	12
Allows resource recovery*		5.3		1+2-liquidCH_4	3	16
Plant reliability		8.3		Very well known processes	5	42
Maintenance		7.6		Low	4	30
Plant flexibility		7.3		Allows 2 process variations	3	22
					Total	**416**

Flowsheet 3: ABR + anMBR + chemical treatment

Objective	**Unit**	**Weighting *W***	**In**	**Out**	**Balance/ removal**	**Ranking *R***	***W* x *R***
Flow	m^3/day		2500.0	2478.6			
Energy balance	kW/m^3	8.6	0.013	0.347	-0.334	4	34
Efficiency:							
BOD		9.1	313.2	6.3	98.0	5	46
COD	%	9	971.3	41.0	95.8	5	45
Solids	%	8.7	589.2	2.3	99.6	5	44
Total nitrogen	%	8.6	65.5	1.5	97.8	5	43
Total P	%	8.6	10.4	0.3	97.0	5	43
Other parameters:							
CAPEX	£	6.6			£5,200,494	3	20
OPEX	£/year	8.3			£126,416	3	25
Sludge production	kg/day	4.7			74	1	5
Plant footprint	m^2	3.9			701	3	12
Allows resource recovery		5.3		1+2+3+4		5	27
Plant reliability		8.3		ABR + anMBR not well tested		2	17
Maintenance		7.6		medium		3	23
Plant flexibility		7.3		Allows 3 process variations		4	29
						Total	**411**

Flowsheet 4: ABR + UASB + biological treatment

Objective	**Unit**	**Weighting *W***	**In**	**Out**	**Balance/ removal**	**Ranking *R***	***W* x *R***
Flow	m^3/day		2500.0	2469.6			
Energy balance	kW/m^3	8.6	0.013	0.370	-0.357	4	34
Efficiency:							
BOD		9.1	313.2	2.0	99.4	5	46

COD	%	9	971.3	81.3	91.6	4	36
Solids	%	8.7	589.2	11.0	98.1	5	44
Total nitrogen	%	8.6	65.5	10.0	84.7	4	34
Total P	%	8.6	10.4	0.7	93.3	4	34
Other parameters:							
CAPEX	£	6.6			£5,652,021	2	13
OPEX	£/year	8.3			£80,079	4	33
Sludge production	kg/day	4.7			96	1	5
Plant footprint	m^2	3.9			847	2	8
Allows resource recovery		5.3		1+2-liquidCH_4		3	16
Plant reliability		8.3		ABR not well known processes		4	33
Maintenance		7.6		Medium		3	23
Plant flexibility		7.3		Allows 2 process variations		3	22
						Total	**381**

Flowsheet 5: 1mm screen + anMBR + chemical treatment

Objective	**Unit**	**Weighting *W***	**In**	**Out**	**Balance/ removal**	**Ranking *R***	***W* x *R***
Flow	m^3/day		2500.0	2478.6			
Energy balance	kW/m^3	8.6	0.008	0.309	-0.301	4	34
Efficiency:							
BOD		9.1	313.2	11.0	96.5	5	46
COD	%	9	971.3	52.1	94.6	5	45
Solids	%	8.7	589.2	0.0	100.0	5	44
Total nitrogen	%	8.6	65.5	3.0	95.4	5	43
Total P	%	8.6	10.4	0.5	95.5	5	43
Other parameters:							
CAPEX	£	6.6			£3,287,597	4	26
OPEX	£/year	8.3			£119,031	3	25
Sludge production	kg/day	4.7			61	2	9
Plant footprint	m^2	3.9			239	5	20
Allows resource recovery		5.3		1+2+3+4		5	27
Plant reliability		8.3		anMBR and Anion Exchanger not well tested		3	25
Maintenance		7.6		Medium		3	23
Plant flexibility		7.3		Allows 3 process variations		4	29
						Total	**438**

Flowsheet6: Conventional aerobic flowsheet

Objective	Unit	Weighting *W*	In	Out	Balance/ removal	Ranking *R*	*W* x *R*
Flow	m^3/day		2500.0	2364.6			
Energy balance	kW/m^3	8.6	0.01	0.98	-0.97	2	17
Efficiency:							
BOD		9.1	313.2	7.9	97.5	5	46
COD	%	9	971.3	34.0	96.5	5	45
Solids	%	8.7	589.2	40.2	93.2	4	35
Total nitrogen	%	8.6	65.5	0.2	99.7	5	43
Total P	%	8.6	10.4	1.0	90.4	4	34
Other parameters:							
CAPEX	£	6.6			£5,304,819	3	20
OPEX	£/year	8.3			£106,498	3	25
Sludge production	kg/day	4.7			96	1	5
Plant footprint	m^2	3.9			641	3	12
Allows resource recovery		5.3		1		2	11
Plant reliability		8.3		Very well known processes		5	42
Maintenance		7.6		Low		4	30
Plant flexibility		7.3		No		1	7
						Total	**371**