Improvement of pulp-mill wastewater for anaerobic digestion

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This dissertation is dedicated to my wife.

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DECLARATION

I, Richard Bence, hereby declare to be the sole author of the report entitled:

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ABSTRACT

Anaerobic digestion is the most cost-effective biological-treatment process available for generating energy. However, the use of certain pulp-mill wastewater streams for anaerobic digestion is not commonly implemented, mainly due to toxic and recalcitrant compounds. By using certain pre-treatment methods, most of the toxic and recalcitrant compounds can be removed, which improves the suitability of these streams for anaerobic digestion. In this work, sulphite evaporator condensate (SEC) was pre-treated and evaluated at the substrate level, after which the pre-treatments were evaluated using bench-scale upflow anaerobic sludge blanket (UASB) reactors.

Characterisation of the SEC showed a high chemical oxygen demand (COD) concentration (19 000 mg/L) and was largely composed of volatile fatty acids (VFAs), furfural, lactic acids, polyphenols and lignosulphonate. Additionally, the wastewater consisted of a high concentration of carbonate alkalinity and had a low pH. The high concentration of VFAs, furfural, lactic acids and carbonate alkalinity were favourable for methane production and the stability of anaerobic digestion. Polyphenols and lignosulphonate are inhibitory to anaerobic digestion and high sulphate concentrations may reduce methane production.

The pre-treatment methods were therefore focused on removing the polyphenols and lignosulphonate, without affecting the potential substrates for anaerobic digestion. Laccases and coagulants were used during the pre-treatments due to the effectiveness on phenol-containing compounds, with few side-effects. Laccase11 was the best-performing enzyme and increased the molecular weight of lignosulphonate by 60%, and the removal was 34% and 33% for lignosulphonate and polyphenols, respectively. Polydiallyldimethylammonium chloride (PolyDADMAC) was the best-performing coagulant and removed 62% lignosulphonate and 57% polyphenols. However, PolyDADMAC also removed 50% of the VFAs.

Three batch pre-treatments were performed on the SEC, which were used as feed to the reactors. The SEC in each batch was adjusted to a pH of 7 and treated with PolyDADMAC, Laccase11 or a control. Characterisation of the batches revealed that Laccase11 removed more than 30% and PolyDADMAC more than 50% of the inhibitory compounds from the SEC. The biological oxygen demand did not change significantly. Additionally, PolyDADMAC removed 34% sulphate.

These pre-treatments enabled higher volumetric hydraulic loading (VHL) rates to the respective reactors to achieve the same organic loading rate (OLR) as the control. Treatment of SEC with PolyDADMAC was the most effective, allowing the VHL to increase by 1.13 times to obtain the same OLR as the control. At all OLRs tested, the PolyDADMAC reactor removed the most COD

and had the highest specific methane yield. At the highest OLR where all three reactors were stable (13kg_{COD}/m^3d), the PolyDADMAC reactor had a COD removal efficiency of 60%, a specific methane yield twice the value of the control and the methane produced was more than double. At the highest OLR tested (16 kg_{COD}/m^3d), the COD removal efficiency of the reactors using Laccase11- and PolyDADMAC-treated effluent was below 55%, with the PolyDADMAC reactor performing 7% better. At the same OLR, the reactor fed with the control batch went “sour”, with less than 28% COD removed. Therefore, PolyDADMAC was the most effective pre-treatment and may be a financially feasible option to enhance anaerobic digestion.

Keywords: coagulation, condensate, laccase, lignosulphonate, polyphenols, sulphite evaporator, upflow anaerobic sludge blanket (UASB) reactor, wastewater,

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CHAPTER 1. INTRODUCTION

1.1 Background and motivation

In recent years, both environmental awareness and wastewater legislation have improved substantially. Given the pressure of maintaining good relationships with customers and the public, many industries – including the pulp industry – have been prompted to improve their wastewater quality. The pulp industry is a large producer of wastewater and its global treatment requirement is set to increase by up to 60% by 2020 (Meyer and Edwards, 2014). The industry faces challenges in meeting the treatment requirement because of the high cost of wastewater treatment facilities, the unique composition of pulp-mill wastewater, and stringent wastewater regulations (Kamali and Khodaparast, 2015).

The wastewater regulations that must be complied with generally relate to colour, odour, pH, temperature, biological oxygen demand (BOD), chemical oxygen demand (COD), suspended solids content, and toxicity (Pokhrel and Viraraghavan, 2004). Pulp-mill wastewater generally consists of chlorinated organics, organic acids, suspended solids, fatty acids, cellulose, hemicellulose, lignin, phenolic compounds, and sulphur-containing compounds (Ali and Sreekrishnan, 2001). Most of these compounds contribute to high concentrations of BOD, COD and toxicity, that cause considerable damage to the environment (Kamali and Khodaparast, 2015). Fortunately, most of these compounds are potential sources of energy and can be used to reduce treatment costs. For instance, compounds of lignin and its derivatives may be valuable as fuel sources, whereas cellulose, hemicellulose, fatty acids and certain sulphur-containing compounds can be used in biological-treatment processes (Elliott and Mahmood, 2007).

Anaerobic digestion is the most cost-effective biological-treatment process available for generating energy and the method is environmentally friendly (Zheng *et al.*, 2014). Anaerobic digestion could thus be a suitable treatment process for most pulp-mill wastewaters. However, certain pulp-mill wastewater streams are not widely used for anaerobic digestion. This is largely due to toxic chlorinated and phenolic compounds, recalcitrant compounds such as lignin, and the complex structures of cellulose and hemicellulose polymers (Himmel *et al.*, 2007). Through certain pre-treatments, most of the recalcitrant and complex compounds can be degraded and the toxic compounds removed. Doing so improves the suitability of these streams for anaerobic digestion. In addition, pre-treating pulp-mill wastewater streams that are already suitable for anaerobic digestion may improve the overall efficiency of anaerobic digestion further.

However, pre-treatment might enhance the anaerobic digestion of pulp-mill wastewater but remain an environmentally and financially unsustainable approach. In this study, the usefulness

of pre-treatments is assessed according to the chemical and energy balance of substrate pre-treatments as well as the overall anaerobic system.

1.2 Objectives of the dissertation

Research was conducted to determine whether certain pre-treatments could be useful for treating pulp-mill wastewater streams to achieve enhanced anaerobic digestion. The research objectives were as follows:

(a) To complete a literature study to investigate the suitability of different pulp-mill wastewater streams and pre-treatment methods for anaerobic digestion.

(b) To characterise a selected wastewater stream from a pulp-mill to determine its suitability for anaerobic digestion.

(c) To conduct pre-treatments on the selected wastewater stream and to analyse and evaluate the effects thereof.

(d) To monitor and evaluate effects of the pre-treatments on a bench-scale upflow anaerobic sludge blanket (UASB) reactor.

1.3 Outline of the dissertation

The research activities performed to fulfil the objectives set out in Section 1.2, are stated below:

In Chapter 1 the general motivation for the research is explained. In Chapter 2 a literature review is provided regarding the constituents of various pulp-mill wastewater streams and their suitability for anaerobic digestion. Pre-treatment methods to improve the suitability of pulp-mill wastewater were reviewed based on the mechanisms by which they alter substrates and how it influences anaerobic digestion on a physical and microbiological level.

In Chapter 3, potential pre-treatment methods were used to treat pulp-mill wastewater suitable for anaerobic digestion. The pre-treatment methods were evaluated on their ability to remove recalcitrant components without affecting potential substrates for anaerobic digestion.

In Chapter 4, three bench-scale upflow anaerobic sludge blanket (UASB) reactors were designed, constructed and commissioned for further evaluation on the pre-treatment methods.

In Chapter 5 the most suitable pre-treatment methods identified from Chapter 3 were used to treat large batches of pulp-mill wastewater and were used on the UASB reactors to evaluate the real effects and usefulness of pre-treatments for a specific application.

In Chapter 6 the conclusions and recommendations are provided.

CHAPTER 2. PRE-TREATMENT OF PULP-MILL WASTEWATER FOR ANAEROBIC DIGESTION: A LITERATURE REVIEW

2.1 Introduction

Anaerobic digestion is viewed as a balanced ecological process where different groups of microorganisms work together, in the absence of oxygen, to convert organic materials. The organic materials are converted for cell growth and to produce products such as carbon dioxide, methane and hydrogen sulphide (Chernicharo, 2007). Of the many treatment methods available, anaerobic digestion might be highly suitable for treating pulp-mill wastewater because it generates energy and has little impact on the environment (Carrère *et al.*, 2010). With the growing human population and demand for energy, many researchers are attempting to optimise anaerobic digestion through increased degradation, higher methane yields, and decreased volumes of solids disposed (Holm-Nielsen *et al.*, 2009). However, to achieve this aim, certain substrate-related obstacles must be overcome.

Anaerobic digestion can be divided into four metabolic stages: hydrolytic, acidogenic, acetogenic and methanogenic. The hydrolytic stage is often reported as the rate-limiting step (Holm-Nielsen *et al.*, 2009). In hydrolysis, large substrates are broken down into smaller, more digestible forms; hence, larger and more complex substrates may take longer to hydrolyse. Many pre-treatment methods for substrates have been studied to increase the rate of hydrolysis (Carlsson *et al.*, 2012). However, the overall performance of anaerobic digestion on a substrate level depends on other substrate-related obstacles as well. The obstacles to overcome include, mechanical issues, such as large solids or dry substrate materials that hinder efficient mixing; the presence of recalcitrant structures that offer limited availability for degradable compounds; the presence of toxic compounds, that hinders all metabolic stages; complex and large substrate particles, that slows down the hydrolytic stage (Carlsson, 2015).

These substrate-related obstacles can be amended by the dilution of dry substrates, removal of unwanted materials, particle-size reduction, and enhancement of complex structures through various pre-treatment methods (Carlsson, 2015). Pre-treatment methods should be carefully selected because some may remove degradable organic material or form inhibitory compounds.

Every wastewater stream in the pulp industry consists of various compositions of substrates. Therefore, several pre-treatments can be used to overcome substrate-related obstacles and achieve improved anaerobic digestion. Monitoring pre-treatment at the substrate level can indicate the extent to which anaerobic digestion can be improved, although the true effects on anaerobic digestion will remain unknown. Pre-treated substrates are usually fed into a batch-

anaerobic process to collect data such as the methane yield and consumption of organic matter (Pokhrel and Viraraghavan, 2004). This information provides an idea of the usefulness of the pre-treatment. However, the results of every pre-treatment are tied to the specific reactor used and to the process conditions (Carlsson, 2015). Therefore, using a pilot-scale reactor and measuring all the chemical and energy inputs may give the best indication of whether a pre-treatment is environmentally and financially sustainable.

2.2 Anaerobic treatability of pulp-mill wastewater

Every wastewater stream in the pulp and paper industry is unique due to the wood species, pulping process, bleaching sequences and chemicals used in each mill. Variations in these factors increase the diversity of available pre-treatment options and anaerobic-digestion configurations. To identify the pre-treatment options that could enhance anaerobic digestion, the principles of anaerobic digestion and substrate-related obstacles must be understood. The effects of the constituents of diverse pulp-mill wastewater streams on anaerobic digestion must also be understood.

2.2.1 Microbiology of anaerobic digestion

Anaerobic digestion generally consists of an organic-matter breakdown phase and a product-forming phase (Lettinga *et al.*, 1996). During the breakdown phase, anaerobic bacteria convert complex substrates into – mainly – volatile fatty acids (VFAs) and hydrogen gas, that are further converted into methane and carbon dioxide gas during the product-forming phase. These two phases can be subdivided into four metabolic stages, represented in Figure 2.1.

Microorganisms are unable to digest complex structures. Therefore, in the first stage of anaerobic digestion (hydrolysis), fermentative bacteria hydrolyse complex organics through exoenzymes into smaller, simpler molecules that can be absorbed by the cell membranes of fermentative bacteria (Chernicharo, 2007, Henze *et al.*, 2008). The products formed during the first stage include monosaccharides, amino acids, fatty acids, alcohols and hydrogen sulphide (Figure 2.1). According to Lettinga *et al.*, (1996) the hydrolysis stage is usually slow and depends on temperature, pH, residence time, particle size and the nature of substrate. Complex polymeric substrates such as cellulose, hemicellulose and lignin take longer to hydrolyse. By degrading complex substrates and removing recalcitrant compounds, the rate of hydrolysis may be increased.

In the second stage (acidogenesis), fermentative bacteria absorb the simple molecules from stage 1 and convert them into new bacterial cells, carbon dioxide, VFAs, ammonia and alcohol. In the third stage (acetogenesis), acetogenic bacteria oxidise the acidogenic products into carbon

dioxide, VFAs and hydrogen. During acetogenesis, abundant hydrogen is produced, which decreases the pH. The reduced pH is managed either by methanogenic bacteria, which use VFAs and hydrogen to form methane, or by the chemical reaction of hydrogen, carbon dioxide and carbon to form propionic acids (Chernicharo, 2007).

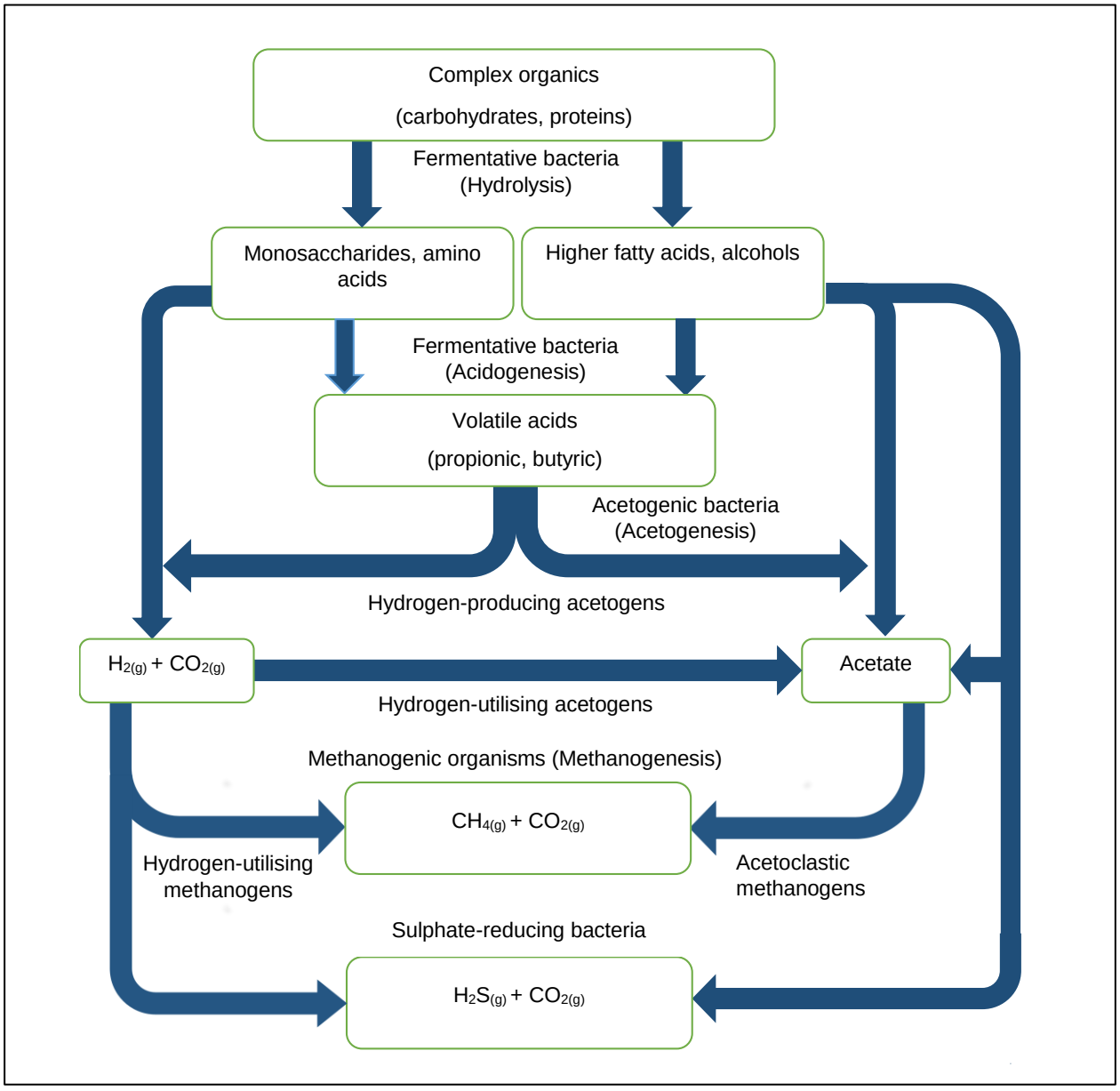

Figure 2.1: Microbial groups and pathways in anaerobic digestion with sulphate reduction, (adapted from Chernicharo, (2007)).

The final metabolic stage of anaerobic digestion (methanogenesis) is governed by mesophilic and thermophilic methanogens. The optimal operating temperature range for mesophilic methanogens is 20°C to 35°C, and for thermophilic methanogens it is 45°C to 55°C (Ferrer *et al.*, 2008). Methanogenic bacteria produce methane and carbon dioxide either by converting VFAs through acetoclastic methanogens or by converting hydrogen and carbon dioxide through

hydrogenotrophic methanogens. Most of the methane produced during anaerobic digestion is a product of VFA conversion by methanogenic microorganisms. When enough methanogenic microorganisms are present, and the environmental conditions are right, VFAs are converted as fast as they are formed and do not exceed the buffering capacity of the natural alkalinity present. However, if conditions are unfavourable and insufficient methanogenic microorganisms are present, VFAs are not converted rapidly and the pH drops. Fortunately, anaerobic processes can acclimatise and produce sufficient methanogenic biomass under the right circumstances to restore the ecological balance.

In anaerobic reactors that treat sulphate-containing wastewater, sulphate-reducing bacteria (SRB) can use sulphate or sulphite as electron acceptors during the oxidation of organic molecules (Lettinga *et al.*, 1996). The molecules that SRB can use as a substrate include VFAs, methanol, ethanol and most polysaccharides. In wastewater containing high concentrations of sulphate or sulphite, many compounds formed during the metabolic stages (Figure 2.1) are consumed. As a result, SRB competes with the fermentative, acidogenic, acetogenic and methanogenic microorganisms, which restricts the amount of methane produced. Reducing the sulphite and sulphate content during pre-treatment may increase the effectiveness of an anaerobic reactor.

2.2.2 Pulp-mill wastewater constituents and digestibility

Anaerobic digestibility is generally measured in terms of anaerobic toxicity assays (ATAs), biochemical methane potential (BMP) and reduction of chemical oxygen demand (COD). The ATA is measured in terms of inhibition indices (*i*), where a value of 1 indicates no inhibition and larger values reflect increasing inhibition of anaerobic microorganisms. The ATA is merely an indication of how toxic a wastewater stream may be, because the assays are generally conducted for unacclimatised cultures (Hall and Cornacchio, 1988). The BMP tests are also merely an indication of the amount of methane that can be produced. Measuring the toxicity of substrates and methane yield in continuous-flow reactors allows bacterial biomass to acclimatise and may yield different results.

The COD in pulp-mill wastewater generally consists of alcohols, VFAs, sugars, chlorinated organics, lignin, resin acids and phenolic compounds (Meyer and Edwards, 2014). Alcohols, VFAs and sugars are easily digested by anaerobic microorganisms, whereas lignin is difficult to digest. Chlorinated organics, resin acids and phenolic compounds are toxic to anaerobic microorganisms. The COD parameter is used to simplify measurements as it would be difficult to measure each of these compounds regularly. If the COD value is low, the quantity of biodegradable compounds is low no matter what the composition. If the COD value is high, there

may be many or few biodegradable compounds, depending on the amount of toxic and recalcitrant compounds present.

Comparing COD reduction and methane yield in an anaerobic process can indicate how efficiently the process works; it also roughly indicates the concentration of digestible compounds in the COD value. However, this value is merely an indication because methane yield also depends on the presence of toxic compounds, temperature, pH, alkalinity and nutrient availability. If all the COD is converted to methane, a theoretical methane-yield coefficient of 0.35 m^3/kg_{COD} can be obtained (Chernicharo, 2007). However, methane-yield coefficients as high as 0.40 m^3/kg_{COD} have been reported (Hall and Cornacchio, 1988, Meyer and Edwards, 2014). Coefficients that are higher than the theoretical maximum could be obtained if no temperature correction factor is used, or if biomass accumulate in the reactor, thus yielding higher methane volumes.

Of all the wastewater streams in pulp and paper mills, only a few have been used to date for full-scale anaerobic treatment because low COD values render treatment uneconomical (Habets and Driessen, 2007). Approximately two-thirds of all anaerobic reactors used in the pulp and paper industry are used to treat paper-mill wastewater (Habets and Driessen, 2007). Such wastewater is preferred as it generally has high COD concentrations and low concentrations of inhibitory compounds; in some cases, it also contains easily digestible starch (Driessen *et al.*, 2000). In pulp mills, anaerobic reactors are mainly used to treat condensate streams from chemical pulping and alkaline peroxide mechanical pulping (Meyer and Edwards, 2014).

In a comprehensive study by Hall and Cornacchio, (1988), 43 streams from 21 pulp mills in Canada were characterised and tested for their anaerobic treatability. The study should still be relevant today since the pulping processes have not changed much since then. The anaerobic treatability was determined in terms of ATAs, BMP and COD reduction. The tested wastewater included streams from kraft, sulphite, thermomechanical and non-sulphur semi-chemical mills. Remarkably, 21 of the tested wastewater streams showed adequate COD concentrations for anaerobic treatment, with little inhibition, even though the anaerobic microorganisms were unacclimatised to their specific environments. The sulphite and non-sulphur semi-chemical pulping effluents had the highest digestibility and the bleaching effluents had the lowest digestibility. The low digestibility of bleaching effluents might be the result of low COD concentrations and high toxicity. Modern high-rate reactors can treat effluents having low COD concentrations, whereas combining streams that have low and high COD concentrations could improve the digestibility (Meyer and Edwards, 2014).

2.2.2.1 Chemical pulping effluents

The main chemical pulping methods focus on kraft and sulphite (Sjöström, 2013), and the effluent from these processes are generally the only chemical pulping effluents treated anaerobically (Pokhrel and Viraraghavan, 2004). Kraft pulping uses sodium hydroxide and sodium sulphide, whereas the sulphite process uses sulphites or bisulphites to remove lignin from the biomass. Most effluent streams from these two processes contain high concentrations of sulphurous compounds (Meyer and Edwards, 2014).

Condensate streams from the digesters and evaporators contain lower concentrations of sulphurous compounds and are generally the only streams from either of these processes that are treated anaerobically (Driessen *et al.*, 2000). Kraft condensates contain up to 620 mg/L sulphide and sulphite condensates contain up to 800 mg/L sulphite (Table 2.1). High COD content, easily digestible alcohols and organic acids in these two condensate streams might contribute to their widespread use in anaerobic reactors. The COD content of kraft condensates can be up to 14 000 mg/L, with ethanol and methanol being main contributors (Table 2.1). Sulphite condensates contain high concentrations of acetic acid and methanol, with COD concentrations up to 27 000 mg/L (Table 2.1). Recalcitrant compounds, such as phenols and lignin, are also present in the effluent streams of both condensate streams. Removing these compounds may improve the rate of anaerobic hydrolysis and serve as an additional revenue stream.

Table 2.1: Composition of condensate effluents from chemical pulping processes and the suitability for anaerobic digestion.

Wastewater type	Composition		Concentration (mg/L)	References	COD removed (%)	Methane yield coefficient (m^3/kg_{COD})
Kraft evaporator condensates	COD		600 – 14 000	a,b,c,d,e	60 – 95	0.20 – 0.35
	Substrate	Ethanol	0 – 200	a,c		
		Methanol	300 – 3 000	a,c		
	Inhibitor	Sulphides	0 – 620	a,c,d		
		Sulphites	3 – 10	c,d		
		Resin acids	28 – 230	c		
		Phenols	1 – 45	c		
Kraft combined condensates	Substrate	Methanol	1 300	c,f	60	0.21 – 0.37
Sulphite evaporator condensates	COD		3 000 – 27 000	b,e,f,g	87 – 90	0.28 – 0.36
	Substrate	Acetic acid	2 000	f		
		Methanol	0 – 250	f		
		Furfural	0 – 250	f		
	Inhibitor	Sulphite	450 – 800	f,g		
		Resin acids	3.2 – 9.3	f,g		
		Lignin	10 000	g		

References: a = Qiu *et al*., (1988); b = Driessen *et al*., (2000); c = Dufresne *et al.*, (2001); d = Xie *et al.*, (2010); e = Hall and Cornacchio, (1988); f = Meyer and Edwards, (2014); g = Ali and Sreekrishnan, (2001)

Kraft and sulphite condensate streams have lower COD values than those of other chemical effluents, such as pre-hydrolysis liquor (PHL), spent sulphite liquor, and sulphite pulping effluent, which has COD concentrations up to 115 000 mg/L (Table 2.2). However, most of the COD in kraft and sulphite condensates consists of easily digestible compounds, with few inhibiting compounds. Higher COD concentrations and high flow volumes, which occur in pulp-mill wastewater, mean that larger anaerobic digesters must be built. Therefore, higher COD concentration does not necessarily make a wastewater stream more treatable.

Kraft PHL is gaining attention as a suitable substrate for anaerobic digestion (Råmark *et al.*, 2012) as it may contain high concentrations of acetic acid, furfural, and monomeric carbohydrates (Table 2.2). Kraft PHL also contains high concentrations of lignin, which can make anaerobic treatment less attractive. However, suitable pre-treatment may render PHL more suitable for anaerobic treatment. Other effluent streams from kraft mills, including contaminated hot water, woodroom effluent and brown-stock decker filtrate, may also be suitable for anaerobic digestion, with high COD removal and methane yields of 0.20 to 0.34 m^3/kg_{COD} (Table 2.2).

Table 2.2: Characterisation of suitable chemical pulping effluents for anaerobic digestion.

Wastewater type	Composition		Concentration (mg/L)	References	COD removed (%)	Methane yield coefficient (m^3/kg_{COD})
Kraft woodroom	COD		1 000 – 7 500	a,b	40 – 90	0.35 – 0.40
Kraft contaminated hot water	COD		3 900	a,b	88	0.34
Kraft brown stock decker	COD		700	a,b	86	0.20
Kraft PHL		COD	14 000– 53 000	c	32 – 90	0.30
	Substrate	Carbohydrates	1 140	c		
		Furfural	7 000 – 10 400	c		
		Acetic acid	11 000– 25 000	c		
	Inhibitor	Lignin	9 600 – 12 000	c		
Spent sulphite liquor	COD		24 000 – 115 000	d,e	24 – 52	0.00 – 0.31
	Inhibitor	Resin acids	40	e		
		Sulphate	5 100	d		
		Sulphite	4 800	b,d,e		
Sulphite pulping effluent	COD		6 300 – 48 000	a,b,e	29 – 38	0.14 – 0.30

References: a = Hall and Cornacchio, (1988); b = Meyer and Edwards, (2014); c = Debnath *et al*., (2013); d = Bajpai, (2000); e = Ali and Sreekrishnan, (2001)

Spent sulphite liquor and sulphite pulping effluent from the sulphite pulping process may also be suitable for anaerobic digestion, with specific methane yields of up to 0.31 m^3/kg_{COD} (Table 2.2). However, COD removal is minimal, with the removal being no more than 52%. The low COD removal may be attributed to high concentrations of sulphurous compounds.

2.2.2.2 Bleaching effluents

The anaerobic digestibility, COD removal and methane yield of bleaching effluents vary widely because diverse bleaching methods are implemented. Bleaching is usually done sequentially using various oxidation, extraction and washing stages. The stages involve applying chlorine dioxide (D), sodium hydroxide (E), hydrogen peroxide (P), oxygen (O), sodium hydrosulphite (Y), and ozone (Z) in varying combinations (Sjöström, 2013). Many studies have reported on the anaerobic digestibility of elemental chlorine-free (ECF) bleaching, which is widely implemented and less toxic than other bleaching effluents (Chaparro and Pires, 2011). Removal of up to 90% of COD and methane yield coefficients of up to 0.38 m^3/ kg_{COD} have been reported (Table 2.3).

Bleaching effluents are not usually treated as they contain high concentrations of chlorinated organic compounds, which are generally toxic to anaerobic digestion (Lettinga, 1996). Interestingly, Chaparro and Pires, (2011) reported that the removal efficiencies for adsorbable organic halides (AOX), phenols and residual lignin were between 20% and 45% during anaerobic treatment. The authors stated that digestion of these toxic compounds could be attributed to an acclimatised microbial consortium. Further research on acclimatisation of anaerobic biomass could help to improve the efficiency of anaerobic treatment of bleaching effluents.

Table 2.3: Composition of bleaching effluents and the suitability for anaerobic digestion.

Wastewater type	Composition		Concentration (mg/L)	References	COD removed (%)	Methane yield coefficient (m^3/kg_{COD})
Kraft ECF bleaching	COD		1 100 – 2 400	a,c	46 – 64	0.00 - 0.22
	Inhibitor	Chloride	420 – 700	a		
		AOX	40 – 45	a		
		Phenols	200 – 600	a		
Chlorine bleaching	COD		650 – 1 500	b,c,d	20 – 67	0.00 - 0.38
	Substrate	Methanol	0.0 – 140	b		
		Acetate	0.0 – 20	b		
		AOX	110	b		
		Chloride	1 300 – 1 600	b		
Kraft alkaline bleaching	COD		300 – 4 300	b,d,e	15 - 90	0.00 - 0.14
	Substrate	Methanol	40 – 75	e		
	Inhibitor	AOX	2.6 – 200	e		
		Chloride	1 200 – 1 400	e		
		Sulphate	170 – 250	e		

References: a = Chaparro and Pires, (2011); b = Meyer and Edwards, (2014); c = Vidal *et al*., (1997); d = Hall and Cornacchio, (1988); e = Qiu *et al*., (1988)

2.2.2.3 Mechanical pulping effluents

The aim of mechanical pulping is to make the fibres easier to separate and refine, rather than to remove lignin as in chemical pulping (Sjöström, 2013). Effluents from mechanical pulping often contain high COD concentrations, easily digestible carbohydrates and acetic acid (Table 2.4). High concentrations of resin acids – up to 10 000 mg/L – are generally included, as are other inhibitors, such as sulphate and sulphite (Table 2.4). Most mechanical pulping effluents are suitable for anaerobic digestion with COD removal of up to 70% and methane yield coefficients of between 0.18 and 0.40 m^3/kg_{COD} (Table 2.4).

Table 2.4: Composition of mechanical pulping effluents and the suitability for anaerobic digestion.

Wastewater type	Composition		Concentration (mg/L)	References	COD removed (%)	Methane yield coefficient (m^3/kg_{COD})
Thermo-mechanical-pulping composite	COD		2 000 – 5 000	b	50 – 70	0.20 – 0.60
	Substrate	Carbohydrates	1 200 – 2 700	a		
		Acetic acid	235	a		
		Methanol	25	a		
	Inhibitor	Sulphate	200 – 800	b		
		Peroxide	200 – 700	b		
		Resin acids	0 – 100	b		
Chemi-thermo mechanical pulping	COD		6 000 – 10 400	b,c	40 – 66	0.18 – 0.31
	Substrate	Carbohydrates	1 000	c		
		Acetic acid	1 500	c		
	Inhibitor	Sulphate	500 – 1 500	c		
		Sulphite	50 – 200	c		
		Peroxide	0 – 500	c		
		Resin acids	50 – 500	c		

References: a = Hall and Cornacchio, (1988); b = Habets and De Vegt, (1991); c = Welander and Andersson, (1985)

2.2.2.4 Semi-chemical pulping effluents

Semi-chemical pulping uses a combination of chemical and mechanical pulping methods (Sjöström, 2013). Less lignin is removed, and higher pulp yields are obtained compared to chemical pulping. Commonly used methods are neutral sulphite semi-chemical (NSSC) pulping and soda pulping (Sjöström, 2013). The NSSC pulping process involves impregnation with sulphite and carbonate, followed by mechanical refining, whereas soda pulping uses sodium hydroxide during cooking (Sjöström, 2013). In NSSC and soda pulping, hardwood species are generally used; therefore, the concentration of inhibitory compounds such as resin acids is typically low (Meyer and Edwards, 2014). Semi-chemical effluents are highly digestible, with COD removal efficiencies of up to 80% and methane yields between 0.20 and 0.35 m^3/kg_{COD} (Table 2.5).

Table 2.5: Composition of semi-chemical pulping effluents and the suitability for anaerobic digestion.

Wastewater type	Composition		Concentration (mg/L)	References	COD removed (%)	Methane yield coefficient (m^3/kg_{COD})
NSSC composite	COD		1 800	a,b,c	50 - 80	0.18 - 0.28
	Substrate	Carbohydrates	610	b		
		Acetic acid	54	b		
		Methanol	9	b		
	Inhibitor	Lignin	500	b		
NSSC spent liquor	COD		28 000 - 40 000	c	70	0.38 - 0.40
	Substrate	Carbohydrates	6 210	c		
		Acetic acid	3 200	c		
		Methanol	90	c		
		Ethanol	5	c		

References: a = Hall and Cornacchio, (1988); b = Arshad and Hashim, (2012); c = Lee *et al*., (1989)

2.3 Pre-treatment options for anaerobic digestion

Pollution of wastewater streams can be minimised by changing outdated internal processes to the best available technology (BAT). An example is chlorine pulp bleaching sequences, which previously released abundant AOX into aquatic systems (Kamali and Khodaparast, 2015). These bleaching sequences have been changed in recent years to elemental chlorine-free (ECF) or total chlorine-free (TCF) bleaching processes, which cause far less environmental harm. Changing internal processes may be seen as a pre-treatment method for wastewater-treatment technologies such as anaerobic digestion. However, changing internal processes is not always viable and wastewater treatment remains essential.

In pulp mills, pre-treatment methods for anaerobic digestion usually focus on waste sludge due to its high content of biodegradable compounds. A few of these methods may be efficient in treating wastewater streams as they contain relatively little biodegradable matter. The pre-treatment methods investigated can be divided into three categories: physical, physicochemical and enzymatic. These pre-treatment methods are based on overcoming the substrate-related obstacles.

2.3.1 Physical pre-treatment

Physical pre-treatments include mechanical, thermal and ultrasonic methods. These treatment methods enhance the hydrolysis rate and anaerobic degradability of sludge, primarily (Mudhoo, 2012, Sawayama *et al.*, 1997). Mechanical pre-treatment is aimed at reducing particle size to

render biodegradable components accessible to microorganisms. Pulp-mill wastewater is already broken down into small particles, which means mechanical pre-treatment may not be applicable.

Thermal pre-treatment increases the partial solubilisation of substrates, improving anaerobic digestion significantly (Appels *et al.*, 2010). The optimal temperature range for partial solubilisation, according to Mudhoo, (2012), is 160°C to 180°C. The optimal operating temperature ranges for the two types of methanogenic species present in anaerobic reactors, namely mesophiles and thermophiles, are 20°C to 35°C and 45°C to 55°C, respectively (Ferrer *et al.*, 2008). Therefore, it can be deduced that the substrate requires heating and cooling before digestion, which in turn requires ample heat energy. The heat may be obtained from surplus mill streams to make this treatment more economical.

Ultrasound is defined as any sound wave having a frequency higher than 20 kHz (Mudhoo, 2012), whereas microwaves comprise electromagnetic waves (Meyer and Edwards, 2014). When sound or magnetic waves are passed at a high enough frequency through a medium, they generate gas bubbles, which continually expand and contract until they implode, causing extreme pressure and temperature at the implosion site (Wu *et al.*, 2001). The intensity (amplitude) is an important factor in causing the implosion. If the intensity is not high enough, the bubbles oscillate without imploding (Mudhoo, 2012, Wu *et al.*, 2001). When an implosion does occur, it ruptures cell walls and increases the amount of soluble COD. Increased soluble COD in turn increases the amount of VFAs released during anaerobic digestion, increasing the methane yield (Saha *et al.*, 2011).

Ultrasound and microwave pre-treatment in the pulp industry have mostly been studied regarding waste sludge. Saha *et al.*, (2011) reported that for waste sludge with relatively low anaerobic digestibility, ultrasound pre-treatment increased the methane yield by 80%, whereas microwave treatment increased it by 90%. However, ultrasound and microwave pre-treatment of pulp-mill wastewater for anaerobic digestion may be uneconomical, as such wastewater contains much less organic compounds that can be broken down to generate methane gas.

2.3.2 Physicochemical pre-treatment

Physicochemical processes are applied to remove suspended solids, floating particles, colour and toxic components. The methods used are sedimentation, flotation, coagulation, precipitation, membranes, adsorption and oxidation (Pokhrel and Viraraghavan, 2004).

2.3.2.1 Coagulation and precipitation

Coagulation is a chemical method used to neutralize particles and bind them together to form flocs. These particles form macromolecules, which eventually become heavy enough to settle out

of suspension. In the pulp industry, coagulation is mainly used in tertiary wastewater treatment (Pokhrel and Viraraghavan, 2004). Various coagulants have been tested and have been shown effective for reducing total suspended solids (TSS), COD, AOX, phenolic compounds and toxicity. Most coagulants used in wastewater treatment are aluminium-based or iron-based. Several studies have shown that these coagulants bind with amino acids, long-chain fatty acids and phenolic compounds, without affecting sugars (Dentel and Gossett, 1982, Razali *et al.*, 2011, Renault *et al.*, 2009). Although the coagulants reduce inhibitory compounds, the ferric and aluminium residues from these coagulants inhibit microorganisms (Yang *et al.*, 2010). Organic-based coagulants, such as polydiallyldimethylammonium chloride (PolyDADMAC) or chitosan, may be effective without adding inhibitory compounds to solution (Yang *et al.*, 2010). However, Saeed *et al.*, (2012) showed that PolyDADMAC and chitosan were not selective towards phenolic compounds, and removed some of the biodegradable components such as hemicellulose as well.

Zhang *et al.*, (1999) compared various coagulants, such as $Al_2(SO4)_3$ and chitosan, alone and in conjunction with the enzyme horseradish peroxidase (HRP). They found that chitosan was the most effective for removing total organic carbon (TOC), AOX and colour from bleaching effluents, and that a higher removal percentage was obtained when chitosan was combined with HRP. Coetzee *et al.*, (2015) found a 12.8% reduction of lignosulphonate in a PHL stream using 2 mg/L chitosan. However, they may have used too little chitosan and the chitosan was not activated.

Yu *et al.*, (2012) used $Ca(OH)_2$ to remove lignosulphonate from a sulphite stream. The results showed that higher concentration, longer residence time and higher temperatures all enhanced precipitation, with 26% lignosulphonates being removed. However, increased concentration also increased hemicellulose removal. By contrast, Coetzee, (2012) reported 90% lignosulphonate removal from a spent sulphite stream using $Ca(OH)_2$, with little hemicellulose removal. However, recovery of lignosulphonate from the formed precipitate required re-acidification, making the process expensive. Treatment with $Ca(OH)_2$ may also add inhibitory components and raise the pH to an unsuitable range for anaerobic digestion. Treatment with $Ca(OH)_2$ may be an option after anaerobic digestion, to reduce COD concentrations.

2.3.2.2 Membrane technologies

Membrane technologies have been widely used in the pulp industry but have been hindered by the economic costs of high input pressures and retentate disposal (Greenlee *et al.*, 2010). By using membranes with larger pores, the frequency of retentate disposal can be reduced and lower input pressures are needed. By increasing the pore size, certain inhibitory compounds are no longer retained. Inhibitory compounds such as lignin can be polymerised to sizes large enough to be retained, whereas most anaerobic substrates – such as sugars – are small enough to pass

through. Ko and Fan, (2010) reported a 60% increase in COD reduction by using laccase polymerization before ultrafiltration, with little effect on the sugar content.

2.3.2.3 Adsorption

Adsorption is a simple method having low operational costs; it is viewed as a relatively new alternative for chemical treatments (Fatehi and Chen, 2016). Various materials are used for adsorption, with activated carbon (AC) being the most common due to its high adsorptive capacity (Namasivayam and Kavitha, 2002). However, production of AC is expensive because of the high temperatures required, and AC is not selective towards only one compound (Das and Patnaik, 2000). Fatehi and Chen, (2016) showed that hemicellulose and furfural were adsorbed on AC. The removal of hemicellulose and furfural as a substrate lowers the methane gas yield in an anaerobic digester. Also, having different compounds adsorbed to AC makes the desorption of valuable compounds difficult. If inexpensive commercial adsorptive materials that are selective towards certain inhibitory compounds are used, this may be a viable option before anaerobic digestion. In a recent study by Jang *et al.,* (2018), adding biochar to the anaerobic reactor stabilised the pH and improved methane gas yield by up to 40%.

2.3.3 Enzymatic pre-treatment

Enzymes can be defined as organic molecules present in living cells, which act as catalysts to change the chemical reactions within substances (Smith, 2004). The advantage of enzymatic treatment over conventional treatments is its operability across varying contaminant concentrations, temperatures and pH values. In the enzymatic process, less sludge is generated (Duran and Esposito, 2000); in addition, enzymes are biodegradable and do not form part of the final product (Smith, 2004).

Commercial enzymatic treatment of waste has received considerable attention due to its ability to function *in vitro* and to target specific substrates (Duran and Esposito, 2000). Commercial enzymes frequently investigated for pulp-mill wastewater treatment include oxidases for polymerisation reactions and cellulases for hydrolysis of complex organic molecules (Duran and Esposito, 2000). Given the increased interest and technological advances, enzymes have become more effective and widely available (Smith, 2004) and may become more so as technology improves further.

2.3.3.1 Oxidase pre-treatment

Two types of oxidative enzymes, peroxidase and laccase, have been studied in the pulp industry, mainly due to their ability to degrade or polymerise lignin (Morozova *et al.*, 2007). Peroxidase

catalyses the oxidation of phenols in the presence of hydrogen peroxide, whereas laccase uses oxygen only. Because hydrogen peroxide is toxic for anaerobic microorganisms, laccase could be more suitable as a pre-treatment option for anaerobic digestion.

Laccase is a member of the blue copper oxidase enzyme group; these enzymes transfer an electron from a substrate molecule to an oxygen molecule while reducing the oxygen molecule to a water molecule (Morozova *et al.*, 2007) (Figure 2.2). A free radical is formed on the substrate molecule, creating a potential site for reaction with other radicals. The process is aided by the four copper atoms in the active site of the laccase protein. One copper atom facilitates the electron removal while the other three copper atoms accept the electron and reduce the oxygen molecule (Morozova *et al.*, 2007). The oxidation of a substrate depends on whether the substrate will fit in the active site of the enzyme as well as the redox potentials of the enzyme and substrate (Riva, 2006). Laccases have a wide range of redox potentials and, therefore, a wide range of substrates can be oxidised. These substrates include phenols and aromatic amine groups (Strong and Claus, 2011).

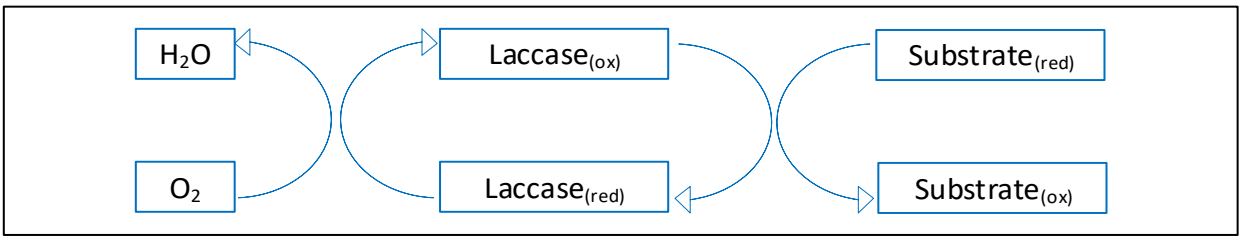

Figure 2.2: Laccase direct oxidation (Riva, 2006)

Various mediators can be used to oxidise an even wider range of substrates. These mediators are oxidised by enzymes, creating free radicals that can oxidize other molecules (Figure 2.3) (Riva, 2006). Previous studies have indicated that 1-hydroxybensotriazol (HBT) or 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) are the best options for mediators. However, they are expensive (Morozova *et al.*, 2007, Strong and Claus, 2011) and may be environmentally hazardous.

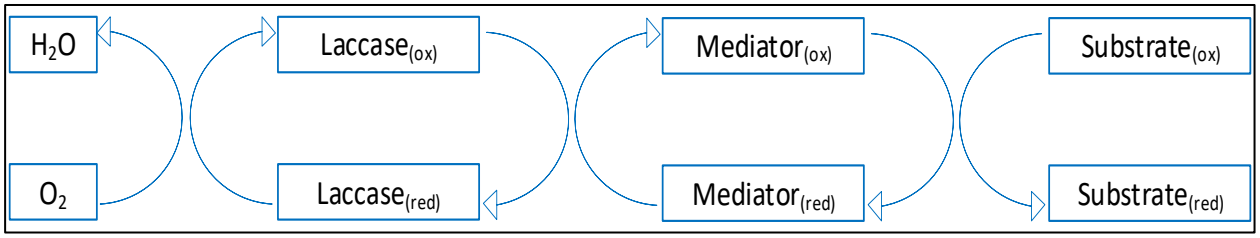

Figure 2.3: Laccase mediator oxidation (Riva, 2006)

After laccase treatment, polymerisation, depolymerisation or internal reactions can occur (Strong and Claus, 2011). Many studies have reported that laccase polymerises phenol-containing compounds, without generating undesirable by-products (Areskogh *et al.*, 2010a, Kim *et al.*, 2009, Ko and Fan, 2010). Polymerised phenol-containing compounds could be removed by physicochemical methods or they could settle out of suspension if the molecular weight is increased enough. The extent to which laccase polymerises its substrate depends on factors such as operating temperature, pH, type of laccase, type of mediator and the laccase concentration used (Strong and Claus, 2011, Morozova *et al.*, 2007).

Wang *et al.*, (2014) studied the effect of temperature, pH, residence time and laccase concentration on lignin removal. The objective of that study was to optimize the removal of lignin from kraft PHL dissolving pulp by laccase-induced polymerisation. The lignin was separated using a nylon membrane. The optimal conditions were found to be as follows: laccase concentration 2 U/mL, residence time of 3 h, temperature of 36°C and pH of 3.6. At these conditions, 55% of lignin was removed, which increased to 65% when PolyDADMAC was added. Although these results were unique to the substrate, the study gave viable ranges for environmental parameters.

Areskogh *et al.*, (2010a) tested the laccases NS 51002 (*Trametes villosa*) and NS 51003 (*Myceliophthora thermophila*) with an optimum pH of 5 and 7.5 respectively. Four lignosulphonate salts were tested, with concentrations ranging from 1 g/L to 100 g/L. Various enzyme concentrations were used, without a mediator present. In all cases, an increase in molecular weight and polydispersity and a decrease in phenolic content were observed. The NS 51002 formulation increased the molecular weight 23-fold at a residence time of 4 h. This enzyme also oxidised non-phenolic compounds and thus increased the molecular weight of lignin more than NS 51003 did. Areskogh *et al.*, (2010a) also found that higher concentrations of lignosulphonate and enzymes yielded higher lignin molecular weights. In a similar study by Ko and Fan, (2010), NS 51003 was used to treat a sample of wastewater at 30°C, pH of 7.5 and residence time of 1 h. The particle size increased from 900 Da (Dalton) to 1300 Da.

Kim *et al.*, (2009) showed that the laccase NS 51003 preferentially polymerised calcium lignosulfonates by binding phenoxy radicals. Interestingly, a period of depolymerisation was observed, followed by a period of polymerisation. A temperature of 50°C and pH of 5 were used as these were determined to be the optimal conditions for NS 51003 laccase activity. The exact increase in molecular weight and degree of polymerisation were not determined.

Li *et al.*, (2010) used laccase to remove petroleum oil from wastewater. A laccase concentration of 3 U/mL, residence time of 6 h, temperature of 30°C and pH of 6 were determined as the optimal

conditions, with 69% of the petroleum oil being removed. Adding chitosan to the solution increased the petroleum oil removal by 13%.

These studies documented that no significant increase in molecular weight was observed when phenolic compounds occurred at a low concentration. In the study by Areskogh *et al.*, (2010a), significant increases in molecular weights were observed only at phenolic-compound concentrations above 10 g/L. Pulp-mill wastewater streams, such as sulphite evaporator condensates and kraft PHL, may thus yield significant increases in lignin molecular weights.

2.3.3.2 Cellulase pre-treatment

Cellulase is a substrate-specific enzyme produced by the bacteria and fungi responsible for hydrolysis in cellulose. The main product of cellulose hydrolysis is the highly digestible glucose (Champagne and Li, 2009). Therefore, wastewater containing high concentrations of cellulose could be an energy source for anaerobic digestion if treated by cellulase. Although bacterial cellulases have high specific activity, they cannot be produced in large amounts (Champagne and Li, 2009). Of all cellulases, the fungal laccase from *Trichoderma reesei* has been studied the most extensively, as it can be produced in large amounts (Sun and Cheng, 2002).

Three main groups of cellulase are involved in the hydrolysis of cellulose: endoglucanase (endo-1,4-D-glucanohydrolase or EC 3.2.1.4); exoglucanase or cellobiohydrolase (EC 3.2.1.91); and β-glucosidase (EC 3.2.1.21). Endoglucanase creates free chain-ends in regions of low crystallinity. Exoglucanase removes cellobiose units from the free chain-ends, and β-glucosidase reduces cellobiose to glucose (Sun and Cheng, 2002, Coughlan and Ljungdahl, 1988). These enzymes work synergistically to reduce cellulose to glucose. Commercial cellulases are highly effective and usually comprise a mixture of these enzymes (Sun and Cheng, 2002). To improve the rate and yield of hydrolysis, factors such as substrate concentrations, reaction conditions and end-product inhibition should be considered.

2.3.3.2.1 Subtrate concentration

Substrate concentration is a main factor that influences the initial rate and yield of enzymatic hydrolysis. At a low concentration of substrate, an increase in substrate may increase the rate and yield of hydrolysis because more points of attack are available (Cheung and Anderson, 1997). Conversely, a high concentration will result in substrate inhibition, lowering the rate and yield of hydrolysis (Huang and Penner, 1991). The rate and yield of hydrolysis also depend on the structural features of the cellulose, such as its crystallinity and degree of polymerization.

2.3.3.2.2 Blend of cellulases

The use of cellulase blends from different microorganisms and other enzymes has been studied extensively to improve functionality (Beldman *et al.*, 1988). For example, the addition of β-glucosidase to cellulase from *T. reesei* achieved more saccharification than cellulase alone (Excoffier *et al.*, 1991). The β-glucosidase enzyme improved hydrolysis by degrading cellobiose, which is an inhibitor of cellulase activity.

Chen *et al.*, (2008) hydrolysed pre-treated maize straw with 20 FPU/g substrate cellulase from *T. reesei* at a residence time of 60 h. After 48 h of hydrolysis, a 65.9% cellulose yield was observed, with little increase after 48 h. Abundant cellobiose existed in the hydrolysate, which inhibited the cellulase reaction. By increasing the β-glucosidase concentration from 1.64 CBU/ g substrate (cellobiase units) to 10 CBU/g substrate, an increase in hydrolysis yield of 15.3% was achieved.

2.3.3.2.3 Surfactants

Hydrolysis by cellulase on cellulose consists of three steps: adsorption of cellulase onto the cellulose surface, degradation of cellulose to monomeric sugars, and the desorption of cellulase (Sun and Cheng, 2002). Cellulase activity is partially decreased during hydrolysis due to irreversible adsorption (Converse *et al.*, 1989). Irreversible adsorption can be minimised by using surfactants that modify the surface of cellulose (Sun and Cheng, 2002). Non-ionic surfactants are believed to be more suitable for enhancing hydrolysis than anionic and cationic surfactants due to the inhibitory effects observed (Ooshima *et al.*, 1986). Using a non-ionic surfactant such as Tween 80 improved the rate of hydrolysis on newspaper by 33% (Castanon and Wilke, 1981). Chen *et al.*, (2008) observed an increase in hydrolysis yield from 81.2% to 87.3% by adding 5 g/L Tween 80 to a cellulase treatment. Other non-ionic surfactants, such as Tween 20, Pluronic F68 and F88, have also yielded significant increases in hydrolysis (Wu and Ju, 1998).

2.3.3.3 Hemicellulase pre-treatment

Hemicelluloses are heterogeneous polymers of pentose, hexose and acids (Saha, 2003). Pentose sugars include xylose and arabinose, whereas the hexose sugars include mannose, glucose and galactose. Hemicellulose from hardwood contains mainly xylans, whereas softwood hemicellulose contains mainly glucomannans (McMillan, 1994, Saha, 2003). Hardwood cellulose consists of a xylopyranose backbone. Besides xylose, xylans also consist of arabinose and acids such as acetic, glucuronic, ferulic and p-coumaric acid (Saha, 2003). For total degradation of xylan, enzymes such as endo-β-1,4-xylanase, β-xylosidase and accessory enzymes such as *ρ*-coumaric acid esterase, ferulic acid esterase, α-L-arabinofuranosidase, α-glucuronidase and acetylxylan esterase are required to hydrolyse the substituted xylans (Saha, 2003).

Hemicellulose has a more complex structure than cellulose and requires more enzymes for its complete degradation. However, hemicellulose does not form the tightly packed crystalline structure of cellulose and is thus easier to hydrolyse enzymatically (Gilbert and Hazlewood, 1993). Commercial hemicellulases usually contain a blend of enzymes and can thus effectively degrade most hemicelluloses (Saha, 2003). Only for optimisation purposes would the addition of individual enzymes be required, which could increase the cost.

2.3.3.4 Cost of enzymes

Enzyme prices have decreased over the past decades. For example, bulk quantities of enzymes for most food applications are now 20 to 35% cheaper than in the mid-1970s (Smith, 2004). More specialised enzymes have increased in use because of improved production methods. Further large-scale application of enzymes will be achieved only if their costs continue to decrease. Recombinant DNA technologies and improved fermentation methods and downstream processing can increasingly reduce production costs, making high-cost enzymes more competitive with other chemical alternatives (Cherry and Fidantsef, 2003).

When enzymes are used as bulk additives, only one or two kg are usually required to react with 1 000 kg of substrate (Smith, 2004). The cost of the enzyme should then be between R50 and R400 per kg, or 10 to 14% of the value of the end-product. Such enzymes are usually sold in liquid formulations and are rarely purified. In contrast, analytical-grade enzymes will generally be used in mg or µg quantities and can cost up to R1 000 000 per kg (Smith, 2004).

2.4 Upflow anaerobic sludge blanket reactors

Treatment with anaerobic reactors is largely applied to animal waste, crop residue, sewage sludge and effluent from food and beverage industries. These waste types have high COD values and low toxicities, yielding high volumes of methane gas (Chernicharo, 2007). Anaerobic treatment of these wastes is thus economically feasible. By comparison, pulp-mill wastewaters generally have lower COD values and are more toxic. Compounding the problem, large volumes of pulp-mill wastewater have to be treated. However, improved technology and anaerobic reactors that can treat large flow volumes in short retention times have made the anaerobic treatment of pulp-mill wastewater more popular (Habets and Driessen, 2007).

The most widely used high-rate anaerobic reactors in pulp and paper mills are the upflow anaerobic sludge blanket reactors (UASBs) (Figure 2.4) and improved versions thereof, such as the expanded granular sludge bed (EGSB) reactor (Habets and Driessen, 2007). These reactors treat large volumes of wastewater in short periods due to long solid retention times (SRTs) and short hydraulic retention times (HRTs). The long SRTs are necessary because of the slow

bacterial growth rates and hydrolysis of biomass that are innate to anaerobic digestion (Lettinga *et al.*, 1996). Therefore, the main feature that distinguishes these anaerobic reactors is the balance between SRTs and HRTs (Chernicharo, 2007).

In UASB reactors, granular sludge with diameters of 0.5 mm to 3.0 mm is formed, with exceptional settling capacities that promote short HRTs and long SRTs (Meyer and Edwards, 2014). Because of the size and high concentration of the granular sludge, UASB reactors can handle volumetric loading rates up to five times higher than normal contact processes in completely stirred tank reactors (Habets and Driessen, 2007).

2.4.1 Principles of a UASB reactor

The reactor is usually inoculated with anaerobic biomass, followed by a low upflow feeding rate during the start-up of the reactor. The feeding rate is gradually increased according to the response of the system. After a few months, a concentrated sludge bed of 4% to 10% total solids per volume is formed in the bottom portion of the reactor (Figure 2.4) (Lettinga and Hulshoff Pol, 1991). The sludge consists of anaerobic granules that are 0.5 mm to 3.0 mm in diameter (Els *et al.*, 2005) and have excellent settling characteristics. The granule sizes depend on the type of wastewater being fed in, and on the operational conditions. The start-up period can last as long as 12 months but can be as little as 2 weeks if granulated sludge is used (Els *et al.*, 2005).

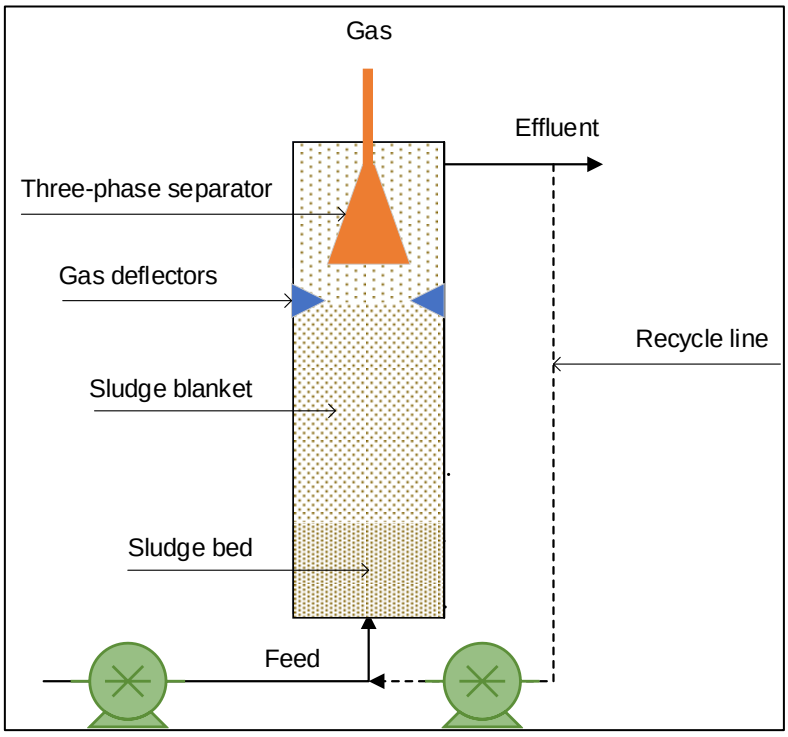

Figure 2.4: Diagrammatic representation of the design of an upflow anaerobic sludge blanket reactor (adapted from, Chernicharo, (2007))

In the area above the sludge bed, a sludge blanket forms (Figure 2.4), which consists of anaerobic biomass with a lower concentration than that of the sludge bed. These biomass granules have slower settling velocities and are generally well mixed by the upflow of biogas and liquid (Chernicharo, 2007). The sludge is transported all the way up to a separator, where the heavier biomass is stopped and allowed to settle. The lighter biomass leaves the system with the liquid and the gas is allowed to escape. This separation system allows high organic loading rates (OLRs) and its simplicity renders the use of UASBs more attractive than other high-rate reactors.

2.5 Conclusions

To increase the efficiency of anaerobic digestion of wastewater, inhibitory compounds should be reduced, and complex sugars degraded for energy. Physical pre-treatments are effective for degrading biomass, although they might be economical for anaerobic digestion only if the biomass concentration is high enough to produce abundant methane. The biomass concentrations in pulp-mill wastewater are generally low; hence, physical pre-treatment may not be a viable option.

Physicochemical treatments are effective for removing compounds from wastewater. Some methods can remove recalcitrant compounds, such as lignin, but they also remove digestible biomass such as cellulose. Organic coagulants, such as PolyDADMAC and chitosan, can effectively remove lignin and other phenolic compounds without adding toxic compounds.

Enzymes may be suitable for anaerobic digestion because they are substrate-specific and biodegradable. Laccase polymerises lignin and phenolic compounds to a suitable molecular weight for membrane separation. Combining chitosan with laccase and membrane separation increases the lignin removal. The hydrolytic enzymes called cellulase and hemicellulase can effectively degrade polysaccharides to easily digestible forms. Thus cellulase, hemicellulose, laccase, organic coagulants and membranes may all be used as suitable pre-treatments for anaerobic digestion.

CHAPTER 3. SCREENING OF METHODS FOR PRE-TREATMENT OF PULP-MILL WASTEWATER ON BENCH-SCALE

3.1 Introduction

Sulphite evaporator condensate (SEC) has been identified as a suitable stream for anaerobic digestion. Streams of SEC display high chemical oxygen demand (COD) concentrations of up to 27 000 mg/L (Driessen *et al.*, 2000, Meyer and Edwards, 2014, Pokhrel and Viraraghavan, 2004), which includes easily digestible alcohols, volatile fatty acids (VFAs) and soluble sugars (Meyer and Edwards, 2014). Therefore, using SEC for full-scale anaerobic treatment may be financially sustainable. However, SEC also contains high concentrations of inhibitory compounds, such as lignosulphonate, polyphenols (soluble lignin) and sulphates (Ali and Sreekrishnan, 2001). These substances may reduce the stream's suitability for anaerobic digestion. The current chapter presents an evaluation of various pre-treatment methods on their ability to reduce inhibitory compounds. The enzyme laccase and coagulants, polydiallyldimethylammonium chloride (PolyDADMAC) and chitosan were identified as suitable pre-treatments for SEC, because of their ability to remove phenol-containing compounds, with little effect on potential substrates for anaerobic digestion.

3.2 Materials and methods

3.2.1 Characterisation of sulphite evaporator condensate

Wastewater (2 m^3) was collected from an SEC stream at a sulphite pulp mill. The SEC was transported to Sappi Technology Centre in Pretoria in a closed flow-bin to prevent contamination and was stored at room temperature. The SEC was characterised for polyphenols, lignosulphonate, volatile fatty acids and alkalinity. The concentration of polyphenols was measured with a UV-visible spectrophotometer (Unicam Helios B) according to TAPPI, T222 om-02 standard, (2002). The sample was filtered with a 0.45-µm polytetrafluoroethylene (PTFE) syringe filter and diluted 600 times with high-performance liquid chromatography (HPLC) grade water. The sample was then dispensed into a quartz cuvette and the absorbance was measured at 205 nm. Concentrations of polyphenols were calculated as described in the standard.

Lignosulphonate concentrations were measured according to a method developed by Mahler and Coetzee, (2017). Lignosulphonic acid sodium salt (Sigma Aldrich) with a molecular weight of 52 000 g/mol was used to make up a dilution series. The SEC sample was prepared by transferring 9 mL of the condensate into a test tube and adding 1 mL of phosphate buffer (pH 7). The sample was then filtered into a quartz cuvette using a 0.45-µm PTFE filter. The sample was diluted 600 times with a phosphate-buffer solution so that the absorbance would measure within the

calibration range. The calibration curve was determined by measuring the absorbance of the dilution series of the lignosulphonic-acid standards. The absorbance of the standards and samples was measured at 280 nm with a UV-visible spectrophotometer (Unicam Helios B). The concentrations of lignosulphonate were calculated from the calibration curve.

Alkalinity and VFAs were measured according to the method described by Van der Laan and Hobma, (1978). The SEC sample was centrifuged at 3214 × g. A portion (5 mL) of the sample was pipetted into a beaker and made up to 100 mL by adding HPLC-grade water. The solution was titrated with HCl (0.1 M) to pH 3. The solution was boiled in a reflux condenser for 3 min and then allowed to cool to room temperature. The cooled solution was titrated with NaOH (0.1 M) to pH 6.5. The titration volumes were used to calculate the respective concentrations of VFAs and alkalinity.

The SEC was further characterised by Waterlab (Pretoria) and the Biorefinery Department at Sappi Technology Centre. Waterlab analysed the five-day biological oxygen demand (BOD_5), total COD, sulphate and phenols. The following methods were used, respectively: WLAB018, WLAB020, WLAB046 and WLAB041, and WLAB046. Biorefinery analysed for lactic acid, total and free sugars, furfural, soluble COD, and pH according to the following methods: BIOSC/M006, BIOSC/M012, BIOSC/M007, CHEM/M015, and CHEM/M001, respectively.

3.2.2 Screening of enzyme formulations

The SEC in the flow-bin was mixed before a sample (20 L) was drawn for all enzyme experiments. The sample was then adjusted to pH 5 by adding approximately 250 mL NaOH solution (20% w/v). Aliquots (40 mL) of the SEC were then dispensed into centrifuge tubes and pre-heated in a water bath to 50°C. Each aliquot was then treated with an overdose (100 µL) of different laccase formulations (Table 3.1), mixed and incubated at 50°C for 1 h and 12 h respectively. The incubation temperature and pH were selected based on the experimental conditions used by Areskogh *et al.*, (2010a). Untreated aliquots of effluent were used as controls and incubated under similar conditions. The samples were placed on ice after treatment to reduce enzyme activity. Each treatment combination was replicated three times in a randomised factorial experiment.

Table 3.1: Enzyme formulations screened in experiments to pre-treat SEC.

Formulation	Description	Supplier	Operating range
Laccase3	Experimental enzyme	AB Enzymes	Data unavailable
Laccase11	Experimental enzyme	AB Enzymes	Data unavailable
Novozym® 51003	Commercial enzyme from Aspergillus oryzae	Novozymes	pH 5 – 7; temperature 50°– 70°
DeniLite® II S	Commercial enzyme from Aspergillus oryzae	Novozymes	pH 4 – 6; temperature 40°– 70°

After treatment, the samples were centrifuged at 721 × g to form a cohesive pellet. The supernatant was then decanted for spectrophotometric determination of polyphenols at 205 nm and lignosulphonate at 280 nm, as described earlier. The data were subjected to analysis of variance (ANOVA) with two factors, namely enzyme formulation and incubation period. Treatment means were compared with Tukey's test at $p \leq 0.05$ (Winer, 1962).

A portion of the treated samples was not centrifuged; it was used to determine the molecular weight of lignosulphonate through gel permeation chromatography (GPC). The molecular weight of lignosulphonate was determined on a Perkin-Elmer Flexar (Shelton, CT) high-performance liquid chromatography (HPLC) system, operated through TotalChrom software. The HPLC consisted of an autosampler, isocratic liquid-chromatography pump, column oven, degasser, and refractive index (RI) detector. Two Agilent PolarGel L columns (7.5 x 300 mm, 8µm particle size) were used. Samples were filtered through 0.45-µm PTFE syringe filters before injection. The injection volume was 100 µL and sample concentration was 8 mg/mL. A flow-rate of 0.4 mL/min was used and the oven temperature was set to 55°C. The eluent used was DMSO/water (9:1, v/v) containing 0.05 mol/dm^3 LiBr. The system was calibrated with pullulan standards (Sigma-Aldrich) of MPeak max (g/mol) of: 342, 1080, 6100, 9600, 21 100, 47 100 and 107 000. The data were processed according to the methods of Gavrilov and Monteiro, (2015) and Shortt, (1993).

3.2.3 Optimisation of Laccase 11 treatments

A factorial experiment with two factors, namely dosage of enzyme and pH, was performed using similar procedures to those described above. Enzyme dosages of 0.00 (control); 0.05; 0.10; 0.50; 1.00 and 3.00 µL/mL were tested at pH 5 and pH 7. The concentrations of polyphenols and lignosulphonate, and the molecular weights of lignosulphonate, were determined as before. The data were subjected to ANOVA and Tukey's test at $p \leq 0.05$ (Winer, 1962).

3.2.4 Coagulation treatments

The SEC sample was collected as described for enzymes and adjusted to pH 7 with an NaOH solution (20% w/v). Because PolyDADMAC and chitosan have a wide operation range (Saeed *et al.*, 2012, Singh *et al.*, 2000), the pH of the sample was adjusted to be compatible with anaerobic digestion. The charge densities of the SEC sample, PolyDADMAC and chitosan were measured using a particle-charge detector (Mütek-PCD-03) according to SCAN-W 12:04 Scandinavian standards, (2004). PolyDADMAC (20 % w/v) with a molecular weight of 100 000 g/mol (Sigma-Aldrich) and Chitosan (Poly(D- glucosamine)) with a molecular weight of 50 000 g/mol (Merck) were used.

Another factorial experiment with two factors, namely coagulant formulation and coagulant dosage, was performed. Dosages of 0.0 (control); 0.1; 0.5; 1.0 and 2.0 $mg_{coagulant}/L_{effluent}$ of PolyDADMAC and chitosan (1% w/v) were used to treat aliquots (40 mL) of the SEC sample, which had been dispensed into centrifuge tubes. The chitosan solution was made by dissolving chitosan powder in acetic acid (1% w/v). The treatments were then mixed at room temperature briefly with a vortex mixer. Each treatment was replicated three times in randomised experiments. Untreated aliquots of effluent were used as controls under similar conditions. The coagulation treatments were then centrifuged at 721 × g and the supernatant was decanted for the determination of polyphenol, lignosulphonate and VFA concentrations. The data were subjected to ANOVA and the means were compared using Tukey's test at $p \leq 0.05$ (Winer, 1962).

3.3 Results and discussion

3.3.1 Sulphite evaporator condensate

During the characterisation of SEC, a high total-COD concentration of 19 000 mg/L was found. This level of COD falls within the range of SEC measured by Driessen *et al.*, (2000) and Hall and Cornacchio, (1988). A small portion of the total COD was insoluble. A BOD_5 concentration of 14 200 mg/L was recorded, indicating that most of the total COD was bio-available. Table 3.2 shows the substances and concentrations recorded during the characterisation of SEC.

Table 3.2: Characteristics of sulphite evaporator condensate used to evaluate various enzymes and coagulants.

Composition	Value
Chemical oxygen demand (total) as O_2 (mg/L)	19 000
Chemical oxygen demand (soluble) as O_2 (mg/L)	17 800
Biochemical oxygen $demand_{(5)}$ as O_2 (mg/L)	14 200
Volatile fatty acids (mg/L)	5 209
Lactic acid (mg/L)	4 184
Total sugars (mg/L)	650
Free sugars (mg/L)	650
Furfural (mg/L)	1 250
Phenols (mg/L)	1
Sulphate as SO_4 (mg/L)	2 342
Polyphenols (mg/L)	4 800
Lignosulphonate (mg/L)	4 400
Alkalinity as $CaCO_3$ (mg/L)	1 600
pH	1.8

BOD_5 does not provide enough information on the digestibility of substrates. On further investigation, large concentrations of lactic acid and VFAs were measured (Table 3.2), which might have contributed to the total COD. Lactic acid and VFAs are produced during the first

metabolic stage of anaerobic digestion and are potential substrates for the anaerobic stages that follow (Lettinga, 1996). Lactic acid can enhance biogas formation (Satpathy *et al.*, 2017) and VFAs are the main substrates used by methanogenic microorganisms to produce methane (Chernicharo, 2007).

Other substrates measured included free and total sugars and furfural (Table 3.2). All the sugars appeared to be free; therefore, additional treatment to degrade oligomeric sugars would be ineffective. The furfural concentration in the SEC (1250 mg/L) appeared to fall in the ideal range for methane production. Furfural concentrations of up to 1000 mg/L have been shown to increase methane yield (Barakat *et al.*, 2012), whereas inhibition of methanogens has been observed at concentrations above 1400 mg/L (Costa *et al.*, 2014).

Possible inhibitors present in the SEC included phenols, polyphenols, sulphate and lignosulphonate (Table 3.2). The polyphenol and lignosulphonate concentrations were the highest and might have contributed the bulk of total COD. By contrast, phenol concentrations were almost negligible. During anaerobic digestion, sulphate metabolism results in the formation of H_2S, thus inhibiting methanogens (Lettinga *et al.*, 1996). This inhibition increases substantially at [COD]/[SO_4] ratios below 7, but depends strongly on the pH. In this particular stream, the [COD]/[SO_4] ratio was slightly above 8 and the high sulphate content could reduce the effectiveness of anaerobic digestion. Reduction of the sulphate, polyphenols and lignosulphonate concentrations of this stream might enhance anaerobic digestion.

For the SEC, the pH of 1.8 was far below the range of pH 6 to pH 8 recommended by Chernicharo, (2007) for anaerobic biomass to function effectively. Therefore, the pH was adjusted for the set of experiments. According to Tchobanoglous *et al.*, (2003), maintaining a pH of 7 in an anaerobic reactor at 35°C requires at least 1500 mg/L of $CaCO_3$ alkalinity. The alkalinity in the SEC stream (Table 3.2) was above the required concentration, which might be beneficial because additional buffering agents should not be required.

3.3.2 Screening of enzyme formulations

During the screening experiments, overdoses of the respective laccase formulations were used to limit any influence that pH and temperature might have on enzyme activity. When the laccase formulations were compared at the two incubation periods, no significant differences were observed in polyphenol and lignosulphonate concentrations or molecular weights of lignosulphonate, for all the enzyme formulations tested (Appendices A to C). The results were thus averaged over the incubation periods to evaluate the influence of the enzymes (Figure 3.1).

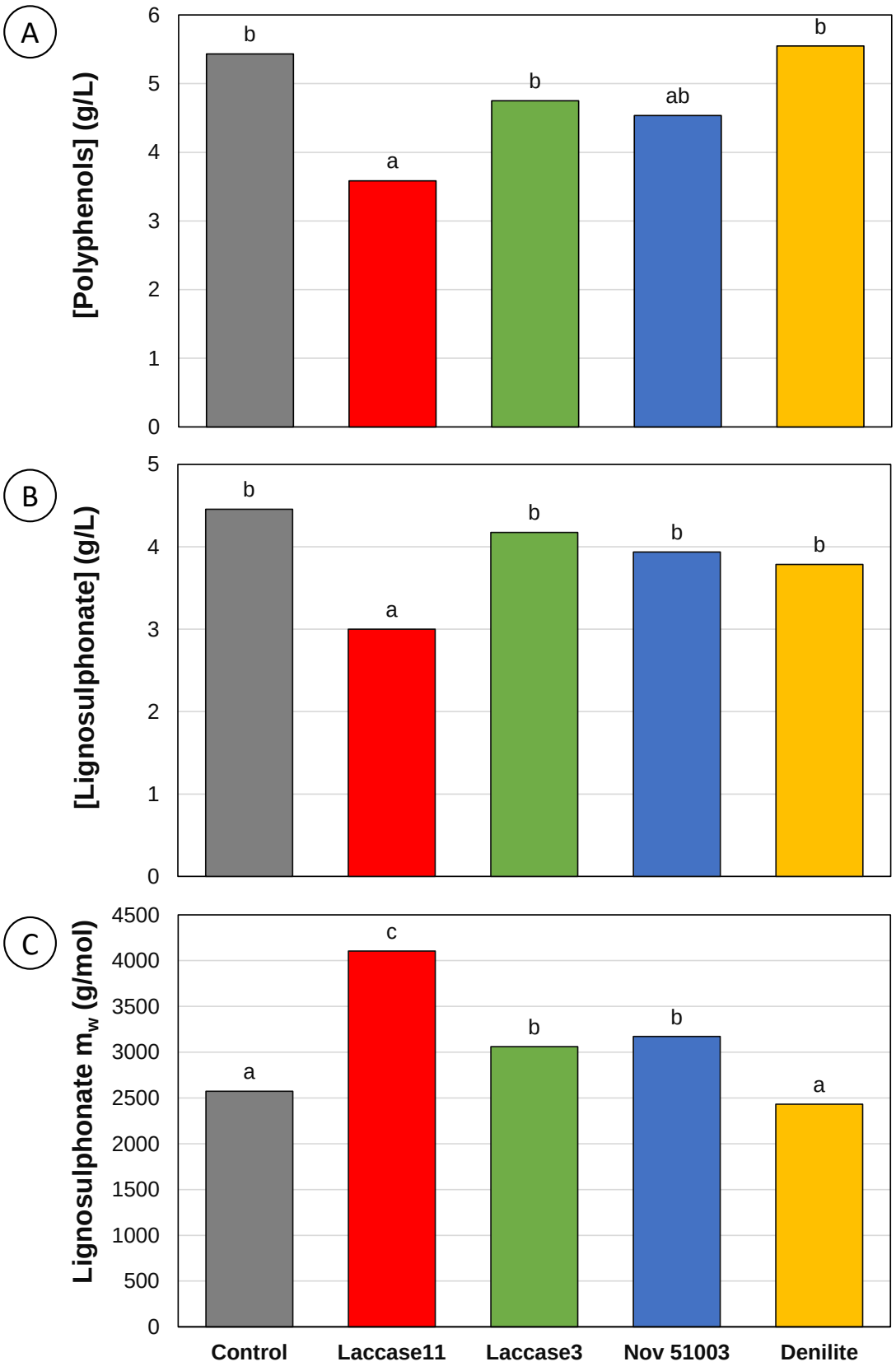

Figure 3.1: Influence of various enzymatic treatments on concentration of: polyphenols (A) and lignosulphonate (B) and molecular weights of lignosulphonate (C). Averaged data for 1 h and 12 h are presented. Bars with the same letters did not differ significantly at $p \leq 0.05$.

Treatment of SEC with Laccase11 resulted in the only significant reduction of polyphenols (34%) and lignosulphonate (33%) relative to the control (Figure 3.1 A, B). Further examination showed that Novozym® 51003, Laccase3 and Laccase11 increased the molecular weight of lignosulphonate significantly, compared to the control (Figure 3.1 C). Furthermore, a significant difference was seen in the increase of molecular weight when Laccase11 was compared to Novozym® 51003 and Laccase3. Laccase11 increased the molecular weight the most (60%), followed by Novozym® 51003 (23%) and Laccase3 (19%). The greater effectiveness of treatment with Laccase11 may reflect a higher redox potential that enables the enzyme to oxidise phenolic and non-phenolic end groups, to achieve a higher degree of polymerisation (Areskogh *et al.*, 2010a). Areskogh *et al.*, (2010b) reported that Novozym® 51003 oxidised only phenol end groups, which might account for the smaller increase in molecular weight compared to Laccase11. The same may be true for the lower efficiency of Laccase3.

An inverse relationship was noted between the molecular weight of lignosulphonate and the concentration of lignosulphonate after treatment with Laccase11. This relationship might have resulted from the increased molecular weight of lignosulphonate, which would have increased the settling during centrifugation (Figure 3.1 B, C). The increase in molecular weight was likely caused by the mode of action of laccase on lignosulphonate. Laccase oxidises phenolic end groups into radicals that subsequently undergo coupling, through which various bonds are formed (Areskogh *et al.*, 2010a). These bonds form intramolecular linkages within the molecules as well as linkages between the molecules; therefore, an increase in molecular weight is observed. The decrease in polyphenol content (Figure 3.1 A) can also result from increased molecular weight due to the oxidation of phenolic end groups.

Treatment with DeniLite® II S appeared to be the least effective method, with no significant increase in molecular weight of lignosulphonate and no decrease in polyphenol and lignosulphonate concentrations (Figure 3.1). Although no significant changes were observed, a small decrease in the molecular weight and concentration of lignosulphonate, compared to the control, was noted (Figure 3.1 B, C). These decreases could result from the degradation of lignosulphonates (Kim *et al.*, 2009) into smaller polyphenol sub-units. The degradation of lignosulphonate could also explain the increase in polyphenol concentration (Figure 3.1 A).

3.3.3 Optimisation of Laccase11 treatments

Laccase11 was identified as the most effective enzyme formulation for SEC treatment. During the optimisation experiments with this enzyme, no significant difference was noted between pH 5 and pH 7 for the measurements of polyphenols, lignosulphonate and molecular weight of lignosulphonate (Appendices D – F). The results were, therefore, averaged over the two pH

treatments. Significant differences in concentrations of polyphenols and lignosulphonate, as well as molecular weights of lignosulphonate, relative to the control were noted for dosages of 0.5 µL/mL or more (Figure 3.2). With increased dosages of Laccase11, the concentration of polyphenols and lignosulphonate decreased, and the molecular weight of lignosulphonate increased (Figure 3.2). It appeared that the most efficient dosage was 0.5 µL/mL as no significant differences were observed at higher dosages. At 0.5 µL/mL, 40% of polyphenols and 32% of lignosulphonate were removed and the molecular weight of lignosulphonate increased by 63%. In a study by Madad *et al.*, (2013) which used lignosulphonate of a similar concentration, an increase in molecular weight of only 50% (approximately) was measured at an overdose of laccase.

Several studies have shown that the main factors in the increased molecular weight of lignosulphonate are the initial concentration of lignosulphonate and the dosage of laccase (Areskogh *et al.*, 2010a, Madad *et al.*, 2013, Wang *et al.*, 2014). In a study by Areskogh *et al.*, (2010a), a 23-fold molecular weight increase was observed, by using a highly concentrated lignosulphonate blend (100 g/L) with an overdose of laccase. Therefore, in the present study, the molecular weight and removal of lignosulphonate could potentially be further enhanced by increasing the concentration of lignosulphonate. Because laccase has the same effect on both polyphenols and lignosulphonate, the molecular weight of polyphenols should also increase with increased concentrations of polyphenols.

Temperature is another factor that can enhance enzyme activity and thus increase the molecular weights of polyphenols and lignosulphonate. However, the temperature was not varied in the present experiments; instead, a suitable temperature for enzyme activity and anaerobic systems was used consistently.

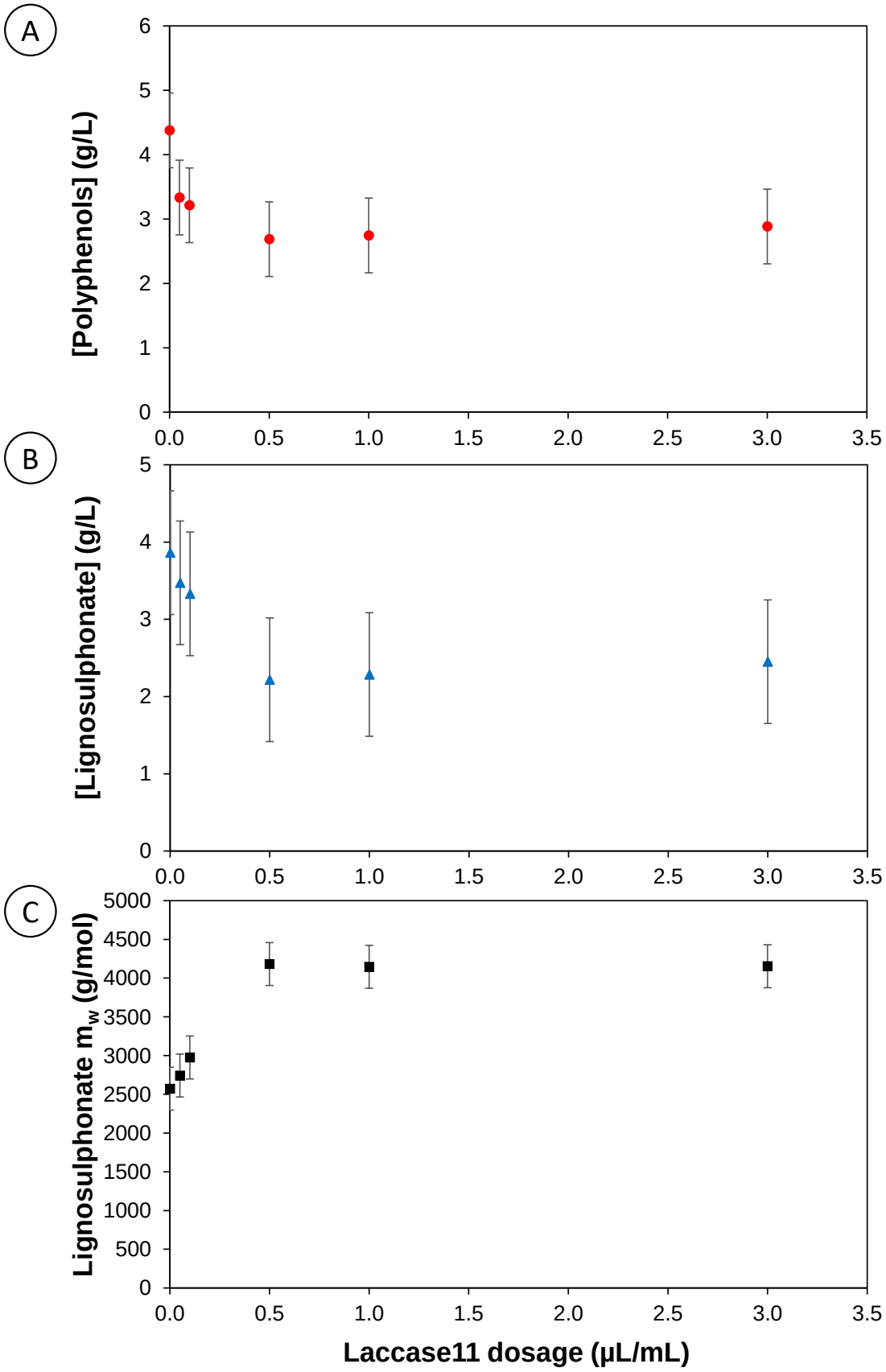

Figure 3.2: Influence of enzyme dosage on concentration of polyphenols (A) and lignosulphonate (B) and on molecular weights of lignosulphonate (C). Averaged data for pH 5 and pH 7 are presented. Error bars represent Q-values at $p \leq 0.05$.

3.3.4 Chitosan and PolyDADMAC treatment

Different concentrations of PolyDADMAC and chitosan were used to remove inhibitory compounds from SEC without affecting potential substrates for anaerobic digestion. The charge densities of PolyDADMAC, chitosan and the SEC sample were 7.0, 4.8 and -0.07 meq/g, respectively. The anionic charge density of the SEC sample can be ascribed to the carboxyl and sulphonyl groups attached to the polyphenols and lignosulphonate present in SEC (Saeed *et al.*, 2012).

During the coagulation experiments, no significant difference was observed between treatment by chitosan versus PolyDADMAC for removing polyphenols (Appendix G). However, significant decreases in polyphenol concentration relative to the control were seen at dosages of 1.0 and 0.5 mg/L for chitosan and PolyDADMAC, respectively (Table 3.3). At these dosages, chitosan and PolyDADMAC both removed 62% of the initial polyphenol content. These values correspond to those reported by Saeed *et al.*, (2012) in a similar study. No significant changes relative to the control were seen at lower or higher dosages.

Table 3.3: Influence of chitosan and PolyDADMAC dosage on concentration of polyphenols in sulphite evaporator condensate.

Coagulant dosage (mg/L)	[Polyphenols] (g/L)	
	Chitosan	PolyDADMAC
0.0	5.37 B	5.37 b
0.1	5.06 Ab	4.10 ab
0.5	3.10 Ab	2.06 a
1.0	2.05 A	2.49 ab
2.0	4.15 Ab	3.98 ab

ab Polyphenol concentrations in same column with the same letters did not differ significantly at $p \leq 0.05$.

Significant reductions of lignosulphonate were noted at dosages of 0.5 mg/L or more, for both coagulants (Table 3.4). At a dosage of 1.0 mg/L, chitosan and PolyDADMAC both reached their maximum effectiveness and removed 41% and 57% of lignosulphonate, respectively. PolyDADMAC removed significantly more polyphenols than chitosan at both 1.0 and 2.0 mg/L (Table 3.4; Appendix H). At dosages above or below 0.5 mg/L, less lignosulphonate was removed for both coagulants.

Table 3.4: Influence of chitosan and PolyDADMAC dosage on concentration of lignosulphonate in sulphite evaporator condensate.

Coagulant dosage (mg/L)	[Lignosulphonate] (g/L)	
	Chitosan	PolyDADMAC
0.0	3.99 C	3.99 c
0.1	3.88 Bc	4.18 c
0.5	3.31 B	3.17 b
1.0	2.34 a*	1.81 a*
2.0	3.56 bc*	2.59 b*

abc Lignosulphonate concentrations in same column with the same letters did not differ significantly at $p \leq 0.05$.

* Concentrations between rows differed significantly at $p \leq 0.05$.

The smaller decrease in lignosulphonate and polyphenol content resulting from chitosan treatment may be attributed to the neutral pH used in this study. Fredheim and Christensen, (2003) showed that all complex formation by chitosan stopped at pH 8 and the optimum occurred at roughly pH 4.5. PolyDADMAC is regarded as operational at most pH values, and was shown to be effective at pH 7 (Razali *et al.*, 2011).

The coagulants tested here removed significant amounts of VFAs at dosages of 0.5 mg/L or more (Table 3.5). Chitosan and PolyDADMAC removed the most VFAs at 0.5 mg/L. At dosages above 0.5 mg/L, VFA removal was significantly decreased for both coagulants. Saeed *et al.*, (2012) reported that compounds having lower molecular weights – such as VFAs, furfural and sugars – formed the most complexes with PolyDADMAC and chitosan at dosages below 0.5 mg/L. At higher PolyDADMAC and chitosan dosages, more complexes were formed with larger molecules, such as polyphenols.

No significant differences between chitosan and PolyDADMAC were noted during the VFA measurements (Appendix I). Therefore, it appears that both coagulants had similar effects on VFAs, and dosages above 0.5 mg/L would remove fewer VFAs. Treating the SEC with dosages above 0.5 mg/L would also enhance the removal of polyphenols and lignosulphonate.

Table 3.5: Influence of chitosan and PolyDADMAC dosage on concentration of volatile fatty acids in sulphite evaporator condensate.

Coagulant dosage (mg/L)	[Volatile fatty acids] (g/L)	
	Chitosan	PolyDADMAC
0.0	5.27 b	5.27 b
0.1	5.06 ab	4.10 ab
0.5	3.10 ab	2.06 a
1.0	2.05 a	2.49 ab
2.0	4.15 ab	3.98 ab

ab VFA concentrations in same column with the same letters did not differ significantly at $p \leq 0.05$.

CHAPTER 4 DESIGN, CONSTRUCTION AND COMMISSIONING OF BENCH-SCALE UPFLOW ANAEROBIC SLUDGE BLANKET REACTORS

4.1 Introduction

In this chapter, the design, construction and commissioning of three identical upflow anaerobic sludge blanket (UASB) reactors are described. The reactors were built to evaluate the effects of pre-treatments on UASB-reactor performance. During the design stage, a main consideration was the need to develop and maintain granular sludge with excellent settling capacity. The design was thus adapted to the specific type of effluent, with consideration of the chemical oxygen demand (COD), toxic, inhibitory and biodegradable compounds. The design factors included the following points: 1) maintaining an upward flow to ensure maximum contact between the anaerobic biomass and the sulphite evaporator condensate (SEC); 2) designing a distribution system to allow effective mixing without blocking the flow; and 3) designing a separation unit to effectively separate biomass, liquid and gas. The design of the reactors is discussed in Section 4.2, followed by the commissioning of the three reactors in Section 4.3. During commissioning, the reactors were inoculated with anaerobic biomass and acclimatised to the SEC. The parameters of the reactors were monitored to ensure that the reactors performed effectively.

4.2 Design of the reactors

4.2.1 Reactor volume and dimensions

The reactor volume and dimensions of a UASB depend on the organic loading rate (OLR), the upflow velocity, and the effective liquid volume (Tchobanoglous *et al.*, 2003). The design was based on a maximum OLR of 16 kg_{COD}/m^3d, as recommended by Lettinga and Hulshoff Pol, (1991) for wastewater (18 kg_{COD}/m^3) with few suspended solids at an operational temperature of 30ºC.

4.2.1.1 Effective liquid volume

The effective liquid volume (V_e) is the actual volume that is occupied by anaerobic biomass (Figure 4.1). This value (V_e) was calculated using equation 4.1. The equation is valid for COD concentrations above 5 g/L (Lettinga *et al.*, 1996).

$$V_e = \frac{Q \times S_0}{L_{org}} \quad (4.1)$$

where:

V_e = effective liquid volume of the reactor (m^3)
Q = flowrate into the reactor (m^3/h)
S_0 = COD of the wastewater (kg_{COD}/m^3)
L_{org} = organic loading rate (kg_{COD}/m^3d)

The reactor was designed to handle SEC at a maximum flowrate of 6 L/d, at a COD concentration of 18 kg/m^3 and OLR of 16 kg_{COD}/m^3d. The calculated V_e was 6.7 L.

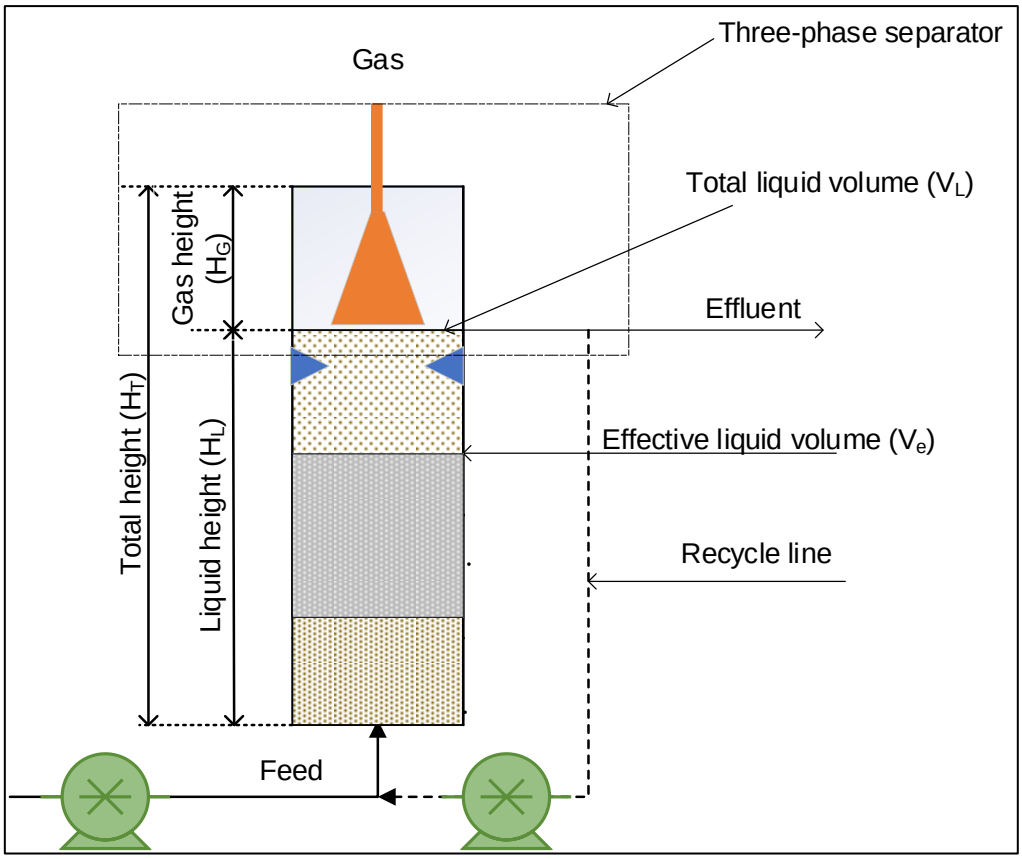

Figure 4.1: Diagrammatic representation of a UASB and basic dimensions used in its design (adapted from, Chernicharo, (2007)).

4.2.1.2 Total liquid volume

The total volume of the liquid in the reactor (Figure 4.1) was calculated using an effectiveness factor of 0.85 (Tchobanoglous *et al.*, 2003) and the following formula:

$$V_L = \frac{V_e}{E} \quad (4.2)$$

where:

V_L = total liquid volume of the reactor (m^3)
E = effectiveness factor

The V_e calculated earlier was used in this equation, and a total liquid volume of 7.84 L was calculated.

4.2.1.3 Liquid height

The liquid height (Figure 4.1) of the reactor was then calculated from the following equation:

$$H_L = \frac{V_L}{A} \tag{4.3}$$

where:

A = cross-sectional area of the reactor (m^2)

and

$$A = \pi r^2 \tag{4.4}$$

where:

r = radius of the reactor (m)

A cross-sectional area of 0.016 m^2 was calculated, using an inside diameter of 0.15 m. For the maximum volumetric flowrate (6 L/d) into the reactor, a much smaller cross-sectional area would be required to achieve a high upflow velocity. This would have been impractical from a construction viewpoint. Therefore, the diameter was chosen for practicality and availability of space. Using the total liquid volume (V_L) calculated above, the liquid height of the reactor was calculated as 0.48 m.

4.2.1.4 Total height

For the total volume of the reactor, a provision was made for collection of gas. The total height was calculated using the following formula:

$$H_T = H_L + H_G \tag{4.5}$$

where:

H_T = total height of the reactor (m)
H_G = height to accommodate gas collection (m)

The height required to provide for the gas phase (H_G) was calculated as 30% of the liquid height (H_L), as recommended by Chernicharo, (2007). The calculated H_G was 0.14 m and the total height of the reactor was 0.63 m.

4.2.1.5 Upflow velocity

The upflow velocity was calculated according to the following equation:

$$v = \frac{Q}{A} \tag{4.6}$$

where:

v = upflow velocity (m/h)
A = area of the reactor (m^2)

Lettinga and Hulshoff Pol, (1991) recommended that an average upflow velocity of 0.5 m/h be used for wastewater that contains few suspended solids. The upflow velocity in the present study, calculated as 0.02 m/h, was far below that recommendation due to the relatively large cross-sectional area of the reactor and low flow-rate (Q) into the reactor. Therefore, a recycle stream was added to increase the upflow velocity to 0.5 m/h.

4.2.2 Design of the heating jacket

A heating jacket, with water as the heat source, was added to the reactor design to maintain optimal operating temperatures inside the reactor (Figure 4.2). The inflow to the jacket was placed on the opposite side of the outflow and at the bottom of the reactor. This placement achieved an even heat distribution. The height of the jacket is equal to H_L and the inside diameter is 0.3 m. Acrylic tubing was used as the construction material. The water to the heating jacket was supplied by a recirculating water bath (Thermo Haake DC10). The recycle was set to maximum velocity to maintain a constant temperature profile in the jacket.

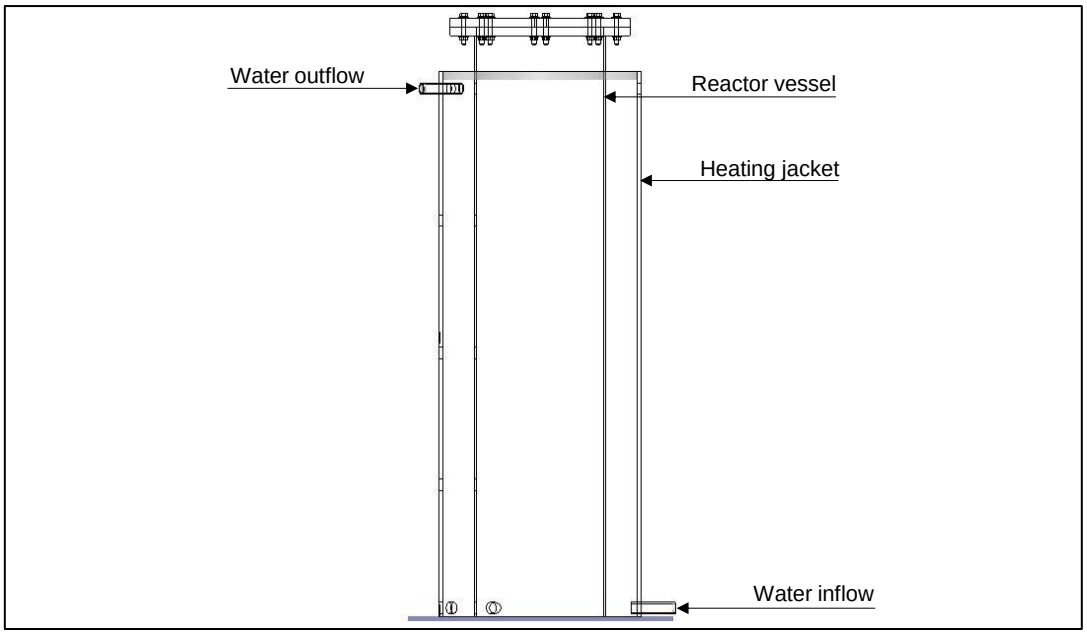

Figure 4.2: Heating jacket with inlet and outlet ports and position relative to reactor vessel.

4.2.3 Design of the inflow-distribution manifold

The design of the manifold was expected to ensure good contact between the substrate and biomass. To avoid short-circuiting, three distribution tubes were equally spaced (40 mm apart) at the bottom of the reactor floor (Figure 4.3). The cross-sectional diameter of each pipe (12 mm) was calculated to prevent a flow velocity above 0.2 m/s (Van Haandel and Lettinga, 1994), so that occasional air bubbles could flow back into the settler. The diameter was also chosen to prevent solids from obscuring the flow. Each aperture at the top of the tubes was 4 mm in diameter, to increase the velocity directly above the tubes for better mixing with the sludge bed. The apertures were evenly distributed along the tubes.

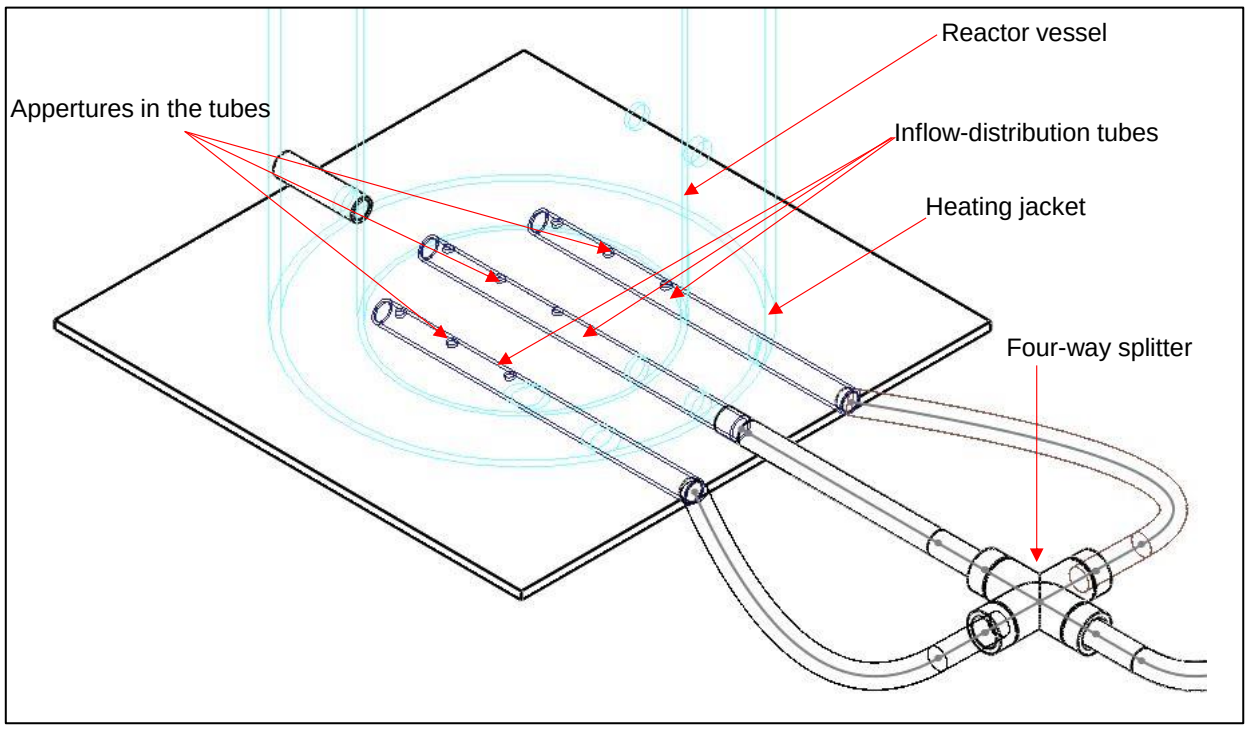

Figure 4.3: The inflow-distribution manifold at the bottom of the UASB.

4.2.4 Design of the three-phase separation unit

The main function of the three-phase separator was to maintain a high sludge age and to separate gas from liquid effectively. The design included the separation of the three phases in the reactor, and a settling unit for increased solid and liquid separation (Figure 4.4). In the reactor, most of the larger granulated sludge was retained by a perforation plate (Figure 4.4 f) with apertures 2 mm in diameter and spaced 100 mm below the effluent pipe (Figure 4.4 d). The settler (Figure 4.4 e) and recycle line (Figure 4.4 g) were used to retain the small solids that passed through the perforation plate. It was determined that most particles of the bio-sludge settled at a velocity of 6 m/h or higher; therefore, a settler with a cross-sectional diameter of 200 mm and volume of 1 L

was sufficient to retain most particles at an inflow rate of 8 L/h. The inflow rate of 8 L/h was based on the recycle volume to create an upflow velocity of 0.5 m/h.

The gas was separated from the solid and liquid phases in the gas-collection chamber (Figure 4.4 c). The separation was accomplished by choosing the diameter of the effluent pipe (10 mm) (Figure 4.4 d) to allow the outflow rate of solids and liquids in the reactor to be less than the inflow rate to the reactor. Thus, a liquid level was established above the effluent pipe, and gas could escape only through the gas pipe (Figure 4.4 a). The reactor was sealed at the top with a flange and lid to avoid gas from escaping (Figure 4.4 b).

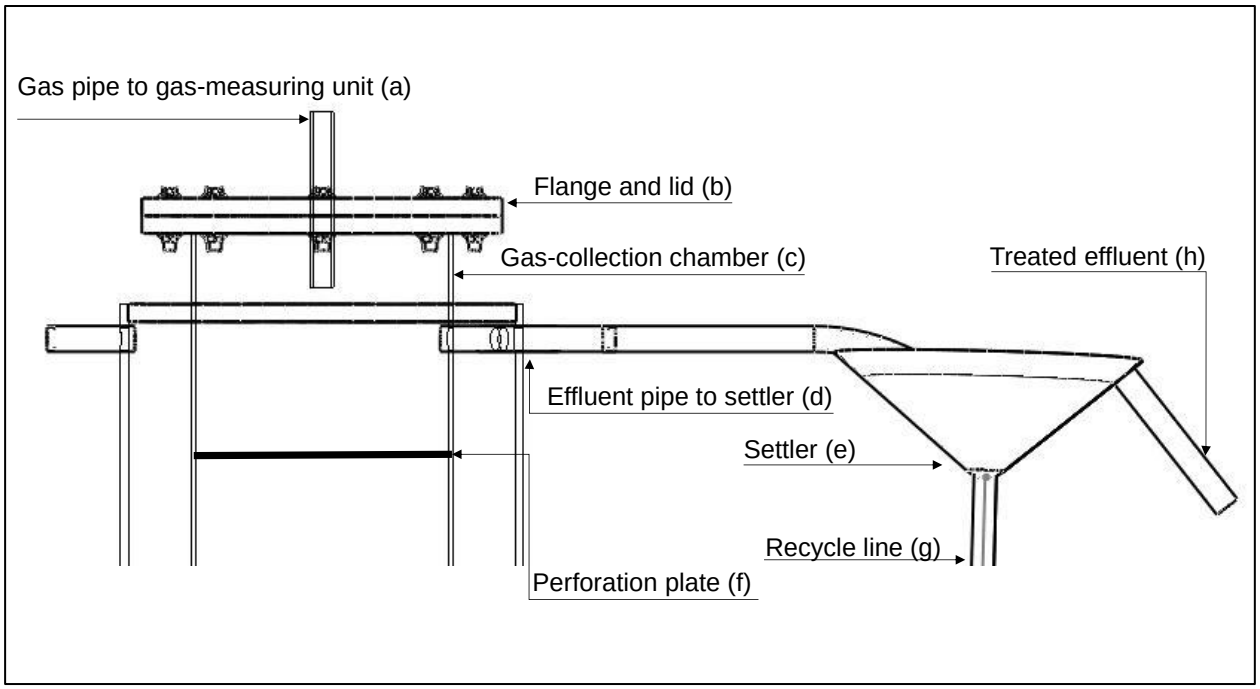

Figure 4.4: Schematic representation of the three-phase separation unit of the UASB.

4.2.5 Design of the recycle system and SEC-feed line.

The recycle line was designed to create an upflow velocity of 0.5 m/h inside the reactor. For the area of the reactor, and accounting for the volumetric load of the SEC feed (Figure 4.5), a recycle volumetric rate of 1.84 L/h was necessary to achieve the upflow velocity of 0.5 m/h. Peristaltic pumps (Watson Marlow 520u and 320s) were used in the recycle and SEC feed line, respectively, to accurately control the volumetric flowrates.

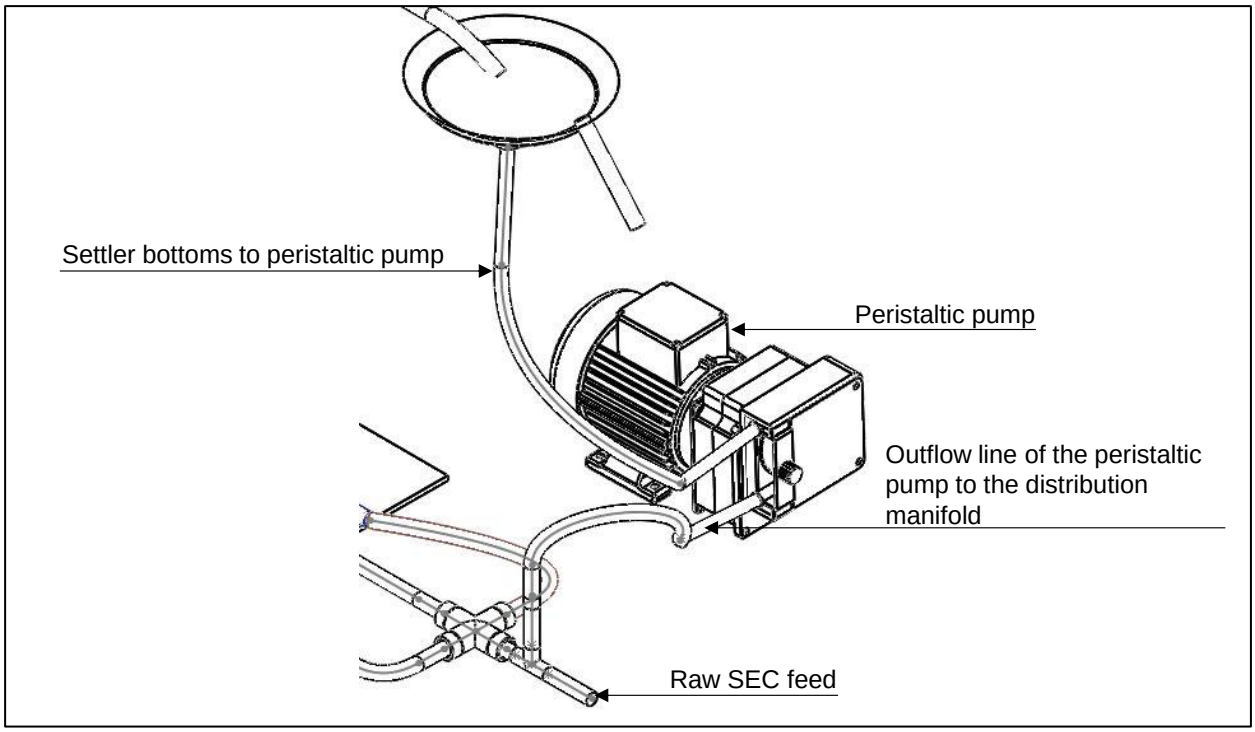

Figure 4.5: Diagrammatic representation of the recycle and feed line to the UASB.

4.2.6 The design of the water-displacement unit

A 10-L container (Figure 4.6 A) with an open top was placed inverted into an 8-L container (Figure 4.6 B) with an open top. The gas-outlet pipe from the reactor (Figure 4.4 a) was tightly attached to container A. The two containers were then attached to each other with string and container B was filled with water. The design worked on the principle that as the reactor generated gas, container A would be filled with gas and would consequently displace water from container B. The water volume that was displaced was measured and assumed to be equal to the gas volume.

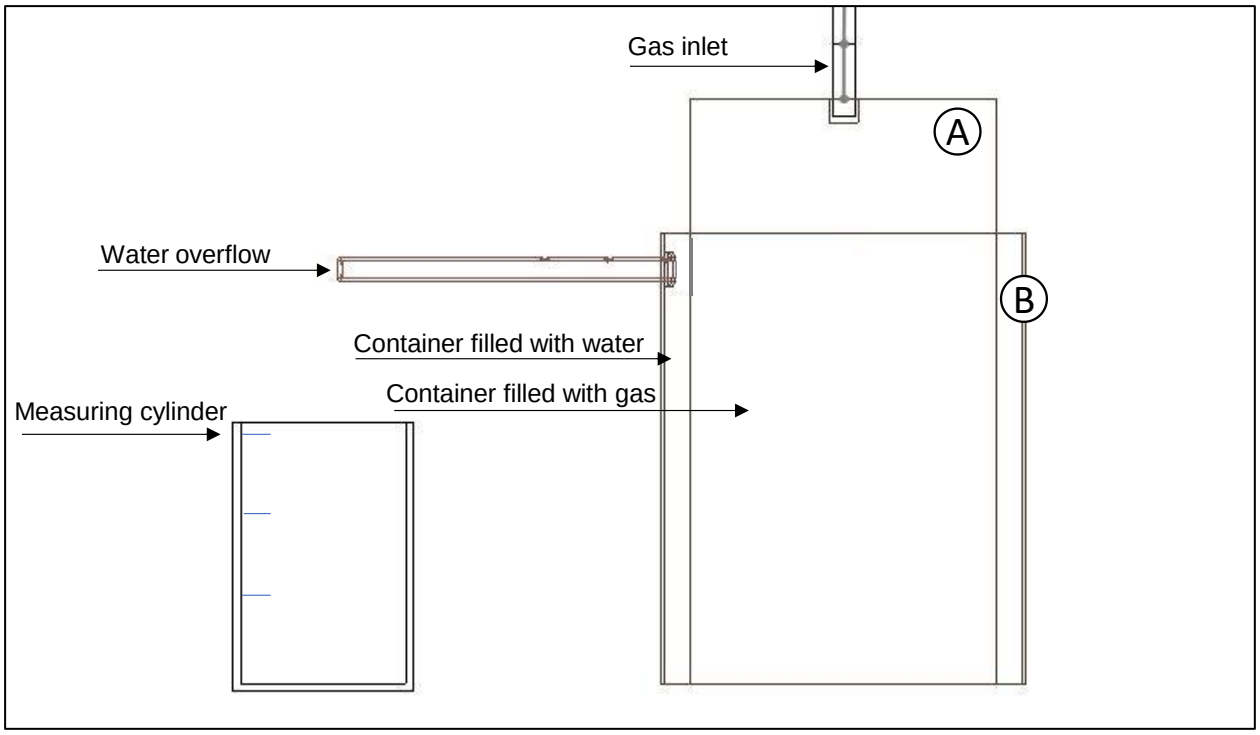

Figure 4.6: The water-displacement unit used to measure gas generated in the UASB.

4.2.7 Reactor assembly

The design of the reactors was drawn on SolidWorks and transferred to the computer of a laser cutter to accurately cut acrylic tubes and sheets to scale. These tubes and sheets were used to assemble the first reactor and its components (Figure 4.7), after which the reactor was tested for leakages. The recycle line, pumps, settler, three-phase separation unit, inflow manifold and water displacement chamber were also tested before two more reactors were built. The greatest concern was the water-displacement unit. Although it worked well and was pragmatic, a gas meter might have been a better option.

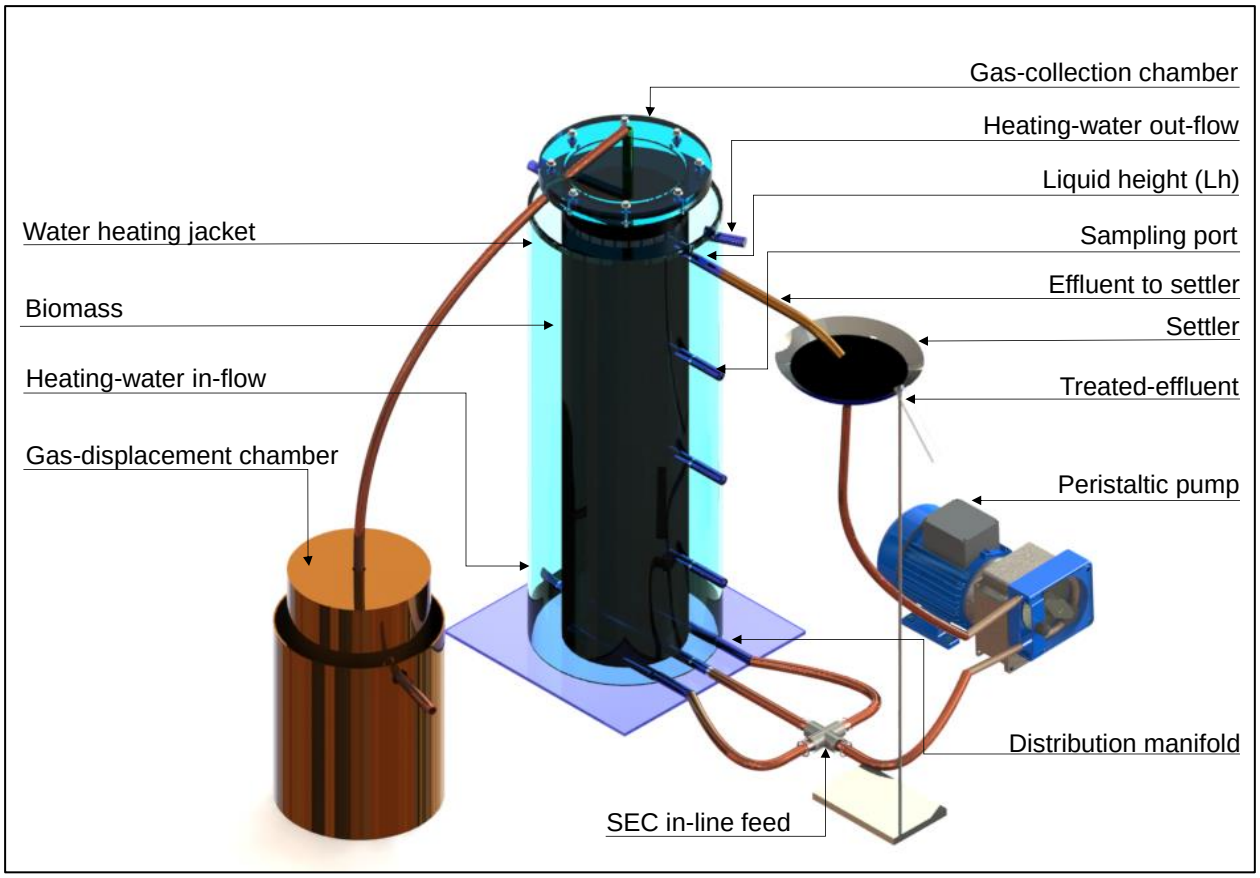

Figure 4.7: A rendered representation of the UASB design and relevant components.

4.3 Commissioning and start-up of the UASB reactors

4.3.1 Materials and methods

Granulated sludge (≤1 mm in diameter) was collected from a UASB that treated brewery wastewater. The three reactors were each inoculated with 2.45 L of the sludge, to reach the recommended biological loading rate of 0.5 $kg_{COD}/kg_{VS.d}$ during start-up (Chernicharo, 2007). The volume of the sludge required was calculated using Equations 4.7 and 4.8. Equation 4.7 was used to determine the required mass of volatile solids (Chernicharo, 2007) and Equation 4.8 to determine the granular-sludge volume.

$$L_s = \frac{Q \times S_0}{M_{vs}} \tag{4.7}$$

where:

L_s = biological loading rate ($kg_{COD}/kg_{VS}d$)
Q = flowrate into the reactor (m^3/d)
S_0 = COD of the wastewater (kg_{COD}/m^3)
M_{vs} = Mass of the volatile solids in the reactor (kg_{VS}/m^3)

The maximum flowrate into the reactor for the design was used (6 L/d), and S_0 was 18 000 mg/L and L_s as described above. The mass of the volatile solids was calculated as 0.21 kg_{vs}/m^3.

$$V_{sl} = \frac{M_{vs} \times V_e}{C_{vs}} \quad (4.8)$$

where:

V_{sl} = volume of the granulated sludge (m^3)
V_e = effective liquid volume of the reactor (m^3)
C_{vs} = concentration of the volatile solids (kg/m^3)

The concentration of the volatile solids (0.58 kg/m^3) was supplied by the brewery. The volume of the sludge was then calculated as 2.45 L.

After inoculation, the water bath was started and the water supply to the heating mantels of the reactors was opened (Figure 4.7). The temperature inside the reactors was regulated at 35ºC for the duration of the experiments. After starting up the heating jackets, the reactors were filled to the total liquid height (H_L) with control SEC diluted to 6000 mg/L. The control SEC (pH 7) was made by adding approximately 1.5 kg NaOH to 400 L of the SEC obtained from a sulphite pulp-mill, as described in Chapter 3.

After 24 h, peristaltic pumps used for the recycle streams (Figure 4.7) were started and set to create upflow velocities of 0.5 m/h in the reactors. After 24 h, the supernatant of each settler was analysed for pH, alkalinity, VFAs and total COD (day 0). The alkalinity and VFA analyses were conducted as described in Chapter 3. The COD was measured using Spectroquant® cell tests (Merck) and the pH with a portable pH meter (Hanna®). The temperature of each reactor was measured inside the effluent pipe with a mercury thermometer.

The control SEC was then fed to each reactor, starting with an OLR of 2 kg_{COD}/m^3d (day 1). The treated effluent was analysed every three days for pH, temperature, alkalinity, VFAs and COD (starting at day 1). The first OLR of 2 kg_{COD}/m^3d was kept constant until all the operational and performance parameters were within the ranges defined by Lettinga *et al.* (1984). These ranges include, a pH between 6.8 and 7.8; temperature and alkalinity above 30°C and 2000 mg/L, respectively; VFAs below 1000 mg/L; and COD removal above 60%. The OLR to each reactor was then increased to 4 kg_{COD}/m^3d (day 9) and then to 6 kg_{COD}/m^3d (day 18) when the parameters above were reached again. During the start-up, the reactors were seeded with nitrogen and phosphorous at a ratio of COD:N:P = 350:5:1 (Lettinga *et al.*, 1996). Urea and potassium dihydrogen orthophosphate were used for the nitrogen and phosphorus demands, respectively.

4.3.2 Results and discussion

During the start-up phase of the anaerobic reactors, the OLR was gradually changed according to the system response. Fortunately, the biomass that was used was already granulated and acclimatised to industrial wastewater with high VFA concentrations. Therefore, the start-up period was considerably shorter than it would be for UASBs without granulated sludge (Els *et al.*, 2005).

Initially, a low OLR of 2 kg_{COD}/m^3d was fed to each reactor for 9 days. During the 9-day period, the percentage of COD removed increased gradually and was higher than 60% after the ninth day (Figure 4.8). The low COD removal at the beginning can be ascribed to the anaerobic biomass being unacclimatised to the SEC.

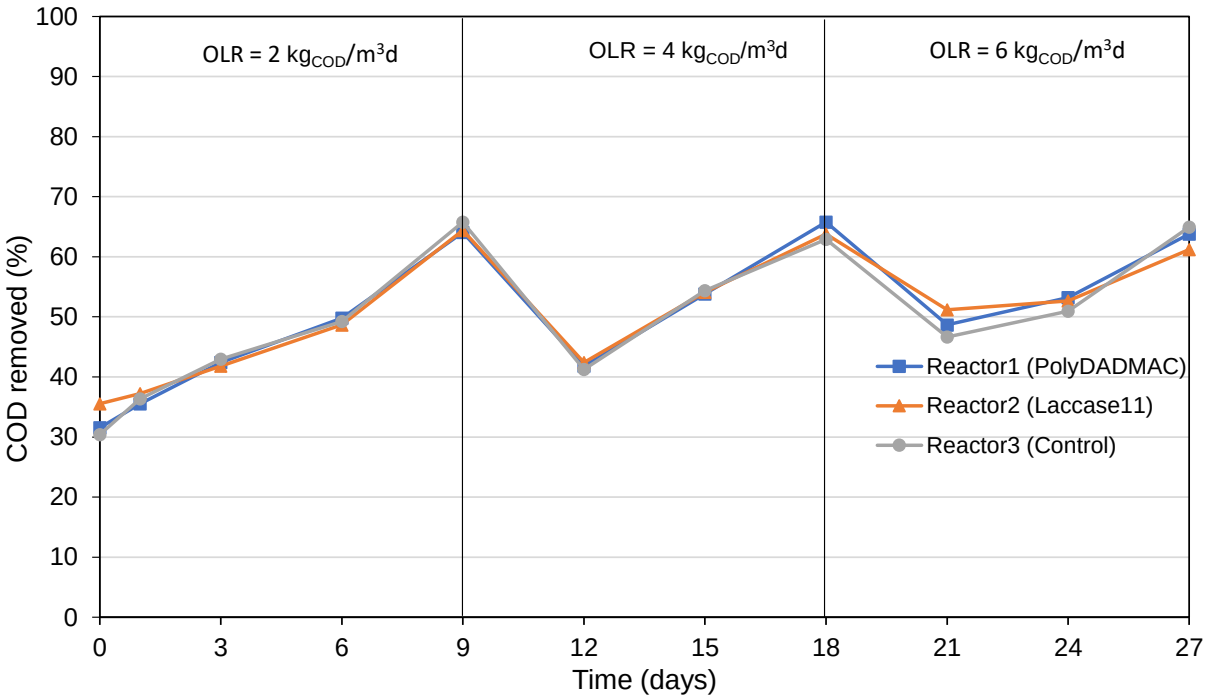

Figure 4.8: Influence of various OLR on COD content of the three reactors during start-up.

Also during the 9 days, a gradual decrease in VFA concentrations and a slight increase in pH and alkalinity were noted (Figure 4.9). The decrease in VFAs could have resulted from VFA conversion to methane gas and the acclimatisation of the anaerobic biomass. The increase in pH and alkalinity was possibly due to the formation of bicarbonates during the breakdown of organic matter and the reduction of VFAs (Tchobanoglous *et al.*, 2003).

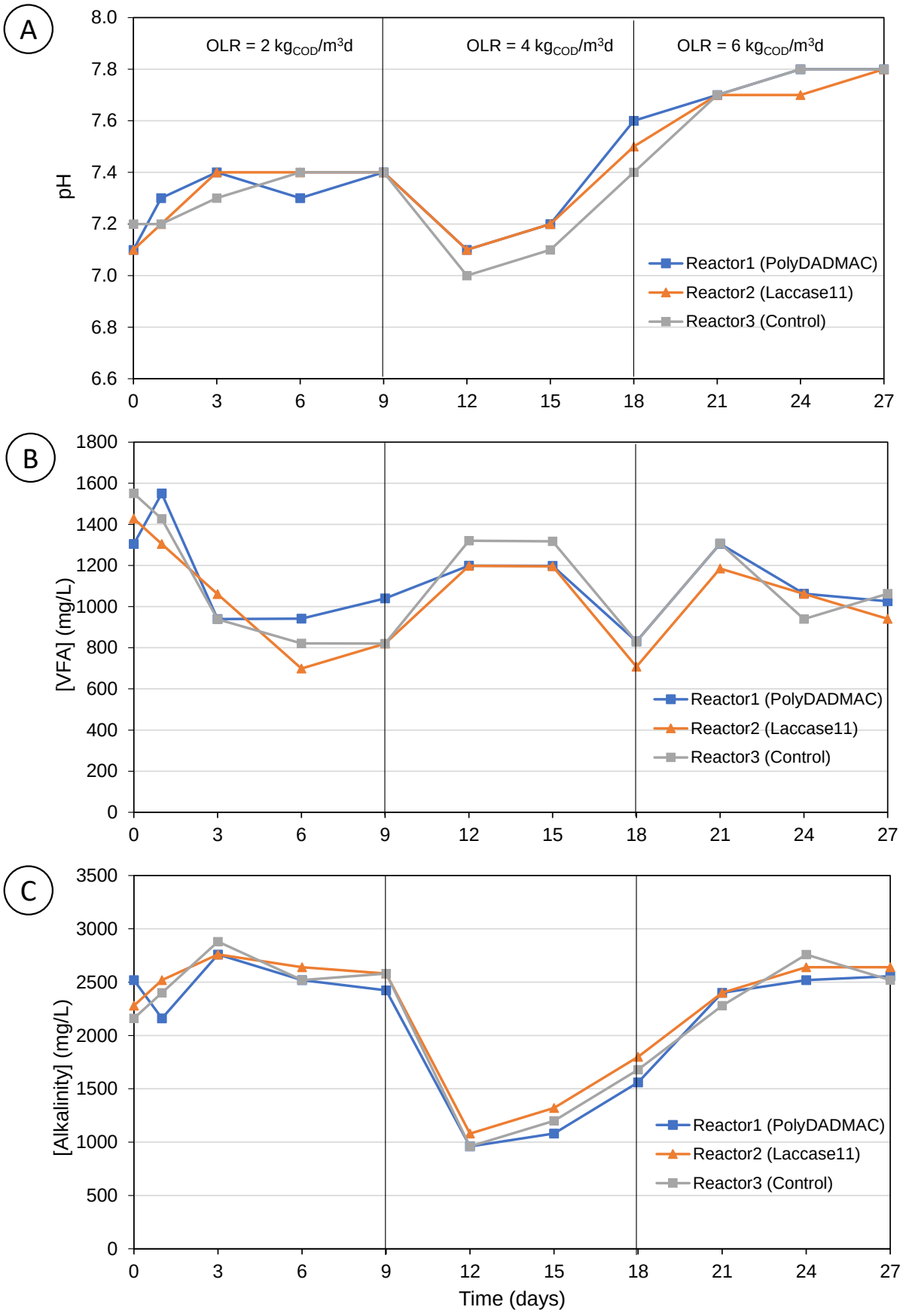

Figure 4.9: The influence of different OLRs on the pH, volatile fatty acids and alkalinity of the three reactors during start-up.

After 9 days, the OLR was increased to 4 kg_{COD}/m^3d because the operational parameters were within the suggested ranges described by Lettinga *et al.*, (1984). After this increase in OLR, an increase in VFAs was noted, along with a decrease in pH and alkalinity (Figure 4.9). The increase in VFAs measured could be ascribed to the larger amount of VFAs fed to the reactor and the inability of the methanogens to consume all the VFAs fed and produced. The decline in alkalinity was related to the buffer reactions with the VFAs, and the decline in pH was related to the increase in VFAs. With the optimal pH range of methanogens between 6.8 and 7.6 (Chernicharo, 2007) the decline in pH could have also decreased the ability of methanogens to reduce VFAs.The decrease in COD removed after the OLR change (Figure 4.8) might have resulted from the rapid change in pH and the inability of the anaerobic biomass to consume all the substrates. After the pH, alkalinity and removed-COD declined, the reactors recovered and reached the ranges necessary for another OLR increase.

During the final OLR increase (18 days), the same trend was observed for COD removal as in the previous OLR change (Figure 4.8). However, the increase in OLR and VFAs did not appear to affect the pH and alkalinity as much as during the previous OLR change (Figure 4.9). The temperature was stable for the entire start-up period with a variation of ±1°C (Figure 4.10).

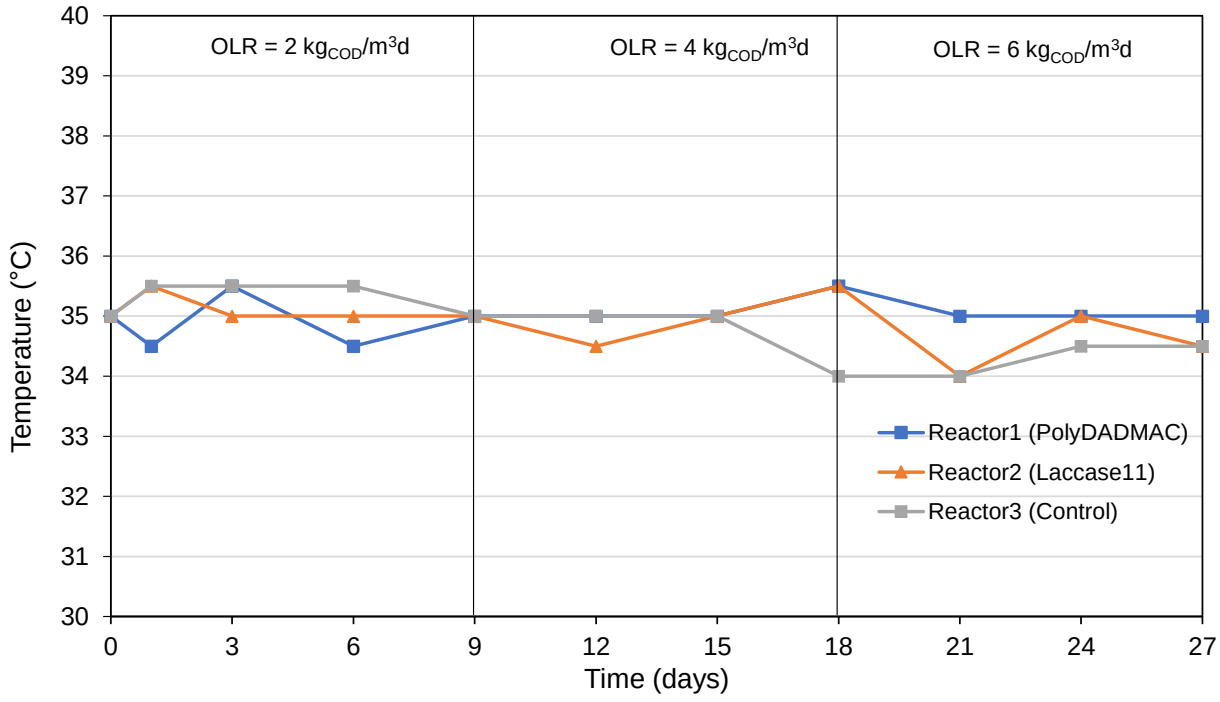

Figure 4.10: Temperature profile of each reactor during the start-up period.

CHAPTER 5 EVALUATION OF PRE-TREATMENT METHODS FOR SULPHITE EVAPORATOR CONDENSATE THROUGH ANAEROBIC DIGESTION

5.1 Introduction

Monitoring pre-treatment methods at a substrate level may indicate the degree to which anaerobic digestion can be improved. However, the real effects of any pre-treatment are tied to the specific anaerobic reactor and process conditions (Carlsson, 2015). In the pulp industry, large volumes of sulphite evaporator condensate (SEC) have to be treated daily. Therefore, the efficiency of pre-treated (SEC) was measured on bench-scale upflow anaerobic sludge blanket (UASB) reactors and the results are reported in this chapter. In Section 5.2, details are provided of the materials and methods used in pre-treating SEC and the operation and monitoring of the three UASBs. In Section 5.3, the results of the pre-treatments and reactor treatments are discussed.

5.2 Materials and methods

5.2.1 Batch pre-treatments of sulphite evaporator condensate

To evaluate the effect of pre-treatments on the performance of the anaerobic reactors, large batches of pre-treated SEC were required for the experiments. The pre-treatments were performed by decanting three batches (400 L each) of the untreated SEC provided by the sulphite pulp mill (Chapter 3) into containers. The pH of the untreated SEC in each container was adjusted to pH 7 by adding approximately 1.5 kg NaOH pellets (Merck) per container.

The SEC in the first container (400 L) was dosed with polydiallyldimethylammonium chloride (PolyDADMAC) solution (1 mg/L) and mixed with a bench disperser. The second batch (400L) was decanted into a large heating vessel and heated to 50°C. The batch was then treated with a Laccase11 dosage of 0.5 µL/mL, stirred with a bench disperser and incubated for 1 h at 50°C.The third batch (400 L) was left untreated at room temperature. All three batches were allowed to settle for 24 h, where after the contents of each container were filtered through a Buchner funnel using Ahlstrom filter paper (220 mm/30 min).

After filtration, the three batches were characterised for five-day biological oxygen demand (BOD_5), total chemical oxygen demand (COD), volatile fatty acids (VFAs), alkalinity, furfural, lactic acid, polyphenols, lignosulphonate, sulphate and pH. The COD and sulphate concentrations were measured using the respective Spectroquant® cell tests (Merck). The BOD, VFAs, alkalinity, furfural, lactic acid, polyphenols, lignosulphonate and pH were measured as described in Chapter 3.

5.2.2 Evaluation of effects of pre-treated SEC on reactor performance

When the conditions of the reactors during start-up became stable after an organic loading rate (OLR) of 6 kg_{COD}/m^3d was achieved (Chapter 4), the three batches of pre-treated SEC were fed to the reactors in a randomised manner at an OLR of 6.5 kg_{COD}/m^3d. The feed configurations were as follows: SEC treated with PolyDADMAC and Laccase11 were fed to Reactor 1 and Reactor 2, respectively, and the control batch was fed to Reactor 3.

The reactors were sampled as shown in Table 5.1. The pH, temperature, COD, VFAs and alkalinity were measured using the same methods as in the start-up. The sulphate content was measured using Spectroquant® cell tests (Merck). The gas volume was determined by measuring the amount of water displaced by the gas-displacement chamber (Chapter 4). The composition of the gas was measured with CO_2(g) and H_2S(g) Dräger-tubes, and the remaining volume was assumed to be CH_4(g). Once the reactors were stable, the OLR to the reactors was increased, first to 13 kg_{COD}/m^3d and then to 16 kg_{COD}/m^3d.

Table 5.1: Parameters measured during anaerobic treatment, showing frequency and position of measurements.

Parameter	Monitoring points and frequency	
	Point A	Point B
Treatment efficiency		
COD		Every 3 days
Biogas production	Daily	
Operational stability		
Temperature		Daily
pH		Daily
Alkalinity		Every 3 days
VFA		Every 3 days
Biogas composition	Every 3 days	
Sulphate		Weekly

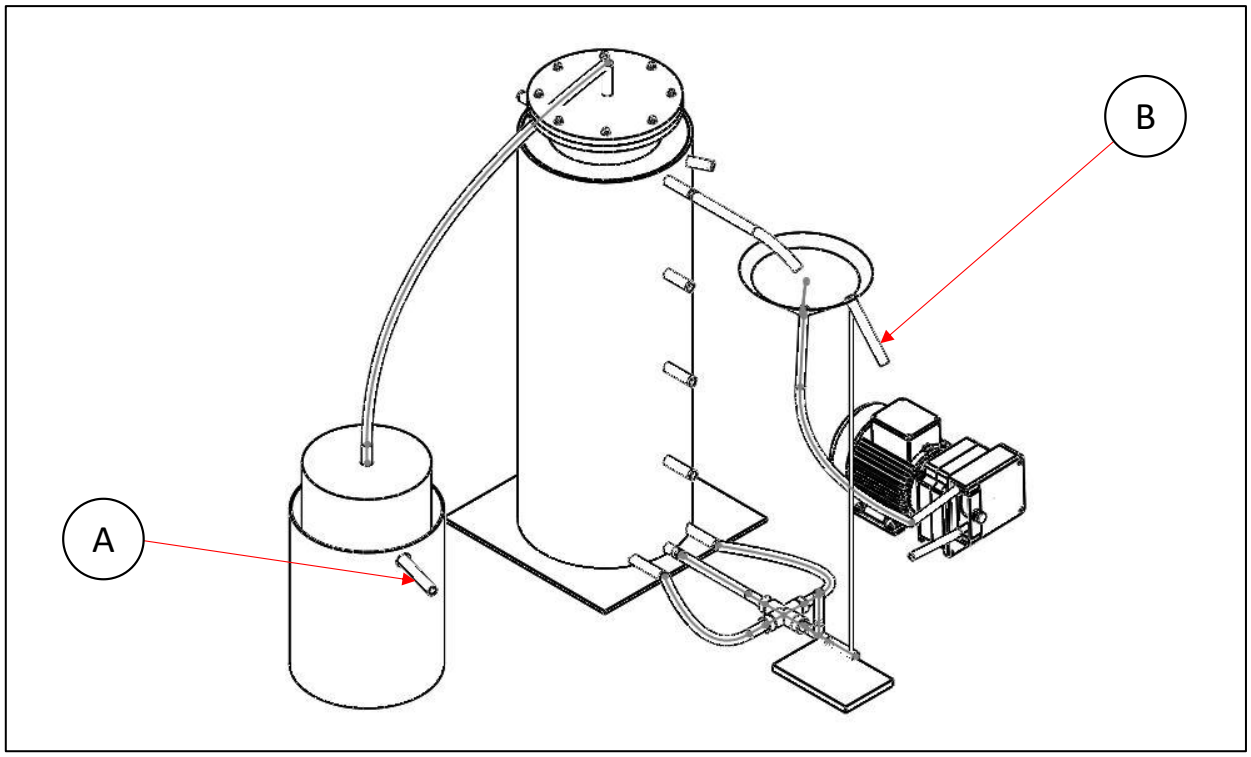

Figure 5.1: Sampling points used on UASB for evaluation of pre-treatments.

5.3 Results and discussion

5.3.1 Batch pre-treatments of SEC

Treatment with Laccase11 and PolyDADMAC reduced the COD concentration by 11% and 18%, respectively, compared to the control (Table 5.2). In turn, the control relative to the untreated SEC, was associated with an 8% decrease in COD concentration due to the change in pH. Therefore, it appears that Laccase11 and PolyDADMAC treatments reduced the COD concentration only by 3% and 10%, respectively. The BOD, lactic acid and furfural concentrations showed no substantial differences among the three batches and the untreated SEC. As the BOD consists mostly of VFAs, furfural and lactic acid, it appears if most of the COD reduction might have been related to the removal of lignosulphonate and polyphenols.

The alkalinity did not vary notably among the batches and untreated SEC (Table 5.2), although Laccase11 removed some alkalinity (420 mg/L) compared to the control. The alkalinity of all three batches remained within the favourable ranges identified in Chapter 3. The VFAs were also reduced by the different pre-treatments but remained within the favourable ranges for methane production. No substantial change in the sulphate content was noted for the pre-treated batches, except for the PolyDADMAC treated batch which removed 36% of the sulphates compared to the control batch. The sulphate decrease may favour methane production during anaerobic digestion, through reducing the competition between sulphate-reducing bacteria (SRB) and methanogens.

Table 5.2: Characteristics of pre-treated SEC for the evaluation of UASB reactors.

Composition	Treatment			
	SEC*	Control**	Laccase11	PolyDADMAC
Chemical oxygen demand (total) (mg/L)	19 000	17 530	15 580	14 310
Biochemical oxygen demand($_5$) (mg/L)	13 200	12 270	12 410	12 620
Lignosulphonate (mg/L)	4 400	4 370	3 080	1 990
Polyphenols (mg/L)	4 800	4 700	3 230	2 120
Alkalinity (mg/L)	1 600	1 720	1 300	1 800
Volatile fatty acids (mg/L)	5 209	4 223	3 774	3 736
Lactic acid (mg/L)	4 184	4 103	4 166	3 630
Furfural (mg/L)	1 250	1 242	1 289	980
Sulphate as SO_4 (mg/L)	2 342	1 935	1 810	1 230
pH	1.8	7.0	7.0	7.0

*SEC as characterised in Chapter 3 as received from the pulp mill.
** SEC from the pulp mill that was adjusted to pH 7.

5.3.2 Evaluation of the effects of pre-treated SEC on reactor performance

To evaluate the effects of PolyDADMAC and Laccase11 pre-treatments on reactor performance, the pre-treatments were compared using the same OLR. The focus was on reducing reactor size rather than improving COD removal and methane yield. For the SEC treated with PolyDADMAC and Laccase11, a volumetric hydraulic loading (VHL) that was 1.09-fold and 1.13-fold larger than that of the control, respectively, were required. These values yielded the same OLR as the control. During the first period (OLR = 6.5 kg_{COD}/m^3d) when the reactors stabilised, Reactors 1 and 2 removed a maximum of 63% of the COD load, and Reactor 3 removed 61% (Figure 5.2). During the first 15 days of treatment, a steady increase in the COD removed in Reactors 1 and 2 was observed, whereas COD removal in Reactor 3 remained steady. The increases could be the result of a smaller concentration of inhibitory compounds applied to these reactors each day, compared to the control.

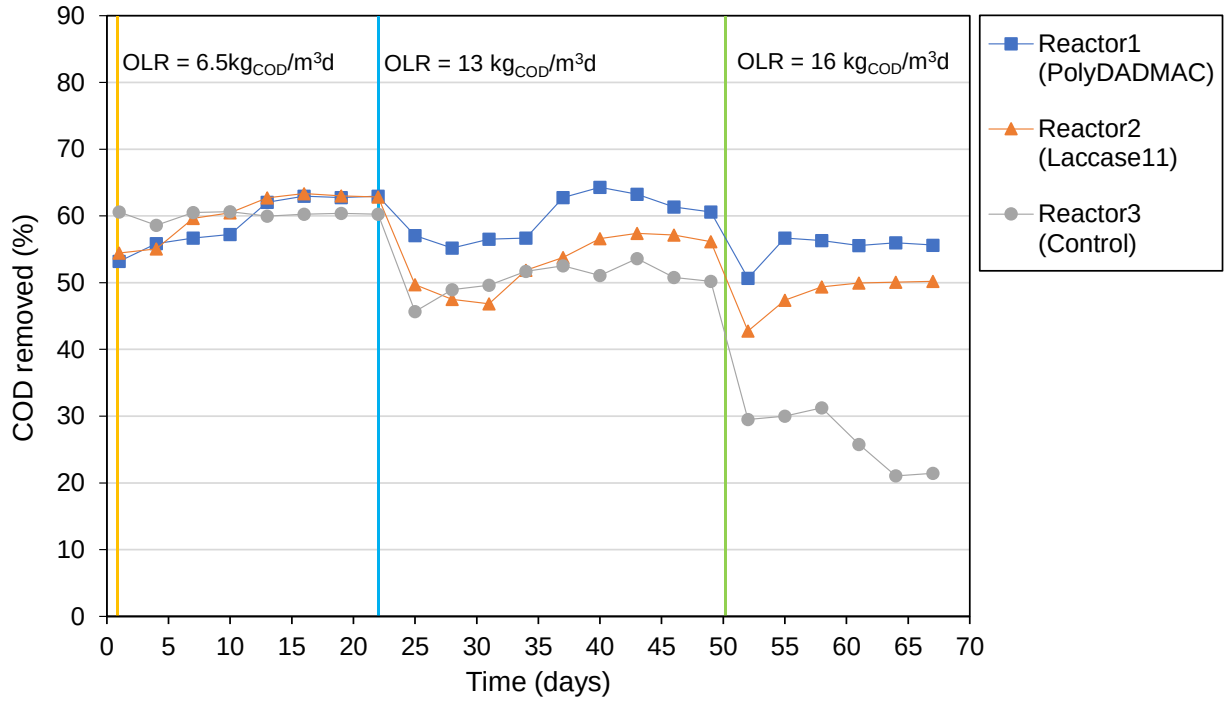

Figure 5.2: Percentage of COD removed in each reactor, for three OLRs of pre-treated SEC.

Also during the first OLR period, the specific methane yield of Reactor 1 reached values of up to 0.35 m^3/kg_{COD}, Reactor 2 up to 0.32 m^3/kg_{COD} and Reactor 3 up to 0.24 m^3/kg_{COD} (Figure 5.3).The specific methane yield of Reactor 1 during this period compared well with the theoretical maximum of 0.35 m^3/kg_{COD} and the results from similar studies on evaporator condensates (Driessen *et al.*, 2000, Hall and Cornacchio, 1988).

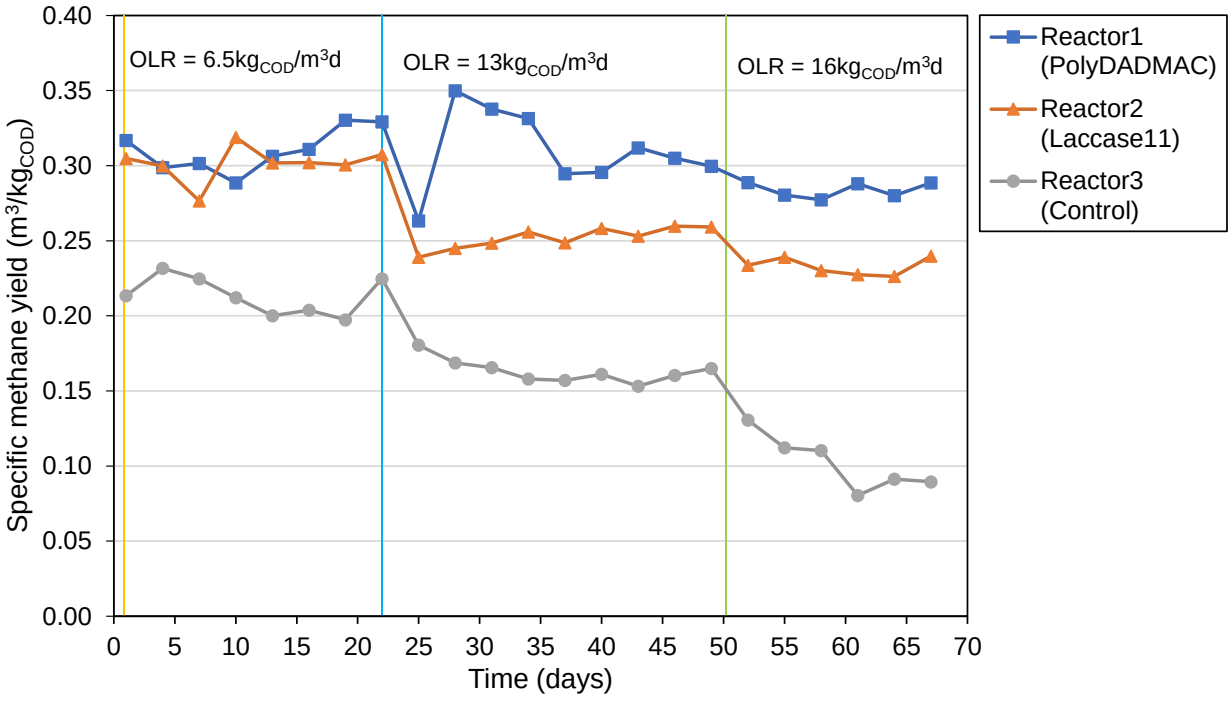

Figure 5.3: Specific methane yield of the three reactors during the different OLR phases.

The lower specific methane yields of Reactor 2 and Reactor 3 could be ascribed to higher quantities of lignosulphonate, polyphenols and sulphate in these reactors, given the higher quantities of these compounds in the respective feeds at the same OLR (Table 5.2). The larger quantity of sulphates increases competition between SRB and methanogens because they use the same substrates to produce sulphite and $H_2S_{(g)}$ (Chernicharo, 2007). This reduces the amount of methane produced and the performance of the anaerobic biomass, due to the toxicity of H_2S dissolved in the liquid.

The percentage of sulphate removed during the first OLR phase was on average the highest for Reactor 1, followed by Reactor 2 and then Reactor 3 (Figure 5.4). The high amount removed by Reactor 1 indicates that the SRB quantity was enough to handle the smaller amount of sulphates fed to this reactor. By contrast, the larger amounts fed to Reactor 2 followed by Reactor 3 were too high for the SRB to reduce effectively. The amount of sulphate that accumulated in these two reactors may thus have reduced their performance.

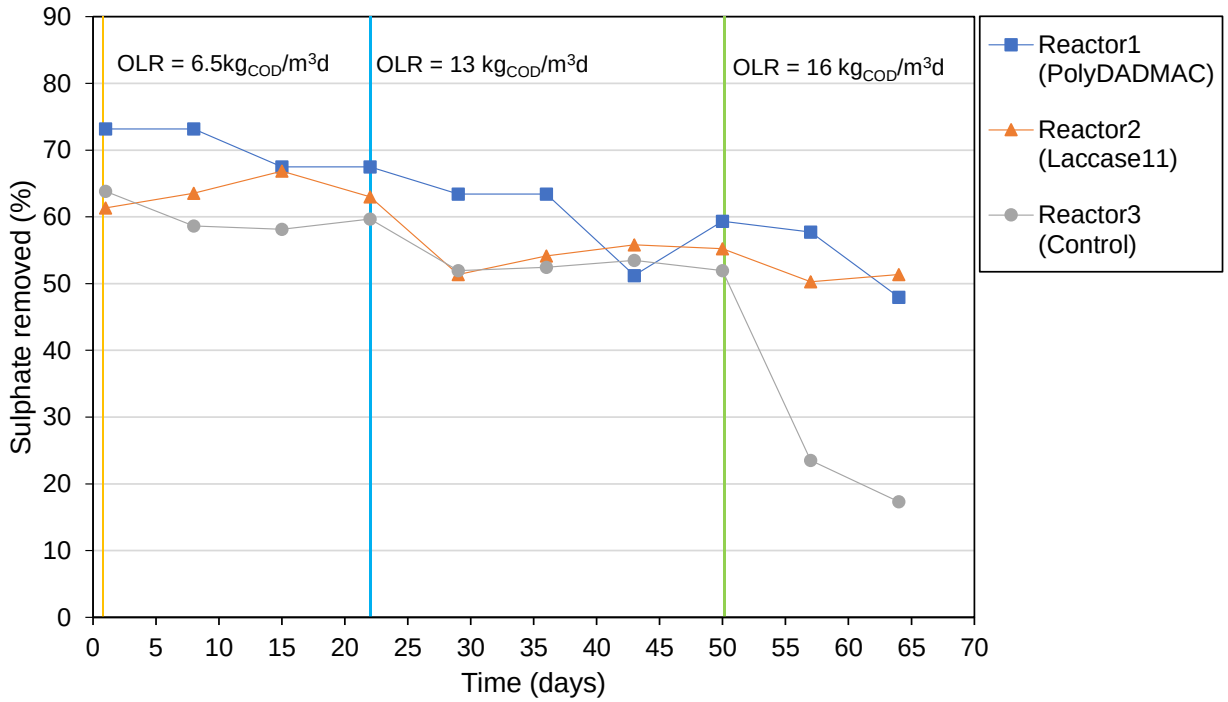

Figure 5.4: Percentage of sulphate removed by each reactor during the OLR phases.

Figure 5.5 illustrates that during the first OLR phase, the amounts of $H_2S_{(g)}$ produced by Reactors 2 and 3 were higher than that of Reactor 1. The increased toxicity from $H_2S_{(g)}$ could partially explain the lower percentages of COD removed in Reactors 2 and 3.

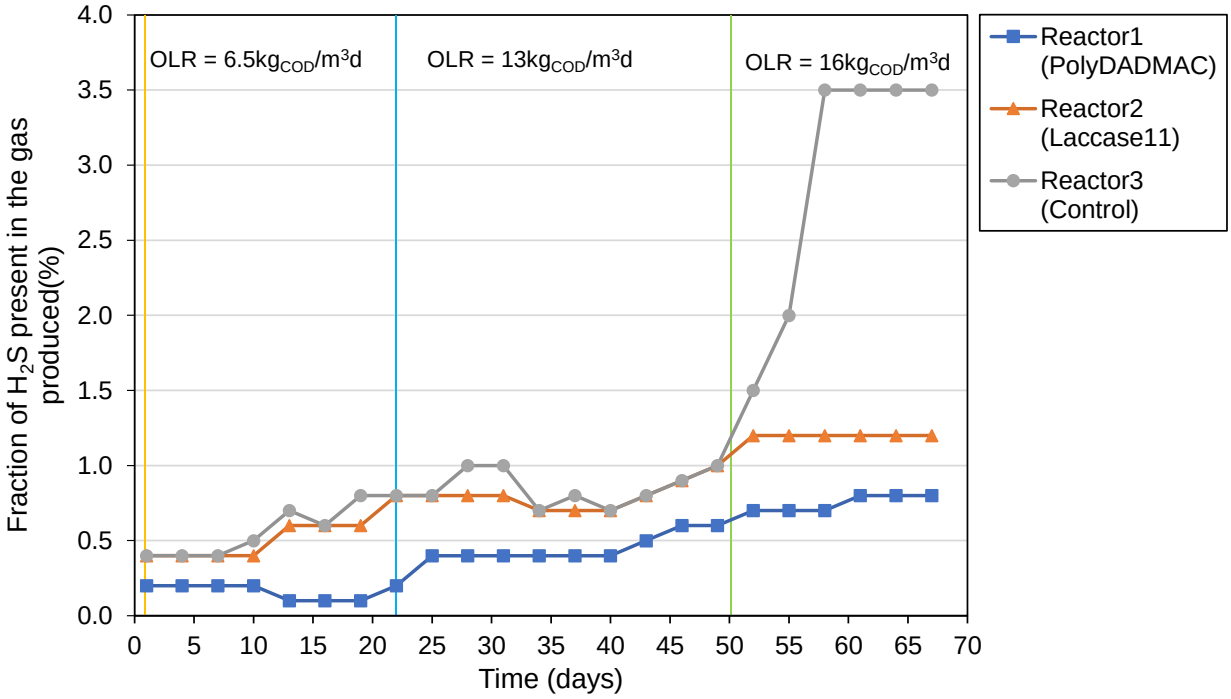

Figure 5.5: The fraction of $H_2S_{(g)}$ produced in each reactor during the OLR phases.

During the second OLR phase, a sharp decline was observed in the amount of COD removed, for all the reactors (Figure 5.2). After 6 days it appeared that all three reactors recovered from the rapid increase in OLR and an increase in COD removal was observed. During this OLR phase, Reactor 1 had an average COD-removal percentage of 60%, Reactor 2 had 52% and Reactor 3 had a removal of 50%. The decline in the percentage of COD removed for all three reactors, compared to Phase 1, might have been due to the higher quantity of sulphates, polyphenols and lignosulphonate fed. The amount of sulphates removed during this period also declined, possibly as a result of a higher load of sulphates fed (Figure 5.4) and increased toxicity caused by $H_2S_{(g)}$ production (Figure 5.5). The specific methane yield also declined during this phase, with the averages being 0.31, 0.25 and 0.16 m^3/kg_{COD} for Reactors 1, 2 and 3, respectively. The decline in specific methane yield could again be related to increased toxicity and competition between the SRB and methanogens. During this OLR, Reactor 1 generated an average of 16.0 L_{CH4}/d, followed by Reactor 2 and 3 with 11.4 L_{CH4}/d and 7.1 L_{CH4}/d, respectively (Appendix K).

The decline in the amount of VFAs removed during Phase 2 (Figure 5.6 B) indicated that the reactors were becoming increasingly ineffective. In addition, during this phase the pH (Figure 5.6 C) and alkalinity (Figure 5.6 A) decreased, as can be expected with an increase in VFA concentrations. Reactor 3 was the most ineffective during Phase 2, this reactor removed on average 45% of VFAs. In studies by Dufresne *et al.*, (2001) and Driessen *et al.*, (2000) on sulphur-containing wastewater, VFA removals were above 90%.

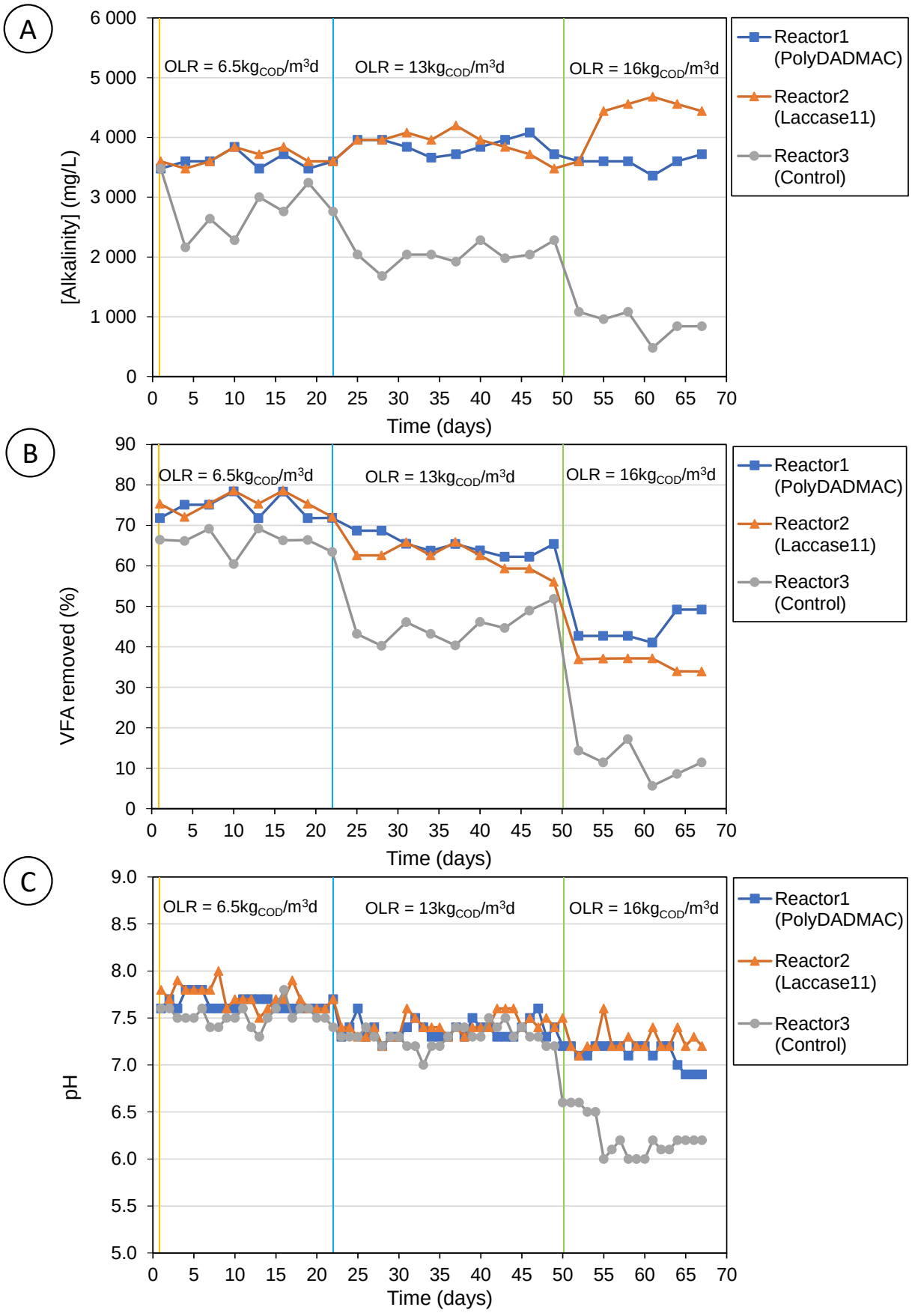

Figure 5.6: The influence of different OLRs on (A) alkalinity, (B) VFAs and (C) pH in the three reactors.

During the last OLR phase, namely a change to 16 kg_{COD}/m^3d, similar relations between the various parameters were noted as for the previous two changes in OLR. The quantity of COD removed declined gradually in Reactors 1 and 2 (Figure 5.2), with Reactor 1 remaining more effective (by 7%). The specific methane yield also declined; averages of 0.28 m^3/kg_{COD} for Reactor 1 and 0.23 m^3/kg_{COD} for Reactor 2 were noted (Figure 5.3). Reactor 3 seemed to become "sour" after the OLR increase. The COD removed in Reactor 3 was on average 28% (Figure 5.2) and the specific methane yield was 0.10 m^3/kg_{COD} (Figure 5.3). The $H_2S_{(g)}$ made up approximately 3% of the gas content in Reactor 3 (Figure 5.5), which was considerably high (Lettinga *et al.*, 1996). In Reactor 3, the VFA reduction was below 10% and thus the pH and alkalinity decreased (Figure 5.6).

CHAPTER 6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

In this study, various pre-treatment methods were evaluated for their capacity to improve sulphite evaporator condensate (SEC) for anaerobic digestion. The focus of the pre-treatment methods used in this study was to increase the molecular weights of polyphenols and lignosulphonate, and thus to remove these compounds from the wastewater. The influence of pre-treatments on the other constituents of SEC was also evaluated. The various pre-treatment methods were then evaluated on bench-scale upflow anaerobic sludge blanket (UASB) reactors to determine the exact effects on anaerobic digestion. In this chapter, the conclusions and recommendations from the research are presented.

6.2 Conclusions

6.2.1 Conclusions from Chapter 3

All laccase formulations except DeniLite® II S have polymerised lignosulphonate and polyphenols at the conditions tested. Of all the enzymes tested, treatment with Laccase 11 resulted in the highest degree of polymerisation and consequently removed the most polyphenols and lignosulphonates. Under optimal conditions and with an enzyme dosage of 0.5 µL/mL, Laccase 11 removed 40% of polyphenols and 32% of lignosulphonates.

Both chitosan and PolyDADMAC removed more than 60% of polyphenols. For treatment with both coagulants at dosages above 0.5 mg/L, significantly less VFAs were removed compared to dosages below 0.5 mg/L. At higher PolyDADMAC and chitosan dosages, polyphenols and lignosulphonate formed larger complexes. Treatment with 0.5 µL/mL PolyDADMAC was the most effective for removing polyphenols (62%), whereas 1.0 µL/mL PolyDADMAC was the most effective for removing lignosulphonate (57%), at the conditions tested. However, no significant differences were noted in the effectiveness of both coagulants. The cost of PolyDADMAC was five times less than that of chitosan per weight unit; therefore, PolyDADMAC appeared more efficient.

Pre-treatment with either Laccase 11 or PolyDADMAC may be beneficial for anaerobic treatment. Laccase 11 removed fewer polyphenols and less lignosulphonate but it works in a substrate-specific manner, whereas PolyDADMAC removed some of the potential substrates for anaerobic digestion. A bench-scale anaerobic test should be performed to determine the exact effects of these pre-treatments.

6.2.2 Conclusions from Chapter 4

During the construction and commissioning of the reactors, many potential problems with leakages, blockages and flow variations were remediated. Different sludges and pipe diameters were used during this phase. The final design and construction of the reactors enabled them to retain the granulated sludge at an upflow velocity of 0.5 m/h; hence, the SEC was effectively mixed with the sludge. The separation unit allowed the three phases to separate effectively, with only a few solids lost to the effluent. The heating jacket worked well and displayed only small deviations (±1°C) from the set temperature of 35°C. The recycle line pumped the correct volumes and the gas chamber seemed to work effectively.

During the start-up of the reactors, all the reactors responded similarly to changes in the OLR. All three reactors could process an OLR of 6 kg_{COD}/m^3d, with at least 60% of the influent COD removed. The changes in VFAs, alkalinity and pH responded as expected, with little deviation among the reactors. As the reactors were allowed to acclimate, VFA removal increased and may be a good indication of the activity of methanogens and the stability of the reactors. Therefore, the reactors were ready for evaluation at higher loading rates and with pre-treated SEC.

6.2.3 Conclusions from Chapter 5

The Laccase11 and PolyDADMAC pre-treatments removed more than 30% and 50%, respectively, of inhibitory compounds from the SEC, without decreasing BOD considerably. These pre-treatments thus enabled higher VHL rates to the respective reactors, compared to the control. The SEC treated with PolyDADMAC allowed the VHL to increase 1.13-fold to yield the same OLR as the control. At the highest and last stable OLR for all three reactors (13 kg_{COD}/m^3d) the SEC treated with PolyDADMAC had a specific methane yield twice the value of the control, and 10% more COD was removed. Also during this period, the PolyDADMAC reactor generated more than double the amount of methane gas compared to the control. Therefore, the SEC treated with PolyDADMAC was the most effective pre-treatment and can decrease the size of a UASB required to treat a SEC stream. Further investigation on pilot-scale should be conducted to consider all the mass and energy balances involved, to determine the financial and environmental feasibility of such a pre-treatment.

6.3 Recommendations

A wider range of laccase formulations and coagulants should be tested for the removal of phenol-containing compounds. The experimental parameters, such as the pH, temperature, incubation period and dosage should be varied in a factorial experiment to determine the optimal conditions.

It was reported many times in literature that the major parameter for the increase in molecular weight of the substrate, was the concentration of the substrate. If it would be practical to increase the concentration of the polyphenols and lignosulphonate in the SEC stream, much higher removal of these two compounds could be observed, when treated with laccase.

Different methods should be developed to analyse the concentration and molecular weight of lignosulphonate in a SEC stream. Large experimental errors were observed (Appendices A – H) and may have been caused by interferences by polyphenol compounds with similar molecular weights as lignosulphonate.

This study only gives an indication of how effective the pre-treatment methods are on a bench-scale. Further evaluation on a pilot-scale treatment plant would be necessary to determine the capital expenditure of a pre-treatment facility and UASB reactor. A mass and energy balance would also be required to determine the feasibility of the pre-treatments.

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APPENDIX A. ANOVA FOR POLYPHENOLS IN ENZYME-SCREENING EXPERIMENTS

Table A.1: Polyphenol concentrations (g/L) measured for three replications of each treatment combination during enzyme screening experiments.

Enzyme formulation	Incubation for 1 h				Incubation for 12 h			
	r1	r2	r3	average	r1	r2	r3	Average
Novozym®51003	5.02	3.89	4.05	4.32	4.47	6.20	3.59	4.50
Laccase11	3.31	3.29	3.50	3.37	3.58	4.13	3.69	3.55
Laccase3	4.96	4.67	4.80	4.81	4.99	4.73	4.34	4.76
DeniLite® II S	6.77	5.87	4.76	5.80	5.47	4.88	5.53	5.58
Control	5.29	5.14	5.69	5.37	5.49	5.39	5.59	5.42

Table A.2: ANOVA for polyphenols in enzyme-screening experiments.

ANOVA	Degrees of freedom	Sum of squares	Mean sum of squares	F (calculated)	F (4;20;0.05)	Significance
Enzymes	4	15.04	3.76	10.48	2,87	*
Time	1	0.04	0.04	0.10	4,35	ns
Interaction	4	0.96	0.24	0.67	2,87	ns
Cells		16.03				
Error	20	7.18	0.36			
Total	29	23.21				

*: Significant difference between treatment means at $p \leq 0.05$
ns: No significant difference between treatment means at $p \leq 0.05$

Tukey's test

q (5; 20; 0.05) = 4.232

Q = 1.03

APPENDIX B. ANOVA FOR LIGNOSULPHONATE IN ENZYME-SCREENING EXPERIMENTS

Table B.1: Lignosulphonate concentrations (g/L) measured for three replications of each treatment combination during enzyme screening experiments.

Enzyme formulation	Incubation for 1 h				Incubation for 12 h			
	r1	r2	r3	average	r1	r2	r3	Average
Novozym®51003	4.02	3.82	3.96	3.93	4.82	3.87	3.13	3.94
Laccase11	3.01	3.32	3.22	3.18	2.23	3.20	3.01	2.82
Laccase3	4.09	3.93	5.02	4.35	4.08	3.87	4.05	4.00
DeniLite® II S	2.88	3.63	3.48	3.33	4.33	3.71	4.69	4.24
Control	4.47	4.17	4.73	4.46	4.47	4.17	4.73	4.46

Table B.2 ANOVA for lignosulphonate in enzyme-screening experiments.

ANOVA	Degrees of freedom	Sum of squares	Mean sum of squares	F (calculated)	F (4;20;0.05)	Significance
Enzymes	4	7.22	1.81	9.29	2.87	*
Time	1	0.01	0.01	0.06	4.35	ns
Interaction	4	1.63	0.41	2.10	2.87	ns
Cells		8.87				
Error	20	3.89	0.19			
Total	29	12.76				

*: Significant difference between treatment means at $p \leq 0.05$
ns: No significant difference between treatment means at $p \leq 0.05$

Tukey's test

q (5; 20; 0.05) = 4.23

Q = 0.76

APPENDIX C. ANOVA FOR MOLECULAR WEIGHT OF LIGNOSULPHONATE IN ENZYME-SCREENING EXPERIMENTS

Table C.1: Lignosulphonate molecular weight (g/mol) measured for three replications of each treatment combination during enzyme screening experiments.

Enzyme formulation	Incubation for 1 h				Incubation for 12 h			
	r1	r2	r3	Average	r1	r2	r3	Average
Novozym®51003	3265.28	3163.83	3136.04	3188.38	3208.34	3322.12	2935.74	3174.24
Laccase11	4120.23	4200.87	4140.25	4153.79	4233.66	3947.55	3988.00	4112.05
Laccase3	2943.75	3010.00	3058.96	3004.24	2875.09	3176.88	3248.70	3045.38
DeniLite® II S	2445.34	2455.85	2428.19	2443.13	2490.60	2376.92	2407.60	2435.37
Control	2498.57	2317.61	2903.38	2573.19	2498.57	2317.61	2903.38	2573.19

Table C.2: ANOVA for molecular weight of lignosulphonate in enzyme-screening experiments.

ANOVA	Degrees of freedom	Sum of squares	Mean sum of squares	F (calculated)	F (4;20;0.05)	Signifi-cance
Enzymes	4.00	10399755.49	2599938.87	87.84	2.87	*
Time	1.00	826.05	826.05	0.03	4.35	ns
Interaction	4.00	29342.47	7335.62	0.25	2.87	ns
Cells		10429924.01				
Error	20.00	592002.94	29600.15			
Total	29.00	11021926.95				

*: Significant difference between treatment means at $p \leq 0.05$
ns: No significant difference between treatment means at $p \leq 0.05$

Tukey's test

q (5; 20; 0.05) = 4.23

Q = 297.25

APPENDIX D. ANOVA FOR POLYPHENOLS IN OPTIMISATION EXPERIMENTS USING LACCASE11

Table D.1: Polyphenol concentrations (g/L) measured for three replications of each treatment combination during optimisation experiments.

Enzyme concentration (µL/mL)	pH 5				pH 7			
	r1	r2	r3	Average	r1	r2	r3	Average
0.00	4.84	4.28	4.01	4.38	4.84	4.28	4.01	4.38
0.05	3.93	3.98	3.56	3.82	3.80	3.61	3.52	3.64
0.10	3.37	3.70	3.21	3.43	3.39	3.46	3.35	3.40
0.50	2.70	2.69	2.80	2.73	2.62	2.75	2.57	2.65
1.00	3.01	3.04	2.23	2.76	2.91	3.15	2.14	2.73
3.00	2.62	2.80	3.31	2.91	2.58	2.72	3.28	2.86

Table D.2: ANOVA for polyphenols in optimisation experiments using Laccase11.

ANOVA	Degrees of freedom	Sum of squares	Mean sum of squares	F (calculated)	F (4;20;0.05)	Significance
Enzymes	5.00	13.29	2.66	25.00	2.62	*
pH	1.00	0.03	0.03	0.31	4.26	ns
Interaction	5.00	0.03	0.01	0.06	2.62	ns
Cells		13.35				
Error	24.00	2.55	0.11			
Total	35.00	15.90				

*: Significant difference between treatment means at $p \leq 0.05$
ns: No significant difference between treatment means at $p \leq 0.05$

Tukey's test

q (6; 24; 0.05) = 4.37

Q = 0.58

APPENDIX E. ANOVA FOR LIGNOSULPHONATE IN OPTIMISATION EXPERIMENTS USING LACCASE11

Table E.1: Lignosulphonate concentrations (g/L) measured for three replications of each treatment combination during optimisation experiments.

Enzyme concentration (µL/mL)	pH 5				pH 7			
	r1	r2	r3	Average	r1	r2	r3	Average
0.00	3.81	3.89	3.79	3.83	3.89	4.11	3.69	3.89
0.05	2.95	4.24	3.40	3.53	2.76	3.80	3.69	3.42
0.10	2.91	3.90	3.32	3.38	2.72	3.62	3.50	3.28
0.50	2.23	2.22	2.35	2.27	2.13	2.29	2.08	2.17
1.00	2.60	2.63	1.67	2.30	2.48	2.77	1.56	2.27
3.00	2.13	2.35	2.96	2.48	2.09	2.25	2.92	2.42

Table E.2: ANOVA for lignosulphonate in optimisation experiments using Laccase11.

ANOVA	Degrees of freedom	Sum of squares	Mean sum of squares	F (calculated)	F (4;20;0.05)	Significance
Enzymes	5.00	14.86	2.97	14.96	2.62	*
pH	1.00	0.03	0.03	0.15	4.26	ns
Interaction	5.00	0.03	0.01	0.03	2.62	ns
Cells		14.92				
Error	24.00	4.77	0.20			
Total	35.00	19.69				

*: Significant difference between treatment means at $p \leq 0.05$
ns: No significant difference between treatment means at $p \leq 0.05$

Tukey's test

q (6; 24; 0.05) = 4.37

Q = 0.80

APPENDIX F. ANOVA FOR MOLECULAR WEIGHT OF LIGNOSULPHONATE IN OPTIMISATION EXPERIMENTS USING LACCASE11

Table F.1: Lignosulphonate molecular weight (g/mol) measured for three replications of each treatment combination during optimisation experiments.

Enzyme concentration (µL/mL)	pH 5				pH 7			
	r1	r2	r3	Average	r1	r2	r3	Average
0.00	2498.57	2317.61	2903.38	2573.19	2511.53	2330.57	2916.34	2586.15
0.05	2683.10	2690.24	2854.24	2742.52	2696.06	2703.19	2867.19	2755.48
0.10	2835.83	2989.13	3101.23	2975.40	2848.79	3002.09	3114.19	2988.36
0.50	4123.81	4298.01	4123.90	4181.91	4136.77	4310.97	4136.86	4194.87
1.00	4001.23	4199.13	4238.96	4146.44	4014.19	4212.09	4251.91	4159.40
3.00	4120.00	4200.00	4140.00	4153.33	4132.96	4212.96	4152.96	4166.29

Table F.2: ANOVA for molecular weight of lignosulphonate in optimisation experiments using laccase11.

ANOVA	DOF	Sum of squares	Mean sum of squares	F (calculated)	F (4;20;0.05)	Significance
Enzymes	5.00	18054477.08	3610895.42	149.28	2.62	*
pH	1.00	1511.24	1511.24	0.06	4.26	ns
Interaction	5.00	0.00	0.00	0.00	2.62	ns
Cells		18055988.32				
Error	24.00	580531.74	24188.82			
Total	35.00	18636520.06				

*: Significant difference between treatment means at $p \leq 0.05$
ns: No significant difference between treatment means at $p \leq 0.05$

Tukey's test

q (6; 24; 0.05) = 4.37

Q = 277.47

APPENDIX G. ANOVA FOR POLYPHENOLS IN COAGULATION EXPERIMENTS

Table G.1: Polyphenol concentrations (g/L) measured for three replications of each treatment combination during coagulation experiments.

Coagulant dosage (mg/L)	Chitosan				PolyDADMAC			
	r1	r2	r3	average	r1	r2	r3	average
0.0	5.33	5.39	5.38	5.37	5.33	5.39	5.38	5.37
0.1	4.98	5.09	5.12	5.06	4.02	4.20	4.08	4.10
0.5	3.68	3.60	2.02	3.10	2.83	2.76	0.58	2.06
1.0	2.59	2.56	1.01	2.05	3.41	3.43	0.64	2.49
2.0	5.36	5.44	1.65	4.15	5.38	5.59	0.96	3.98

Table G.2: ANOVA for polyphenols in coagulation experiments.

ANOVA	DOF	Sum of squares	Mean sum of squares	F (calculated)	F (4;20;0.05)	Significance
Enzymes	4.00	41.67	10.42	5.96	2.87	*
Coagulants	1.00	0.91	0.91	0.52	4.35	ns
Interaction	4.00	2.45	0.61	0.35	2.87	ns
Cells		45.03				
Error	20.00	34.94	1.75			
Total	29.00	79.97				

*: Significant difference between treatment means at $p \leq 0.05$
ns: No significant difference between treatment means at $p \leq 0.05$

Tukey's test between concentrations

q (5; 20; 0.05) = 4.23

Q = 3.23

Tukey's test between coagulants

q (2; 20; 0.05) = 2.95

Q = 2.25

APPENDIX H. ANOVA FOR LIGNOSULPHONATE IN COAGULATION EXPERIMENTS

Table H.1: Lignosulphonate concentrations (g/L) measured for three replications of each treatment combination during coagulation experiments.

Coagulant dosage (mg/L)	Chitosan				PolyDADMAC			
	r1	r2	r3	average	r1	r2	r3	average
0.0	3.95	3.99	4.03	3.99	3.95	3.99	4.03	3.99
0.1	3.84	3.88	3.93	3.88	4.14	4.18	4.21	4.18
0.5	3.27	3.31	3.34	3.31	2.75	3.37	3.41	3.17
1.0	2.35	2.18	2.50	2.34	1.64	1.76	2.02	1.81
2.0	3.03	4.04	3.61	3.56	2.34	2.35	3.09	2.59

Table H.2: ANOVA for lignosulphonate in coagulation experiments.

ANOVA	DOF	Sum of squares	Mean sum of squares	F (calculated)	F (4;20;0.05)	Significance
Coagulants	4.00	15.38	3.84	58.88	2.87	*
pH	1.00	0.54	0.54	8.27	4.35	*
Interaction	4.00	1.44	0.36	5.51	2.87	*
Cells		17.36				
Error	20.00	1.31	0.07			
Total	29.00	18.67				

*: Significant difference between treatment means at $p \leq 0.05$
ns: No significant difference between treatment means at $p \leq 0.05$

Tukey's test between concentrations

q (5; 20; 0.05) = 4.23

Q = 0.62

Tukey's test between coagulants

q (2; 20; 0.05) = 2.95

Q = 0.44

APPENDIX I. ANOVA FOR VOLATILE FATTY ACIDS IN COAGULATION EXPERIMENTS

Table I.1: Concentrations of volatile fatty acids (g/L) measured for three replications of each treatment combination during coagulation experiments.

Coagulant dosage (mg/L)	Chitosan				PolyDADMAC			
	r1	r2	r3	average	r1	r2	r3	average
0.0	5.23	5.29	5.28	5.27	5.23	5.29	5.28	5.27
0.1	5.06	5.02	5.08	5.05	5.10	4.29	5.01	4.80
0.5	3.10	3.29	3.19	3.19	3.06	3.00	3.30	3.12
1.0	3.55	3.84	3.94	3.78	3.49	4.40	3.59	3.83
2.0	4.15	4.10	4.29	4.18	3.98	3.99	3.83	3.93

Table I.2: ANOVA for volatile fatty acids in coagulation experiments.

ANOVA	DOF	Sum of squares	Mean sum of squares	F (calculated)	F (4;20;0.05)	Significance
Enzymes	4.00	18.80	4.70	87.13	2.87	*
Coagulants	1.00	0.08	0.08	1.57	4.35	ns
Interaction	4.00	0.12	0.03	0.54	2.87	ns
Cells		19.01				
Error	20.00	1.08	0.05			
Total	29.00	20.08				

*: Significant difference between treatment means at $p \leq 0.05$
ns: No significant difference between treatment means at $p \leq 0.05$

Tukey's test between concentrations

q (5; 20; 0.05) = 4.23

Q = 0.57

Tukey's test between coagulants

q (2; 20; 0.05) = 2.95

Q = 0.40

APPENDIX J. RAW DATA OF THE THREE REACTORS DURING START-UP

Table J.1: Raw COD, VFA and alkalinity data during start-up of the reactors.

Time (days)	COD (mg/L)			VFA (mg/L)			Alkalinity (mg/L)		
	React1	React2	React3	React1	React2	React3	React1	React2	React3
0	12000	11300	12200	1305	1428	1550	2520	2280	2160
1	11300	11000	11160	1550	1305	1427	2160	2520	2400
3	10100	10200	10000	940	1061	938	2760	2760	2880
6	8800	9000	8900	942	699	821	2520	2640	2520
9	6300	6240	6000	1040	821	821	2424	2580	2580
12	10200	10100	10300	1200	1198	1321	960	1080	960
15	8100	8050	8000	1198	1196	1318	1080	1320	1200
18	6000	6350	6500	831	707	830	1560	1800	1680
21	9000	8560	9350	1306	1185	1307	2400	2400	2280
24	8200	8300	8600	1063	1062	940	2520	2640	2760
27	6350	6800	6150	1026	941	1063	2556	2640	2520

Table J.2: Raw pH and temperature data during start-up of the reactors.

Time (days)	pH			Temperature (°C)		
	React1	React2	React3	React1	React2	React3
0	7.1	7.1	7.2	35	35	35
1	7.3	7.2	7.2	35	36	36
3	7.4	7.4	7.3	36	35	36
6	7.3	7.4	7.4	35	35	36
9	7.4	7.4	7.4	35	35	35
12	7.1	7.1	7.0	35	35	35
15	7.2	7.2	7.1	35	35	35
18	7.6	7.5	7.4	36	36	34
21	7.7	7.7	7.7	35	34	34
24	7.8	7.7	7.8	35	35	35
27	7.8	7.8	7.8	35	35	35

APPENDIX K. RAW DATA FOR THE EFFLUENT OF THE THREE REACTORS DURING EVALUATION

Table K.1: Raw COD, VFA and alkalinity data during reactor evaluation.

Time (days)	COD (mg/L)			VFA (mg/L)			Alkalinity (mg/L)		
	React1	React2	React3	React1	React2	React3	React1	React2	React3
1	6700	7100	6910	1053	931	1416	3480	3600	3480
4	6320	7000	7260	931	1053	1429	3600	3480	2160
7	6200	6290	6920	931	931	1304	3600	3600	2640
10	6120	6160	6900	808	808	1670	3840	3840	2280
13	5430	5810	7020	1053	930	1300	3480	3720	3000
16	5300	5710	6970	809	808	1423	3720	3840	2760
19	5330	5765	6945	1053	931	1419	3480	3600	3240
22	5300	5790	6965	1052	1052	1544	3600	3600	2760
25	6145	7835	9525	1169	1411	2398	3960	3960	2040
28	6415	8180	8950	1169	1411	2523	3960	3960	1680
31	6220	8285	8830	1292	1289	2277	3840	4080	2040
34	6200	7500	8470	1354	1411	2398	3660	3960	2040
37	5330	7200	8320	1293	1288	2520	3720	4200	1920
40	5110	6760	8575	1352	1411	2275	3840	3960	2280
43	5260	6640	8130	1411	1533	2338	3960	3840	1980
46	5530	6680	8630	1410	1535	2156	4080	3720	2040
49	5640	6830	8730	1293	1658	2033	3720	3480	2280
52	7060	8920	12360	2140	2382	3617	3600	3600	1080
55	6200	8200	12270	2140	2374	3739	3600	4440	960
58	6250	7890	12050	2140	2373	3496	3600	4560	1080
61	6360	7800	13010	2203	2371	3986	3360	4680	480
64	6300	7780	13840	1899	2494	3861	3600	4560	840
67	6350	7760	13770	1897	2495	3740	3720	4440	840

Table K.2: Raw data of methane, carbon dioxide and hydrogen sulphide gas generated during reactor evaluation.

Time (days)	$CH_{4(g)}$ (L/d)			$CO_{2(g)}$ (L/d)			$H_2S_{(g)}$ (L/d)		
	React1	React2	React3	React1	React2	React3	React1	React2	React3
1	7.30	7.17	5.59	0.39	1.27	0.30	0.02	0.03	0.02
4	7.23	7.12	5.56	0.38	1.26	0.29	0.02	0.03	0.02
7	7.40	7.11	5.40	0.65	1.26	0.47	0.02	0.03	0.02
10	7.16	8.32	5.56	0.38	1.48	0.29	0.02	0.04	0.03
13	8.24	8.17	5.17	1.12	1.45	0.71	0.01	0.06	0.04
16	8.48	8.25	5.30	0.94	1.47	0.59	0.01	0.06	0.04
19	8.98	8.17	5.13	1.97	1.45	0.71	0.01	0.06	0.05
22	8.98	8.29	4.94	1.98	1.48	0.88	0.02	0.08	0.05
25	12.99	10.23	7.09	2.87	1.82	1.26	0.06	0.10	0.07
28	16.69	10.01	7.09	3.44	1.78	1.27	0.08	0.10	0.08
31	16.50	10.01	7.06	3.89	1.78	1.26	0.08	0.10	0.08
34	16.24	11.43	7.03	4.08	2.03	1.25	0.08	0.09	0.06
37	15.98	11.53	7.10	4.53	2.05	1.27	0.08	0.10	0.07
40	16.43	12.60	7.09	3.62	2.24	1.26	0.08	0.10	0.06
43	17.03	12.50	7.07	3.76	2.23	1.26	0.10	0.12	0.07
46	16.14	12.75	7.00	4.07	2.27	1.25	0.12	0.14	0.07
49	15.66	12.50	7.11	4.45	2.23	1.27	0.12	0.15	0.08
52	15.50	10.53	4.11	5.22	1.89	0.74	0.15	0.15	0.07
55	16.85	11.94	3.59	3.73	2.14	0.65	0.15	0.17	0.09
58	16.56	11.98	3.68	4.18	2.14	0.68	0.15	0.17	0.16
61	16.94	11.97	2.21	3.75	2.14	0.41	0.17	0.17	0.09
64	16.60	11.94	2.05	3.68	2.14	0.38	0.16	0.17	0.09
67	17.00	12.69	2.05	3.77	2.27	0.38	0.17	0.18	0.09

Gas volumes are corrected for temperature and pressure.

Table K.3: Raw pH and temperature data during reactor evaluation.

Time (days)	pH			Temperature (°C)		
	React1	React2	React3	React1	React2	React3
1	7.6	7.8	7.6	38.0	33.0	38.0
2	7.7	7.7	7.6	35.5	34.5	36.0
3	7.6	7.9	7.5	36.0	36.0	36.0
4	7.8	7.8	7.5	36.0	36.0	36.0
5	7.8	7.8	7.5	36.0	36.0	36.0
6	7.8	7.8	7.6	36.0	35.5	35.5
7	7.6	7.8	7.4	35.0	36.0	35.0
8	7.6	8.0	7.4	36.0	36.0	36.0
9	7.6	7.6	7.5	35.0	35.0	35.0
10	7.6	7.7	7.5	35.5	35.0	35.0
11	7.7	7.7	7.6	35.0	35.0	35.0
12	7.7	7.7	7.4	35.0	35.0	35.0
13	7.7	7.5	7.3	35.0	35.0	35.0
14	7.7	7.6	7.5	34.0	33.0	33.0
15	7.6	7.7	7.6	35.0	35.0	35.0
16	7.6	7.7	7.8	36.0	34.0	35.0
17	7.6	7.9	7.5	35.0	35.0	35.0
18	7.6	7.7	7.6	35.0	21.0	21.0
19	7.6	7.6	7.6	32.0	24.0	30.0
20	7.6	7.6	7.5	34.0	34.0	34.0
21	7.6	7.6	7.5	35.0	35.0	35.0
22	7.7	7.7	7.4	35.0	35.0	35.0
23	7.3	7.4	7.3	35.0	34.5	35.0
24	7.4	7.4	7.3	35.0	36.0	34.5
25	7.6	7.3	7.3	35.0	35.0	35.0
26	7.3	7.3	7.4	34.0	35.5	35.0
27	7.4	7.4	7.3	35.0	35.5	35.0
28	7.2	7.2	7.2	35.0	35.5	34.5
29	7.3	7.3	7.3	34.0	35.5	35.0
30	7.3	7.3	7.3	35.0	35.5	35.0
31	7.4	7.6	7.2	35.0	35.0	35.0
32	7.5	7.5	7.2	35.0	35.0	34.5
33	7.4	7.4	7.0	34.5	35.0	35.0
34	7.3	7.4	7.2	35.0	35.0	35.0
35	7.3	7.4	7.2	34.5	35.0	35.0
36	7.3	7.3	7.3	35.0	35.0	36.0
37	7.4	7.4	7.4	35.0	35.0	35.0

38	7.3	7.3	7.4	34.0	35.5	36.0
39	7.5	7.4	7.3	35.0	34.5	35.0
40	7.4	7.4	7.3	35.5	35.0	35.0
41	7.4	7.4	7.5	35.0	35.0	35.5
42	7.3	7.6	7.4	35.0	35.0	35.5
43	7.3	7.6	7.5	35.0	35.0	35.5
44	7.3	7.6	7.3	34.0	34.5	35.5
45	7.4	7.4	7.4	35.0	35.0	35.5
46	7.5	7.5	7.3	35.0	35.0	35.5
47	7.6	7.4	7.3	36.0	35.0	35.0
48	7.3	7.5	7.2	34.0	34.5	35.0
49	7.4	7.4	7.2	35.0	35.0	34.5
50	7.2	7.5	6.6	35.0	35.0	34.5
51	7.2	7.2	6.6	34.0	35.0	34.5
52	7.1	7.1	6.6	35.0	35.0	34.5
53	7.1	7.2	6.5	35.0	34.5	34.5
54	7.2	7.2	6.5	34.5	35.0	34.5
55	7.2	7.6	6.0	35.0	35.0	35.0
56	7.2	7.2	6.1	35.0	35.0	35.0
57	7.2	7.2	6.2	35.5	35.5	35.0
58	7.1	7.3	6.0	35.0	34.5	35.0
59	7.2	7.2	6.0	35.0	34.5	35.0
60	7.2	7.2	6.0	35.0	34.5	35.0
61	7.1	7.4	6.2	35.5	34.5	35.0
62	7.2	7.2	6.1	35.5	34.5	35.0
63	7.2	7.2	6.1	35.5	34.5	35.0
64	7.0	7.4	6.2	35.5	34.5	35.0
65	6.9	7.2	6.2	35.5	34.5	35.0
66	6.9	7.3	6.2	35.5	34.5	35.0
67	6.9	7.2	6.2	35.5	34.5	35.0

Table K.4: Raw sulphate data during reactor evaluation.

Time (days)	Sulphate (mg/L)		
	React1	React2	React3
1	330	700	700
8	330	660	800
15	400	600	810
22	400	670	780
29	450	880	930
36	450	830	920
43	600	800	900
50	500	810	930
57	520	900	1480
64	640	880	1600