Integration of Anaerobic Digestion by UASB into a Hybrid Treatment Process of Waste Streams from Paper Recycling and its Potential for Bioconversion of Carbon Dioxide to Methane

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Abstract

Integration of Anaerobic Digestion by UASB into a Hybrid Treatment Process of Waste Streams from Paper Recycling and its Potential for Bioconversion of Carbon Dioxide to Methane.

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Waste management and renewable energies are both major environmental concerns related to the largest global issue of today, climate change. Anaerobic digestion (AD) systems are able to reduce greenhouse gases (GHGs) that lead to climate change by producing biomethane gas to use as renewable energy, while treating organic waste. AD systems can also serve as carbon sinks because they are able to biologically convert excess carbon dioxide dissolved in the culture, into additional methane gas. Continuous experiments were conducted with five-litre UASB reactors that focused on simulating full-scale operating parameters at a Cascades' paper recycling plant, and employed recycling waste streams as substrates. Experiments compared conditions of single and 2 phase anaerobic digestion (2-PAD), liquid and granular sludge beds, influent wastewater and deinking sludge substrates, temperatures between 25-45 °C, hydraulic retention times between 1 to 5 days and organic loading rates between 0.5-5.0 kg COD/m^3-d. Methane yields across all conditions averaged 0.15 m^3 CH_4/kg COD_{rmv} for total COD and 0.24 m^3 CH_4/kg COD_{rmv} for soluble COD. Removal efficiencies consistently averaged 90% for all conditions. After feeding 2-PAD systems CO_2-infused wastewater, successful bioconversion for soluble COD was observed in all organic loading rate conditions, showing between 5-21% higher methane yields for CO_2 conditions, compared to control. Heavy metals from deinking sludge were monitored though no inhibition was observed. Cost analysis showed that adopting AD as a pretreatment could result in up to 1,733,100 CAD/year in additional revenue. Results of this research provide a solid guideline to pursue developing a pilot scale system.

Acknowledgements

I am very grateful for all the help I received along the way on this very long journey. I know I would not have been able to do it without the kindness and instruction of many. Thank you to Hong Guan and Claire Therrien for all the hours you spent solving problems in the lab, and for all you have taught me. You always go above and beyond. Joe Hrib and Luc Demers provided invaluable aid as well. I would also like to acknowledge all the technicians and professionals who have helped me to gather and develop data throughout; namely, Annie Veilleux from Cascades, Claude Kirouac from Lassonde, Steve Questa from Sciex and Peter McCarthy from ADI Systems. Of course thank you also to my supervisor Dr. Mulligan for all the guidance and mentorship she has offered and especially for her patience and understanding about my progress.

In addition, I would like to thank my parents for their love and support and thank you to all my loved ones, furry and otherwise, who have been there for me. I did not mean to end up here but I am glad I did.

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List of Abbreviations

2-PAD: Two Phase Anaerobic Digestion

AD: Anaerobic Digestion

BOD: Biological Oxygen Demand

COD: Chemical Oxygen Demand

GC: Gas Chromatography

GHG: Greenhouse Gas

GWP: Global Warming Potential

HPLC: High Pressure Liquid Chromatography

HRT: Hydraulic Retention Time

IC: Internal Circulation

ICP-MP: Inductively-Coupled Plasma Mass Spectrometry

IPCC: Intergovernmental Panel on Climate Change

OLR: Organic Loading Rate

PACC: Plan d'action sur les changements climatiques

rmv: removed

RNG: Renewable Natural Gas

sol: soluble

SRT: Solids Retention Time

tot: total

TS: Total Solids

TSS: Total Suspended Solids

UASB: Upflow Anaerobic Sludge Bed

VFA: Volatile Fatty Acids

VS: Volatile Solids

VSS: Volatile Suspended Solids

WAS: Waste Activated Sludge

ww: wastewater

Chapter 1 INTRODUCTION

1.1 Problem Statement

Increasing levels of greenhouse gases (GHGs), creation of renewable energy and waste management are all leading environmental concerns. In addition to exemplifying sustainable resource management, proper treatment of waste streams and use of renewable energy can also alleviate GHG emissions that lead to climate change. Climate change is arguably the most urgent global issue of today. In their 2013 global assessment of climate change science, the Intergovernmental Panel on Climate Change (IPCC) concluded with 95 % certainty that human activity is the dominant cause of observed warming since the mid-20th century (IPCC, 2013) . Evidence of unequivocal and unprecedented changes has been observed in the atmosphere and the ocean, diminishing snow and ice, rising sea levels and increasing concentrations of greenhouse gases. Carbon dioxide, released principally from combustion related emissions, is a major contributor to the greenhouse effect that causes climate change. Figure 1.1 illustrates how GHGs interact in the global atmosphere to lead to global warming that causes climate change.

Climate change is a long-term challenge, but one that requires urgent action. This action can take the form of adequate research and development, implementation of sustainable systems, and supportive political policy. Some governments are more mobilized than others on this issue. Fortunately, Quebec has been a leader in North America, and even the world, for its initiatives to reduce greenhouse gas emissions and prevent the effects of climate change. They have done so by setting a number of ambitious sustainability goals, backing a Climate Change Plan of Action (PACC 2013-2020) and establishing a carbon market, among other efforts. As such, this political climate provides a supportive context in which to establish sustainable systems and solutions.

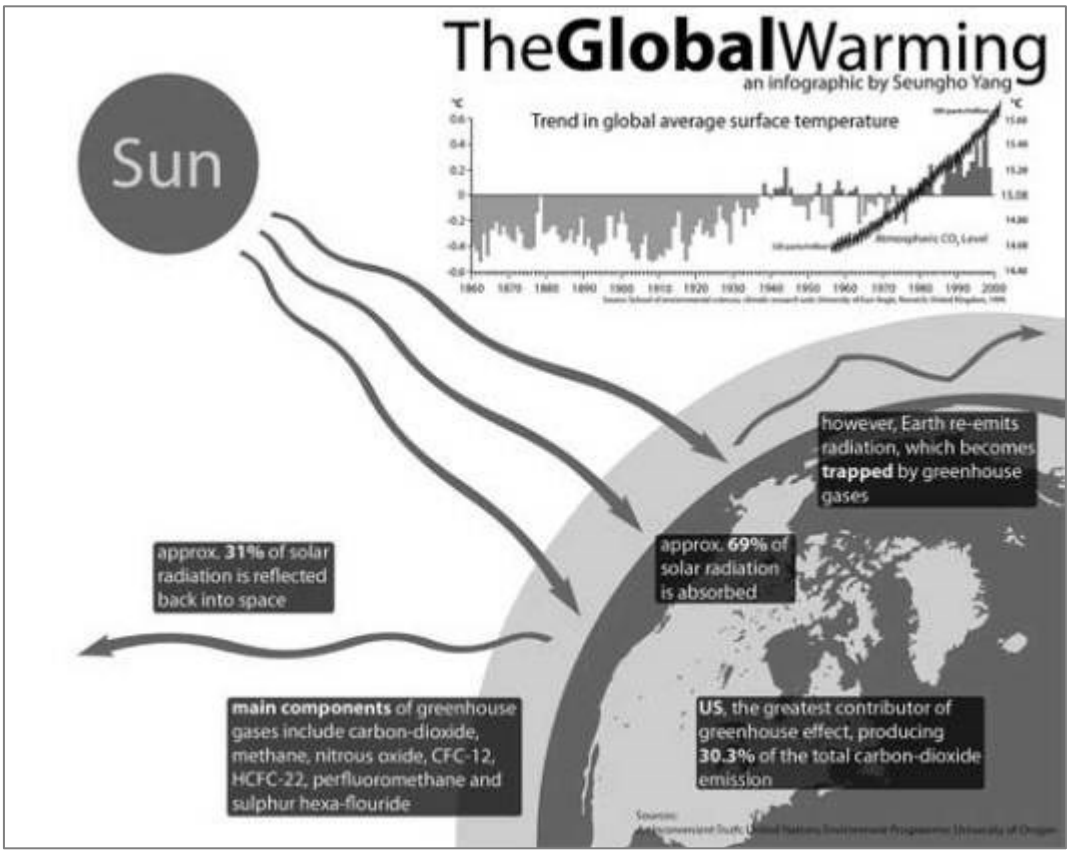


Figure 1.1 Greenhouse gases and interactions with the atmosphere that cause global warming and lead to climate change. Chart illustrates the increase in temperature proportional to increased CO_2 concentrations in the atmosphere in the recent century (Altenergy Shift, 2012).

1.2 Problem Mitigation

There is no one solution to these problems; actions must be distributed across all factions of modern life. Industry has the opportunity to play a substantial role in leading the transition towards sustainability by modifying their methods to help alleviate these problems. Notably, anaerobic digestion (AD) offers industries a unified and comprehensive means to manage these environmental concerns through treatment of waste streams. Anaerobic digestion is a form of biological waste treatment that uses specially cultivated anaerobic bacteria to degrade the organic waste and convert it into biomethane gas. This project proposes the integration of an anaerobic digestion system into the existing wastewater treatment facility of a paper recycling plant.

Anaerobic digestion uses bacteria to biologically convert organic matter into a source of biomethane in a renewable and carbon neutral system. In fact, this system can even serve as a

sink for excess carbon dioxide (CO_2) emissions, which would make it carbon negative. This work is part of a series of studies investigating anaerobic bacteria's potential for biochemical conversion of CO_2 into methane (CH_4,) in different types of substrate wastewater. The anaerobic digester could serve as a carbon sink for CO_2 emissions, and then the bacterial processes in the digester could convert the CO_2 into additional CH_4 (Alimahmoodi & Mulligan, 2008; Salomoni et al., 2011; Abedi, 2015).

Anaerobic digestion does have some disadvantages. Typically, the process does not reduce the oxygen demand of wastewaters to very low levels. Where regulations require low discharge concentrations, it must be used in conjunction with another form of treatment (Habets & Driessen, 2002; Habets & Driessen, 2007). Some additional disadvantages include poor practical and operational stability, high sensitivity to changes in environmental conditions and toxicants, long retention and start-up times, and undesired sludge dewaterability in some conditions (Amani et al., 2010). However, it is the complexity of the process that is often the largest impediment to its wide implementation. Adequate system design must thoroughly consider all these aspects.

On the other hand, anaerobic digestion of wastewater offers many advantages over more commonly employed aerobic treatment, such as waste activated sludge (WAS). Beyond the creation of calorific biogas, AD has a minimal sludge-feed waste ratio, requires less energy and less space, creates less GHGs, usually has a lower operational cost, and can even decontaminate waste streams of certain pollutants in the proper conditions (Jan et al., 2002; Yenigun & Demirel , 2002; Habets & Driessen, 2007; Liu et al., 2013). The digestate from anaerobic processes is valuable as a safe and fast-acting fertilizer with bioavailable nitrogen (Weiland, 2010). Moreover, previous research has shown that systems combining AD with WAS, known as hybrid processes, showed better and more efficient removal of contaminants as well as reduced GHG emissions and energy costs (Ashrafi et al., 2015). Table 1.1 shows the advantages in energy savings that have been observed for these combined systems used to treat wastewater at a paper recycle mill.

Table 1.1 Typical energy balance comparing complete-aerobic treatment with combined anaerobic–aerobic treatment, both applied on recycle paper effluent (Habets & Driessen, 2007).

	Complete aerobic treatment (MJ/ADT)	Combined anaerobic/aerobic (MJ/ADT)	Energy savings difference (MJ/ADT)
Energy production	0	275	275
Energy consumption	90	20	70
Total balance	−90	+255	345

1.3 Lifecycles of Energy Sources

Overall, anaerobic digestion and the biogas it produces present a very good sustainable solution. This advantage is especially pronounced relative to other types of energy that are typically used, such as hydroelectric power and natural gas. Even sources of energy that are considered sustainable or relatively less carbon intensive can be more environmentally damaging than expected. The Cascades Paper Recycling treatment plant in Kingsey Falls, Quebec currently uses hydroelectricity to power equipment motors and natural gas to power building heating.

1.3.1 Hydroelectricity and Dams

Although hydropower is classified as a renewable energy, it actually has a number of disadvantages associated with the creation of dams and reservoirs that make it an environmentally undesirable option. The creation of a dam can cause much ecological damage and ecosystem disruption by altering the river ecology and flooding huge areas of forest (Nilsson & Berggren, 2000). Recent research has demonstrated that these reservoirs also lead to increased GHG emissions from the surface waters due to decomposing organic matter in the water (Matthews et al., 2005; Maeck et al., 2014; Deemer et al., 2016).

1.3.2 Natural Gas

The use of fossil fuel-derived natural gas used for heating is also often cited as an environmentally preferable fuel due to its relatively low CO_2 emissions on combustion, as compared to oil or coal (Figure 1.2). However, this is complicated when one considers how

emissions are measured. The measures of these greenhouse gas emissions are converted to their global warming potential (GWP) value. This GWP is the ratio of each GHG's warming effect relative to CO_2, i.e. one tonne gas χ = Υ tonnes CO_2. Since each GHG has a distinct life, the IPCC provides standard values calculated over specific time-periods: 20, 100 and 500 years. In its 2007 assessment, the IPCC reported methane to have a GWP of 72 for a 20-year time horizon and this value was updated to a GWP of 86 for the same 20-year horizon in the 2013 IPCC assessment report (IPCC, 2013).

The development of new fracturing technology (fracking) has allowed extraction of previously inaccessible reserves of shale gas, coal bed methane and tight gas. As a result, there has been a surge in the proportion of energy provided by natural gas in recent decades. Although this has reduced CO_2 emissions from combustion, recent studies have documented previously unaccounted for increases in emissions of methane that escape during fracking (Howarth et al., 2011; Schneising et al., 2014; Howarth, 2015). Since methane is a much more powerful greenhouse gas than CO_2, these emissions make a significant difference in the GHG evaluation of fractured natural gas.

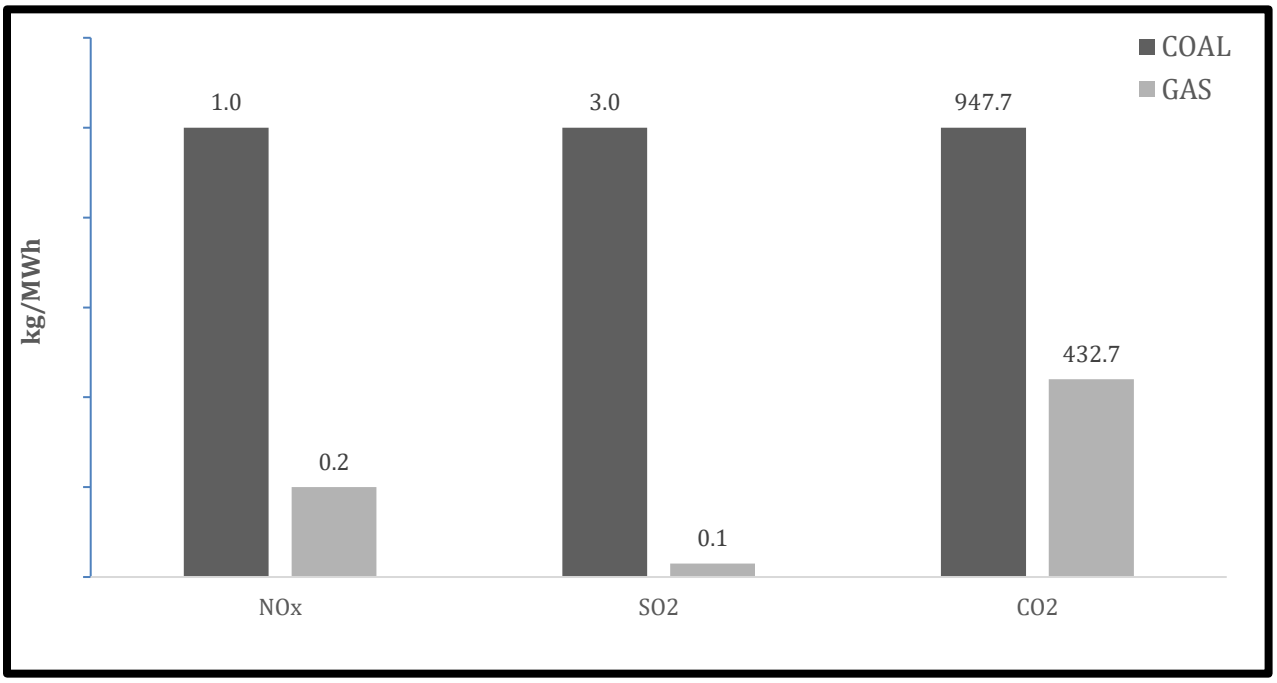


Figure 1.2 Coal vs gas emissions for power generation in 2009 (kg/MWh) (ICF Consulting Canada, 2012).

An accurate assessment of the gases (mostly methane) emitted during these processes requires a lifecycle analysis, i.e., the cumulative quantity of GHGs emitted beginning from feedstock extraction, through distribution, delivery and storage, and up to the use of the finished fuel. Research from recent years, using satellite-based measurements, has provided more accurate estimates of these methane emissions from shale gas extraction (Hayhoe et al., 2002; Schneising et al., 2014). Using a GWP of 86, Figure 1.3 compares the greenhouse gas footprint of shale gas with that of conventional natural gas, oil, and coal by a more comprehensive lifecycle analysis. As illustrated in Figure 1.3 results differ substantially from previous estimates.

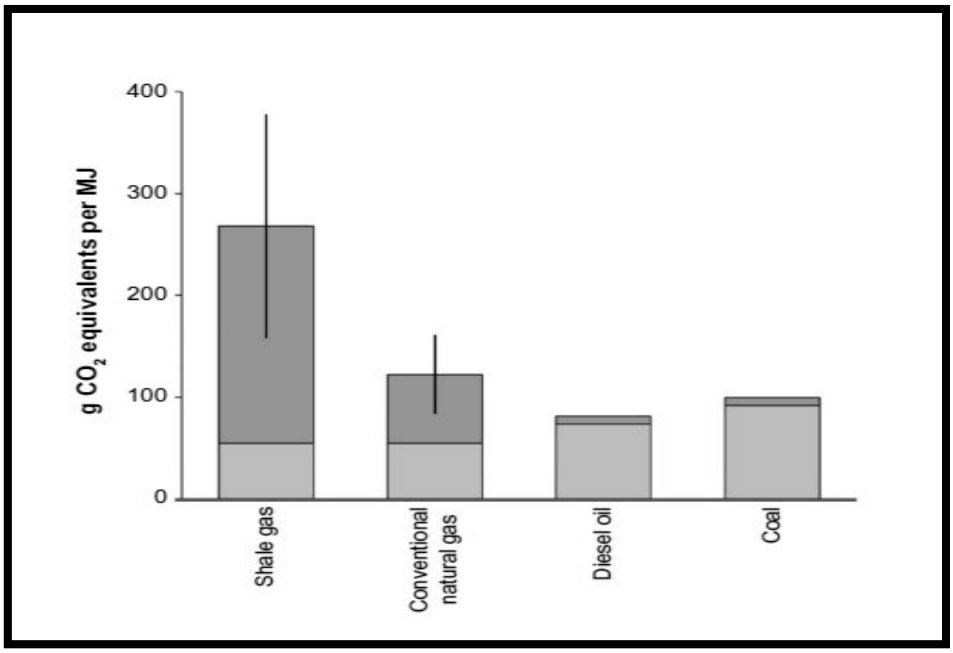


Figure 1.3 GHG footprints of shale gas, conventional natural gas, oil, and coal expressed as g CO_2 equivalents per MJ. Lighter shading indicates direct and indirect emissions of carbon dioxide. Darker shading indicates methane emissions expressed as CO_2 equivalents using a GWP of 86 (Howarth, 2015).

Besides the increased GHG emissions, extraction of shale gas is detrimental to the surrounding environment. This is predominantly due to the fracking fluid required for extraction, which Environment Canada and Health Canada estimate to contain 800 substances, 33 of which have been deemed toxic in other studies (Becklumb et al., 2015). Fracturing operations require mixing these fracking fluids with large amounts of fresh water, which is then contaminated and must be contained or treated. These fracking fluids also endanger

groundwater as they are injected into the shale rock and may leak through fractures into the groundwater above (The Royal Society & the Royal Academy of Engineering, 2012; Expert Panel, 2014).

In this current context, having the capacity to create carbon neutral renewable natural gas onsite, is a premium. Moreover, Quebec's Climate Change Plan of Action includes a ban on organics in landfills beginning in 2021, which aims to prevent GHG emissions from decomposing matter in landfills (MDDEP, 2012; Politique énergétique, 2015). This ban greatly incentivises the development of renewable natural gas (RNG) and its infrastructure. As such, anaerobic digestion is arguably one of the most valuable ways to treat this organic waste. At the same time, the Quebec government has been steadily working to ensure stable and secure access to natural gas across the territory, while Énergir (previously known as Gaz Metro) submitted a request to the Régie de l'énergie to approve a contract to purchase 13-million m^3 renewable biomethane/year for 20 years (Audette, 2016). These factors combine to provide an opportune time to establish this treatment technology.

1.4 General Objective

This research presents a proposal to evaluate the feasibility of integrating anaerobic digestion for industrial waste treatment and bioconversion of CO_2, focusing on waste streams from the paper recycling process.

1.5 Specific Objectives

The specific objectives were designed to find the optimum operating conditions for integrating AD in the wastewater treatment system of Cascades Recycling through experiments with continuous bench-top reactors. These objectives were to:

1) Determine biodegradability of Cascades influent wastewater
2) Determine the biodegradability of Cascades deinking sludge
3) Evaluate and compare operational parameters of the continuous system
4) Evaluate the biomethane potential of the systems

5) Compare the performance of one-phase versus two-phase systems
6) Determine the potential of the system for bioconversion of CO_2 into CH_4
7) Evaluate potential toxicity of the deinking sludge to the AD bacteria and performance
8) Assess the costs and feasibility of such a system.

1.6 Organization of Thesis

This thesis includes 6 chapters as follows:

Chapter 1: Problem Statement and Objectives

Chapter 2: Literature Review

Chapter 3: Methods and Materials

Chapter 4: Results and Discussion

Chapter 5: Economic Analysis

Chapter 6: Conclusions and Recommendations for Future Work

References

Appendices

Chapter 2 LITERATURE REVIEW

2.1 Basic Principles and Mechanisms of Anaerobic Digestion

Anaerobic digestion is one of the most complicated biological processes in the environment (Schink, 1997). The complexity arises from the intricate interrelation of its three multifaceted features: microbiological, operational, and chemical. Anaerobic digestion refers generally to the process where populations of bacteria and archaea decompose and metabolize complex organic molecules found in organic matter, without freely available oxygen. Their enzymatic and metabolic processes ultimately produce gases, the majority of which is methane.

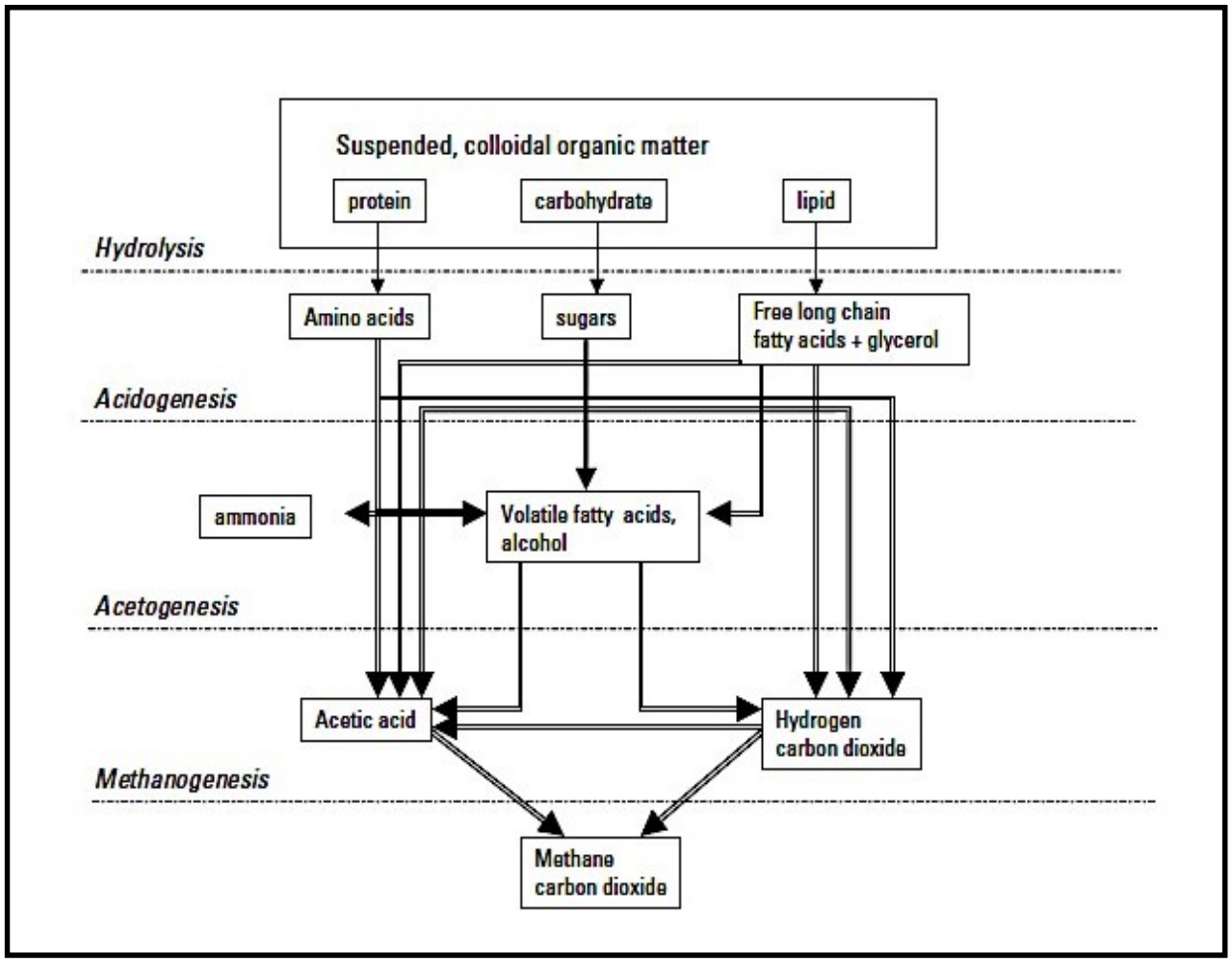


Figure 2.1 Simplified schematic of anaerobic degradation process (Mes et al., 2003)

As seen in Figure 2.1, this process can be broadly divided into four phases, each of which has distinct microbiological populations and processes. These phases are described briefly below (Schink, 1944; Zinder et al., 1984; Dolfing, 1988; Marmara, 2013).

1) Hydrolysis

- Solubilization/liquifaction: cellular lysis of complex and insoluble particles by hydrolytic processes
- Biological decomposition of organic polymers into simpler monomers or dimers, which can pass through cell membranes
- Varies by different parameters such as; (i) particle size (ii) pH (iii) production of enzymes and (iv) diffusion and adsorption of enzymes to particles
- Usually catalyzed by extracellular enzymes through both biological and physico-chemical reactions.

2) Acidogenesis (Fermentation)

- Biodegradation of soluble organic matter into principally: volatile fatty acids (VFAs), alcohols and CO_2 by a heterogeneous microbial population
- Obligatory and facultative anaerobic bacteria are most active
- Most affected by; (i) interspecies hydrogen transfer (ii) pH (iii) hydraulic retention time and (iv) previous acclimation of the anaerobic culture.

3) Acetogenesis

- Oxidation of fermentation products into substrates (acetate, H_2 and CO_2) for methanogens
- Homoacetogenesis: Production of acetate as a sole end product from CO_2 and H_2
- Synthrophic acetogenesis: Anaerobic oxidation of VFAs (such as propionate and butyrate) to acetate and H_2 (requires low H_2 partial pressure)
- Proton reduction.

4) Methanogenesis

- A limited number of organic compounds are used as carbon and energy sources in the production of methane gas (acetate, CO_2 + H_2)
    - Acetotrophic methanogens (produce CH_4 from acetate), almost 65-70%
    - Hydrogenotrophic methanogens (produce CH_4 from CO_2 and H_2)
- Extremely sensitive to temperature, loading rate and pH fluctuations
- Inhibited by a number of organic and inorganic compounds.

Another important process in AD is sulfate reduction. Sulfate (SO_4^{2-}) or sulfite (SO_3^{2-}) can be used by sulphate reducing bacteria (SRB) as an acceptor of electrons released during the oxidation of organic materials under anaerobic conditions. VFAs, several aromatic acids, H_2, methanol, ethanol, glycerol, sugars, amino acids and some phenol compounds are the substrates used in sulfate reduction. The end product is hydrogen sulfide (H_2S). Sulfide can have many roles in AD. The sulfide content of an anaerobic biomass itself is relatively high, approximately 2.5% of a microbial cell, and it is also considered as a micronutrient for methanogens (Amani et al., 2010). Sulfide can also have protective effects against heavy metal toxicity (Lewis, 2010). Two stages of inhibition can exist as a result of sulfate reduction. Primary inhibition is due to competition for common organic and inorganic substrates, which suppresses methane production (Harada et al., 1994). SRB have been show to consistently dominate when in competition with hydrogenotrophic and acetotrophic methanogens (Zinder, 1993; Colleran & Pender, 2002). Secondary inhibition results from the toxicity of sulfide to various bacterial groups (Anderson et al., 1982; Oude Elferink et al., 1994; Colleran et al., 1995; Colleran et al., 1998). The SO_4^{2-}/COD ratio is the critical parameter to monitor for these types of inhibitions. Some interactions of SRB with anaerobic by-products are illustrated in Figure 2.2.

Given its essential role in the process, the performance of an anaerobic digestion system is linked primarily to the structure of the microbial culture in the digester. This microbial community and its performance are a product of three factors, i) the seed sludge used for inoculation, ii) the composition of the feed and iii) the operational process parameters (Guyot et al., 1993; Demirel & Scherer, 2008). It is interesting to note that digesters with a history of good performance and uniform feed, may be more susceptible to upset (caused by high feeding rates or

other operational variability) because they lack the diversity in their microbial community that would facilitate adaptation to varying conditions (McMahon et al., 2001; Meyer & Edwards, 2014).

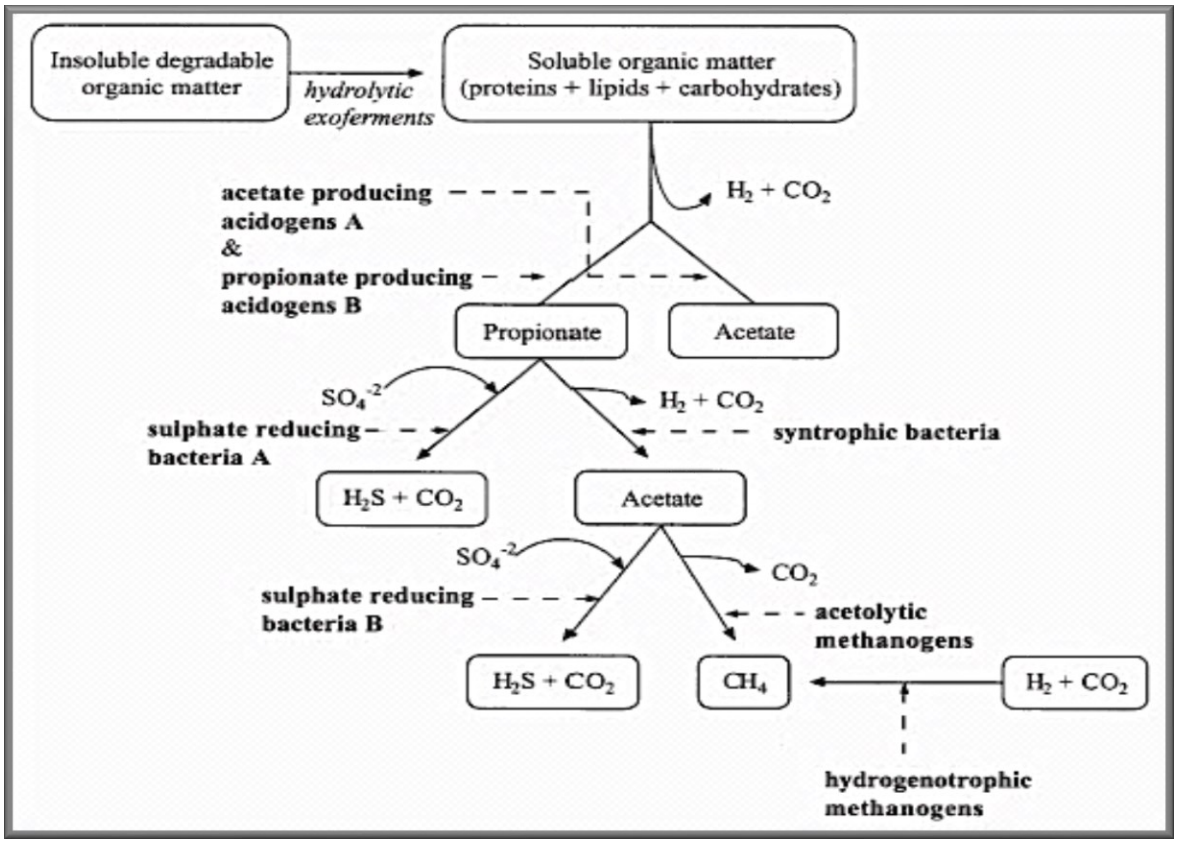


Figure 2.2 Sulphate-reducing bacterial processes (Marmara University, 2013)

Operation of anaerobic digesters usually occurs at either mesophilic or thermophilic temperature ranges. Mesophilic microorganisms are predominantly active between 30 - 38 °C (although also at ambient temperatures between 20 - 45 °C), and thermophilic organisms are active between 50 - 60 °C, even up to 70 °C (Mohd et al., 2015). Mesophilic systems consistently deliver good operational performance, effluent quality and higher stability than thermophilic systems (Parkin & Owen, 1986). They also have lower energy requirements. Thermophilic systems are generally more effective than mesophilic systems at producing high quality biosolids, due to the enhanced hydrolysis of complex organic materials, higher solids destruction efficiency and better dewatering capability (Ahring, 1995; Maibaum & Kuehn,

1999; Kim et al., 2002). Thermophilic conditions are also better able to sustain higher loading rates (Zabranska et al., 2000; Gao et al., 2011; Zheng at al., 2013).

2.2 Types of Anaerobic Bacteria

Decades of research has been devoted to identifying and characterizing the different species of anaerobic bacteria. Their function is very complex and depends not only on the operating conditions but also on their interactions with other competitive or facilitative bacteria. For instance, in the absence of acetotrophic methanogens, which usually convert acetate into CH_4 and CO_2, syntrophic acetate oxidizing (SAO) microbes adopt the role of converting acetate to H_2 and CO_2 which is then converted to CH_4 by a different pathway. Normally, in conditions not limited by hydrogen, these same SAO bacteria reversely produce acetate from H_2 + CO_2 (Zinder, 1994). Amari et al. (2010) list common species of hydrolytic, acidogenic and acetogenic bacteria encountered in anaerobic digestion. An example of these populations can be seen in Table 2.1.

Table 2.1 Products, substrates and an application of typical species of acidogenic bacteria (Amari et al., 2010)

Substrates	Products	Typical species	Gram reaction	Shape	Motility*	Remark
Amino acids	Valerate, Isovalerate, propionate, butyrate,	*Lactobacillus sp.*	+	rod	N	Production of γ-aminobutyric acid
		Eschericia coli	–	rod	M	Synthesis of nitrobenzocyclophospha-mide
	acetate, H_2, Higher fatty acids	*Staphylococcus sp.*	+	sphere	N	
		Bacillus sp.	+	rod	M	
		Pseudomonas sp.	–	rod	M	Biodegradation of mixture of various textile dyes
		Micrococcus sp.	+	sphere	N	
		Eubacterium limosum	+	rod	M	Biotransformation isoflavonoids bio-chanin
		Clostridium sp.	+	rod	M	Hydrogen production from sucrose
		Zymomonas mobiliz	–	rod	M	Ethanol production
Sugars	CO_2, H_2, formate, acetate, butyrate	*Eubacterium sp.*	+	rod	M	Hydrogen production
	CO_2, H_2, formate, acetate, ethanol, lac-tate	*Eschericia coli*	–	rod	M	
	Formate, acetate, etha-nol, lactate	*Bifidobacterium sp.*	+	rod	N	Production of bacteriocin
	Acetate	*Acetobacterium sp.*	–	ellipse	M	Production of vitamin B_{12}
Fatty acids	Valerate, isovalerate, propionate, butyrate, acetate, H_2	*Closrtidium sp.*	+	rod	M	
Alcohols		*Syntrophomonas wolfei*	–	rod	M	Oxidation of fatty acids

*M, motile; N, non-motile.

A representative list of methanogenic bacteria and some identifying characterizations are listed in Table 2.2. Broadly, methanogenic bacteria can be grouped as either hydrogenotrophic (using hydrogen and carbon dioxide as substrates) or acetotrophic (using acetate as a substrate). Figure 2.3 shows examples of each of these types of methanogens. Although there are many more types of hydrogenotrophic methanogens, acetotropic methanogens are much more effective at producing methane (Ferry, 1992).

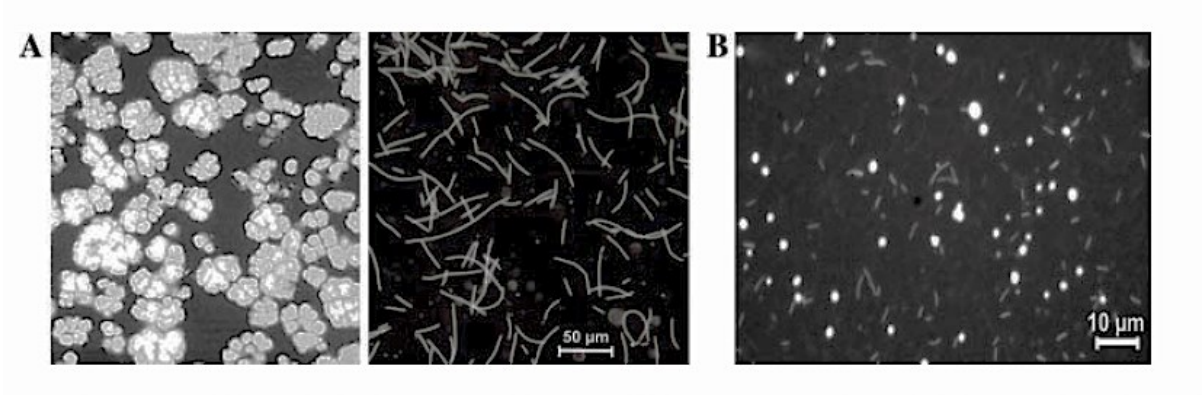


Figure 2.3 Morphology of methanogens by fluorescence microscopy as sampled from a biogas plant. (A) shows acetotrophic methanogens left, *Methanosarcina* and right, *Methanoseata*. (B) shows hydrogeotrophic rods and cocci (Demirel & Scherer, 2008).

2.3 System Performance Parameters

Oxygen demand is an important parameter for determining the amount of organic pollution in water. The chemical oxygen demand (COD) is used to quantify the amount of organic matter in waste streams, to determine the efficacy of treatment, and to predict the potential for biogas production. COD can be separated into several subtypes depending on its characteristics, as illustrated in Figure 2.4. The sum of all products from the AD process must equal the biodegradable COD of the influent substrate. There is no overall reduction of COD in the anaerobic system, only conversion into cellular material or fermentation products, i.e., soluble and gaseous (Eastman & Ferguson, 1981; Boyles, 1997).

Table 2.2 General characteristics of some methanogenic bacteria (Vogels et al. 1988; Boone et al. 1993)

Species	Morphology	Cell width/length (um)	Substrate	Optimal temp (°C)	Optimum pH range
Methanobacterium bryantii	Long rods to filaments	0.5–1.0/1.5	H_2/CO_2	37	6.9–7.2
Methanobacterium formicicum	Long rods to filaments	0.4–0.8/2–15	H_2/CO_2, formate	37–45	6.6–7.8
Methanobacterium thermoalcaliphilum	Rods	0.3–0.4/3–4	H_2/CO_2	58–62	8.0–8.5
Methanothermobacter thermoautotrophicum	Long rods to filaments	0.3–0.6/2–7	H_2/CO_2	65–70	7.0–8.0
Methanothermobacter wolfeii	Rods	0.4/2.4–2.7	H_2/CO_2	55–65	7.0–7.5
Methanobrevibacter smithii	Short rods, short chains	0.6–0.7/1.0–1.5	H_2/CO_2, formate	37–39	n/a
Methanobrevibacter ruminantium	Short rods, short chains	0.7/ 0.8–1.7	H_2/CO_2,, formate	37–39	n/a
Methanothermus fervidus	Short rods	0.3–0.4/1–3	H_2/CO_2, formate	83	< 7.0
Methanothermococcus thermolithotrophicus	Regular to irregular cocci	n/a	H_2/CO_2, formate	65	n/a
Methanococcus voltaei	Regular to irregular cocci	1.5 (diameter)	H_2/CO_2, formate	35–40	6.0–7.0
Methanococcus vannielii	Regular to irregular cocci	1.3 (diameter)	H_2/CO_2, formate	65	7–9
Methanomicrobium mobile	Short rods	0.7/1.5–2.0	H_2/CO_2, formate	40	6.1–6.9
Methanolacinia paynteri	Short irregular rods	0.6/1.5–2.5	H_2/CO_2	40	7
Methanospirillum hungatei	Regular curved rods to long spiral filaments	0.5/7.4	H_2/CO_2, formate	30–40	n/a

Methanosarcina acetivorans	Irregular cocci	n/a	Methanol, acetate	35–40	6.5
Methanosarcina barkeri	Irregular cocci, forming irregular packets	n/a	H_2/CO_2, methanol, methyamines, acetate	35–40	5–7
Methanosarcina mazeii	Irregular cocci, forming cysts and packets	n/a	Methanol, methyamines, acetate	30–40	6–7
Methanosarcina thermophila	Irregular cocci, forming aggregates	n/a	H_2/CO_2, methanol, methyamines, acetate	50	6–7
Methanococcoides methylutens	Irregular cocci	0.8–1.2 (diameter)	Methanol	42	7.0–7.5
Methanosaeta concilii (soehngenii)	Rod	0.8 x 2.5–6.0 (dimensions)	Acetate	35–40	7.0–7.5
Methanosaeta thermophila	Rod	0.8–1.3 x 6.0 (dimensions)	Acetate	55–60	7

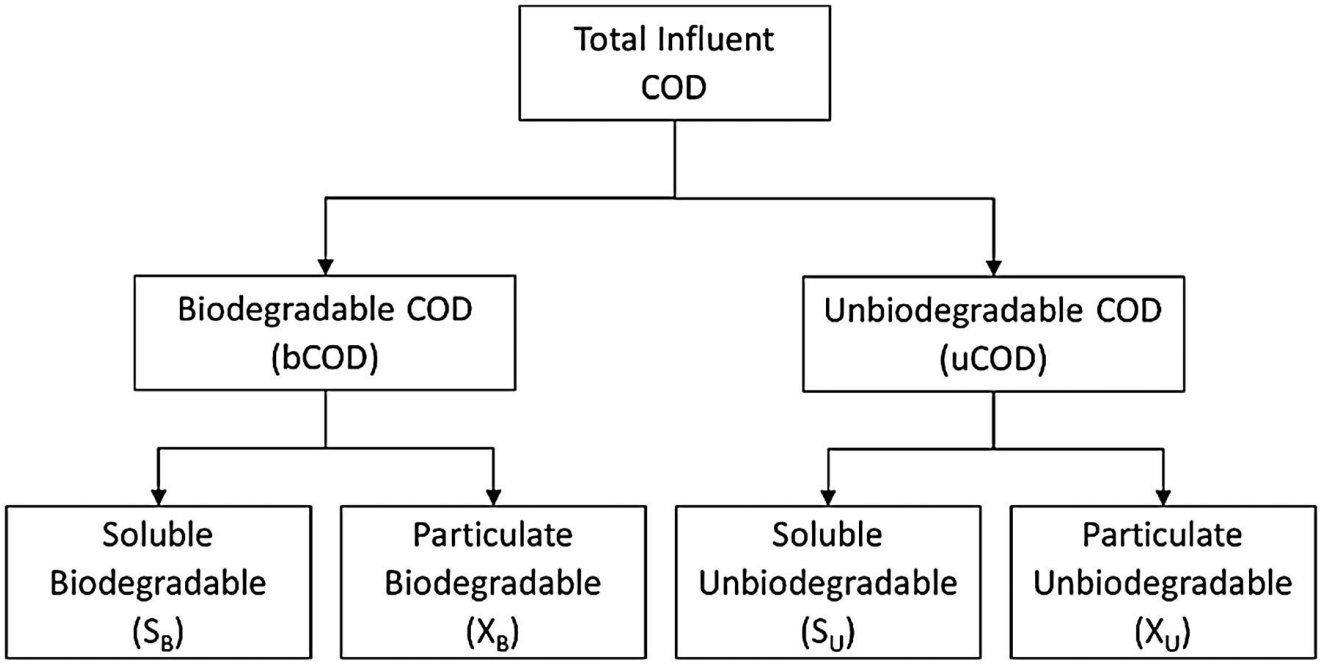


Figure 2.4 Constituent fractions of total COD (Baquero-Rodríguez et al., 2016)

2.3.1 Hydrolysis and Solubilization

However, when the waste stream contains substantial particulate matter or solids, the estimation of biodegradable COD must accurately quantify the liquefaction or solubilization of these solids, represented by Particulate Biodegradable (X_B) in Figure 2.4. This solubilization is accomplished in the initial phases of anaerobic digestion. The hydrolysis step (step one shown in Figure 2.1) degrades both insoluble organic material and high molecular weight compounds such as lipids, polysaccharides, proteins and nucleic acids, into soluble organic substances (e.g. amino acids and fatty acids). The lysis during hydrolysis breaks down of the membranes of cells by enzymatic or osmotic mechanisms. The ruptured cell walls and degraded extracellular polymeric substances are thus made into available organic matter for the acidogenic micro-organisms (Yungin et al., 2010). To maximize the amount of organic carbon solubilized, conditions that favor hydrolytic-enzyme-producing organisms, and the fermentative organisms that support them, must be optimized (Eastman & Ferguson, 1981). The acid fermentation process is illustrated in Figure 2.5.

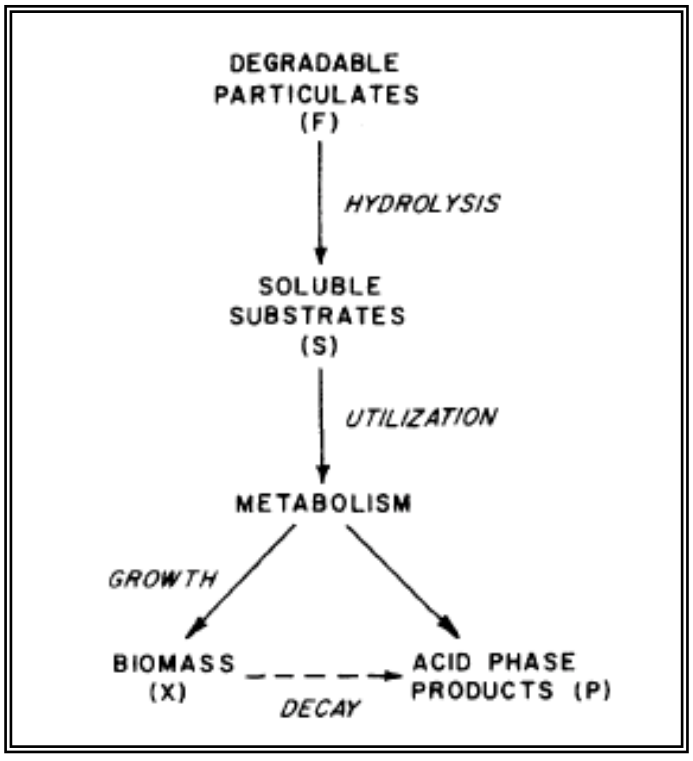


Figure 2.5 Schematic diagram of the acid phase fermentation of particulates (Eastman & Ferguson, 1981).

2.3.2 Biogas and Methane Yield

Theoretical methane yield can be calculated based on the conversion of the biodegradable COD of the feed source. A standard method used to find this yield for engineering purposes is with Equation 2.1. This calculation assumes the COD value of methane to be = 4 g COD, and then determines the gas yield from this amount of COD at standard temperature and pressure. Though this method can be a helpful guideline, it is usually an overestimation of actual operational performance. A more precise means to calculate the theoretical production of biogas is by using the Buswell and Boyle equation for completely degradable organic compounds, according to the chemical composition of the initial influent substrate (Equation 2.2).

$$\mathbf{0.35} * \left(\boldsymbol{COD_{influent}} - \boldsymbol{COD_{effluent}}\right) = \boldsymbol{CH_4} \quad \textbf{Equation 2.1}$$

Typically, biogas is a mixture of methane, 50-60%, carbon dioxide, (20%) and trace amounts of hydrogen sulfide (H_2S), ammonia (NH_3), water (H_2O), nitrogen (N_2), oxygen (O_2), and hydrogen (Table 2.3). However, each substrate produces a different biogas composition (Table 2.4) and each has a different methane yield.

In application, any additional components that may exist in the biogas, such as hydrogen sulfide, carbon dioxide and moisture, must be scrubbed before use. To avoid excessive corrosion and expensive deterioration H_2S should be less than 250 ppm although some estimates allow for as much as 1000 ppm, to prevent damage to gas-utilization units (Wellinger & Lindberg, 1999; Weiland, 2010). Desulfurization is usually done by biological or physical-chemical processes (Schneider et al., 2002; Polster & Brummack, 2009; Siefers, 2010).

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right) \cdot H_2O \Rightarrow$$
$$\left(\frac{a}{2} \frac{b}{8} + \frac{c}{4} + \frac{3d}{b} + \frac{e}{4}\right) \cdot CO_2 + \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} \frac{e}{4}\right) \cdot CH_4 + c \cdot NH_3 + d \cdot H_2S$$

Equation 2.2

Table 2.3 Typical composition of biogas (Wayback Machine, 2018)

Compound	Formula	%
Methane	CH_4	50–75
Carbon dioxide	CO_2	25–50
Nitrogen	N_2	0–10
Hydrogen	H_2	0–1
Hydrogen sulphide	H_2S	0–3
Oxygen	O_2	0–0

Table 2.4 Substrate dependent biogas compositions (Renewable Energy Concepts, 2018)

	CH_4	CO_2	NH_3	H_2S
Carbohydrates (glycerine)	50%	50%		
Fats (tripalmitine)	71%	29%		
Proteins	38%	38%	18%	6%

2.3.3 Nutrients and Micronutrients

Anaerobic bacteria all require certain concentrations of nutrients, minerals and metals to maintain normal cell function. Nitrogen and phosphorous, in soluble form, are required in large quantities by all anaerobic microorganisms. To have a successful anaerobic digestion the ratio of COD to nitrogen to phosphorus (COD:N:P) for high and low strength wastes must be equal or close to the ratios of 1000:7:1 and 350:7:1 (mass: mass:mass), respectively (Speece, 1996). These nutrient concentrations are usually insufficient in industrial wastewater and must be supplemented (Ammary, 2004). Cobalt, iron, nickel, sulfide, selenium, tungsten, molybdenum, barium, magnesium, and sodium are micronutrients required in relatively small quantities by some microorganisms. These micronutrients, however, are usually present in sufficient quantities in municipal and industrial wastes. Nevertheless, the influent should be

analyzed to ensure that sufficient soluble quantities of these nutrients and micronutrients exist before any biological treatment. All deficient minerals and nutrients must be supplemented as necessary.

2.4 Phase separation: Two Phase Anaerobic Digestion

Although they often function collectively in one environment, microbial populations with the anaerobic culture can have distinct ideal conditions. Failure to maintain the balance between these groups of microorganisms is the primary cause of reactor instability (Yenigün & Demirel, 2002). As mentioned briefly, acid forming and methane forming microorganisms differ widely in terms of physiology, nutritional needs, growth kinetics, oxido-reductive activities, pH and sensitivity to environmental conditions (Ghosh et al., 1975; Yenigün & Demirel, 2002; Koutrouli et al., 2009; Salomoni et al., 2011). Thus, it often makes sense for these groups to be cultivated separately, using operating conditions most suited to the distinct bacterial population(s). For these reasons, anaerobic phase separation identifies two distinct population groups. The first includes hydrolytic, acidogenic and acetogenic bacteria, which all thrive under similar conditions, and the second consists of methanogenic bacteria populations that enjoy distinct ideal conditions and are more sensitive to upset. These two groups are commonly called acetogenic and methanogenic bacteria. This is a principle justification for the use of two-phase anaerobic digestion (2-PAD) systems.

These 2-phase systems have shown several advantages over conventional single-stage processes, such as increased stability of the overall process, improved microorganism specialization and better conversion performance (Ghosh et al., 2000; Mata-Alvarez et al., 2000; Oktem et al., 2006; Ponsà et al., 2008; Koutrouli et al., 2009). This is partly due to the shield action against pH and organic load shock provided by the first phase over the methanogenic one, and by a more efficient arrangement of oxido-reductive chemical reactions. Phase separation also allows a more flexible control of retention time, organic loading rate (OLR) and sludge wasting (Demirel & Scherer, 2008; Koutrouli et al., 2009). 2-PAD can also handle high-solid containing waste, since liquefaction occurs along with acidification in the acetogenic reactor, improving COD removal efficiency and solubilization of organics in the

system while reducing the cost of operations (Viéitez et al., 2000; Derbal et al.,2009; Lozano et al., 2009).

In summary, there are 6 main advantages to a 2-PAD system (Ghosh et al., 1975):

1. Optimum environment for each group of organisms
2. Reduction in total reactor volume: reducing capital and operating costs
3. High rate of solid stabilization - increased production rate and methane content in biogas
4. Decreased heat requirement and increased thermal efficiency
5. Suitability for incorporation into existing treatment plant
6. Reduction of nitrogen content of system by de-nitrification of feeds in acid digester.

The disadvantages consist of the need for skilled operation and increased instrumentation for monitoring and control.

2.5 Pulp and Paper Industry

The pulp and paper industry relies primarily on virgin or recovered fibers (RCFs) as raw materials. Although they are usually grouped into one category, the processes for each type of fiber are substantially different. Creating pulp from virgin fibers requires various stages of chemical and mechanical treatments. The process to create pulp from the recovered fibers of existing paper materials is much less resource and energy intensive (Kamali & Khodaparast, 2015).

Motivated by preservation of natural resources, as well as the benefits of reduced emissions and solid wastes, global collection of recovered fibers has increased almost 7 fold since 1970 (FAO, 2012). There are 3 main components to RCF recycling i) pulping, ii) high density screening and iii) deinking (Kamali & Khodaparast, 2015). Pulping converts the waste paper into the RCF by dispersing it in water, forming a pulp and allowing glues and other impurities to separate from the paper or dissolve. Screening removes the high and medium density solids that cannot be processed, such as staples, metal rings, plastics, sand and glass. Once dispersed in the water, the pulp is ready for deinking; the most important step in RCF recycling. The deinking process separates the ink particles from the cellulose fibers through washing or flotation methods and prepares the pulp to be reprocessed into paper products

(Borchardt et al., 1998; Zhenying et al., 2009). If required, fillers are added to supplement the RCF pulp, depending on the quality.

Every year, the pulp and paper industry, as a whole, consumes billions of meters cubed of water and produces huge amounts of highly polluted wastewater that needs to be treated (Toczyłowska-Mamińska, 2017). Figure 2.6 illustrates relative wastewater production by industry around the world. For RCF processing, the pollutants in these waters are directly related to the types of waste papers that are processed. Depending on the source, contaminants can include compounds such paint and ink surfactants, varying amounts of organic/inorganic content, thermoplastic resins and electric-magnetic iron oxide (Zhenying et al., 2009; Guedez & Püttmann, 2014). Moreover, wastewaters often include pulping additive chemicals (such as caustic soda, sodium silicate, hydrogen peroxide and soap) and deinking additives (such as H_2O_2, NaOH, Na_2SiO_3, Na_2CO_3 and surfactants) (Miranda et al., 2009; Monte et al., 2009).

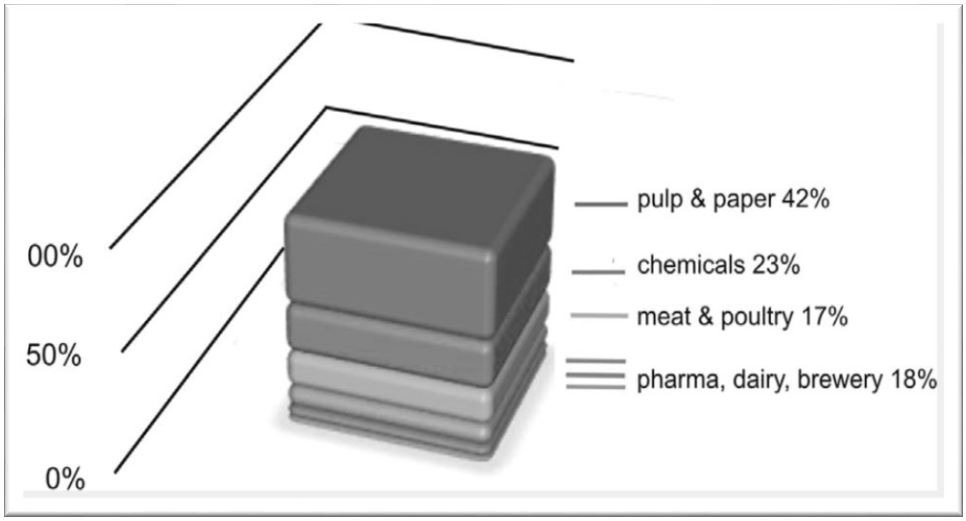


Figure 2.6 Global Industrial Wastewater Production (Urioc, 2015).

2.6 Experimental Set-up

In previous work within the research group, batch tests were conducted with Cascades effluent (Abedi et al., 2015). Batch tests are similar to biomethane potential (BMP) tests, which are helpful to determine the amount of methane, the biodegradability and possibly synergistic

effects of different organic solids and substrates, under different operational conditions. A BMP set-up is illustrated in Figure 2.7. BMP tests can serve as an interesting tool for technical and economical optimization of bio-methane producing plants (Esposito et al., 2012). As such, these batch tests provided a positive indication of the methane potential of the current wastewater and also provided direction about appropriate operating parameters. Subsequently, in the current study research was conducted as continuous experiments using bench-top scale reactors, to simulate operating conditions and parameters of a full size reactor more closely.

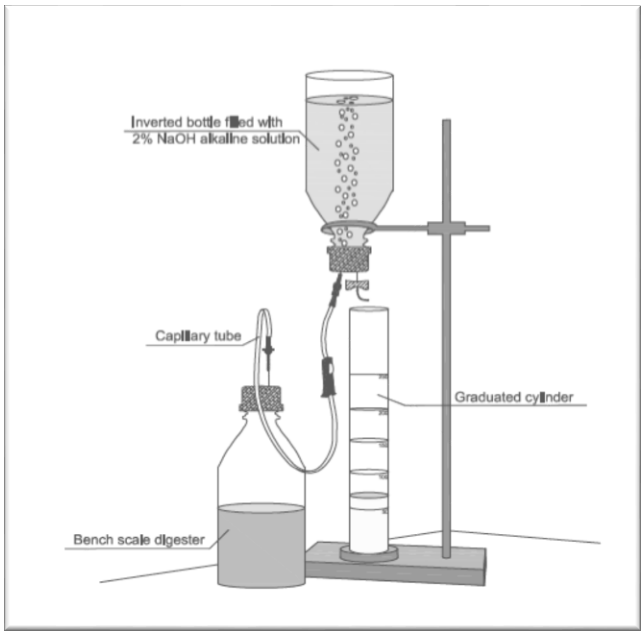


Figure 2.7 Schematic of biomethane potential test set-up (Esposito et al., 2012).

2.7 Types of Anaerobic Digestion Systems and Reactors

There are many types of anaerobic digester systems and different types of sludge that are used in AD treatment methods. Selection of reactor design is a function of the composition and concentration of the waste stream, operational flow, space available and other relevant factors. Figure 2.8 shows a summary of the principle types of reactors used in paper processing treatment. As illustrated in the figure, there are 4 principle types: completely stirred, anaerobic filters, sludge bed reactors and expanded sludge bed reactors. I) Completely

stirred reactors are fully mixed, either mechanically or by gas and may incorporate some sludge recycling. One common type is the bulk volume fermenter (BVF). They can accommodate higher concentrations of solids and usually require minimal sludge wasting (ADI Systems Inc, 2018). II) Anaerobic filters integrate a fixed filter structure inside the reactor on which a biological film of bacteria grow and intercept the passage of water in either an upward or downward flow direction, depending on the model. These reactors are best suited for low to medium concentrations of organic compounds and low concentrations of suspended solids. Flow is usually at a low velocity, and can reverse in direction (Mulligan, 2002). Sludge retention time is very high. III) Sludge bed reactors, usually referred to as Upflow Anaerobic Sludge Blanket (UASB) reactors, have a virtually permanent bed of sludge, usually in granular form, with little to no perceptible sludge growth or wasting. The input is to the bottom of the reactor and flows up first through the sludge blanket, then to a reverse clarifier section of the reactor before exiting from the top of the reactor. Appropriate upflow velocity must be applied. The rising bubbles are usually enough to mix the sludge without the assistance of any mechanical parts. Designs often incorporate sloped walls to deflect material that reaches the top of the tank downwards so that is does not interfere with gas collection. This method can accommodate very high concentrations of organic matter but works best with low concentration of suspended solids, proteins and fats since they may otherwise build-up within the sludge bed and decrease efficiency, although their design can incorporate some degree of fixed solids wasting as particles can settle down through the sludge bed (Habets & Driessen, 2007). IV) The final type of reactor is the expanded sludge bed reactor. This is similar to the UASB except that the flow is at a much higher rate, and occasionally includes mechanical mixing, so that the sludge bed is moved to be perpetually suspended in the reactor and does not have a chance to settle. The elimination of dead zones in the sludge bed and increased points of contact between the wastewater and the sludge combine to result in a more complete degradation (Mulligan, 2002; Water Technologies).

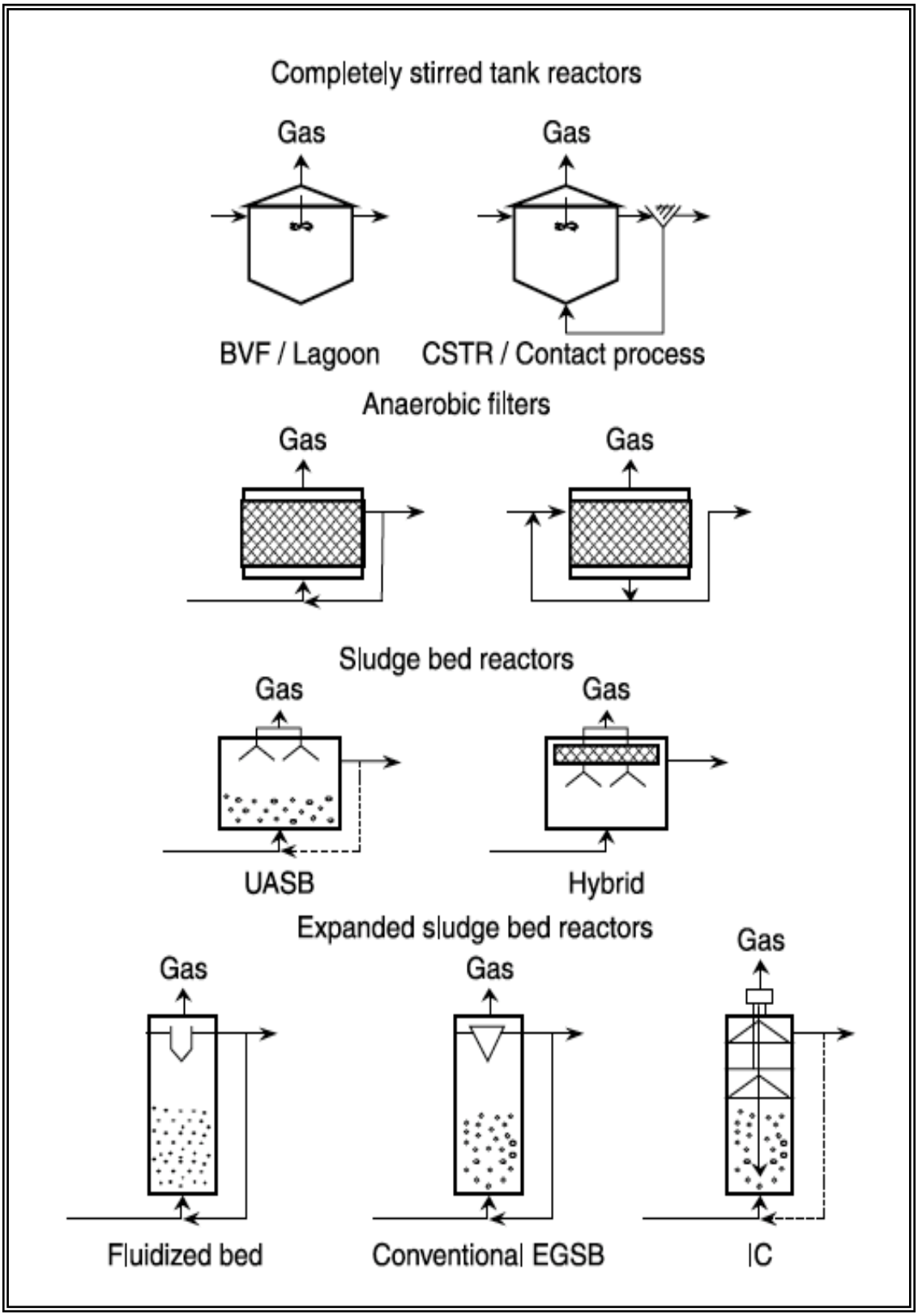


Figure 2.8 Principle types of reactors used in paper processing treatment (Habets & Driessen, 2007)

2.7.1 Upflow Anaerobic Sludge Bed (UASB)

The UASB is a well-established process for large-scale industrial wastewater treatment. The current research will focus on UASB reactors in general, although the discussion here also extends to expanded sludge bed models. These reactors have been shown to be stable and efficient (Liu et al., 2003; Hulshoff Pol et al., 2004; Jeong et al., 2005; Amani et al. , 2010). The success of the UASB reactor concept in the AD treatment of industrial effluents is considered to be due mainly to the formation of anaerobic granular sludge. Due to their weight and the vertical flow direction of the system, these agglomerated microbe granules resist wash out in the upflow and ultimately become the dominant populations in the reactor. This thick and well-built microbial structure has appropriate settleability, high biomass retention time, and slow to no perceivable bacterial growth (Speece, 1996). This is a major advantage as it allows for virtually permanent sludge retention time (SRT) to be coupled with hydraulic retention time (HRT) of as little as a few hours. Figure 2.9 illustrates two examples of UASB reactors with granular sludge.

2.7.2 Granular Sludge

Furthermore, the granules (1 to 3 mm in diameter) that are formed in the sludge blanket protect the anaerobic microorganisms and increase the performance and efficiency of the system (Hulshoff Pol et al., 2004). The initial stages of the formation of anaerobic granules follow the same principles as biofilm formation of bacteria on solid surfaces, though identifying the mechanisms of how precisely this occurs, is subject to much debate (Hulshoff Pol, 1989; Hermanson, 1999; Liu & Tay, 2002; Liu et al., 2003; Jeong et al., 2005; Amani et al., 2010). Granular sludge increases the chemical oxygen demand (COD) removal efficiency by about 70% and the system is able to receive higher loading rates, while being more resistant to toxic shocks (Hulshoff Pol, 1989; Joeng et al., 2005). In fact, volumetric loading rates are usually 2-5 times greater than with contact processes (Habets & Driessen, 2007). Granular sludge shows very high specific methanogenic activity and high degradation rates due to the micro-colonies such as hydrogenotrophic methanogenic archaea which allow efficient interspecies hydrogen transfer (Hulshoff Pol et al., 2004). Disadvantages of granular sludge include the long time for formation (it can take up to several months), and the high purchase cost.

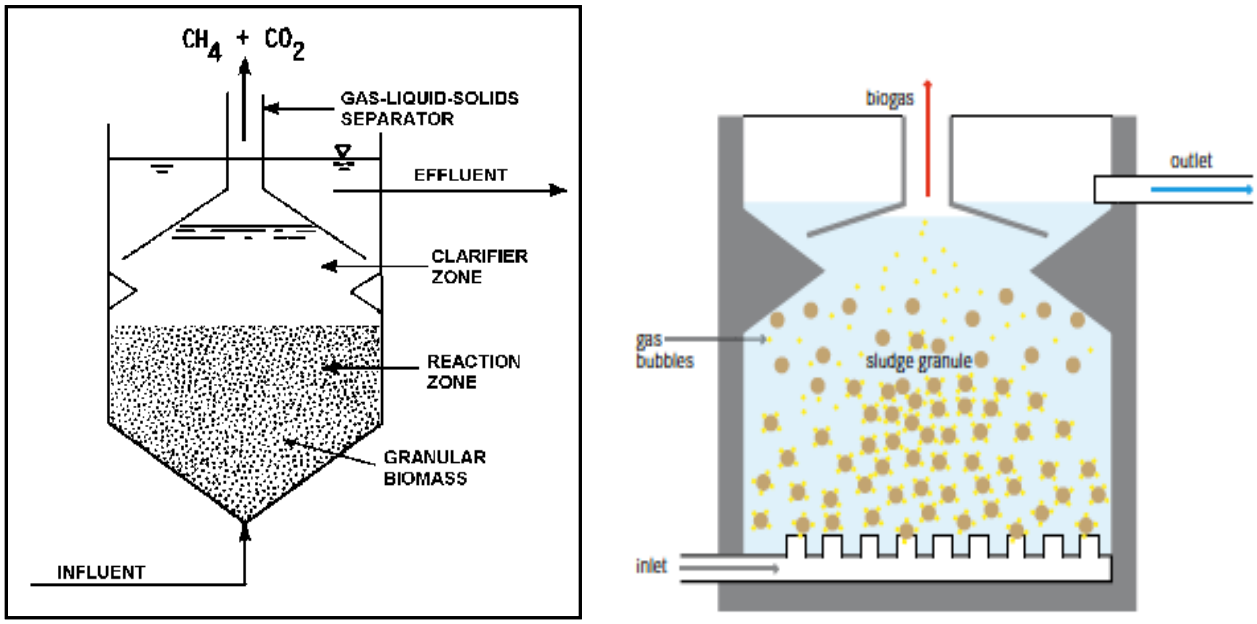


Figure 2.9 Schematic representations of two types of UASB reactors with granular sludge (Engineering Fundamentals, 2018)

Research and application has shown that the UASB system is a suitable form of treatment for paper processing wastewaters. Habets and Driessen (2007) conducted a survey of registered anaerobic treatment plants and found that 2/3 were treating recycled paper mill effluents while 1/3 was treating virgin pulp mill effluents. Figure 2.10 illustrates the distribution of types of treatment that were implemented from the years 1981 – 2005. The figure on the left indicates the totals for this time period, while the figure on the right focuses on developments from 2000-2005. From the figure it is clear that internal circulation (IC) reactors came to dominate the field and this is most likely due to their success in the industry; their efficient design allows for a more complete digestion of lower concentration wastewaters. For the purposes of the current study, the bench top UASB system offers preliminary results as to the potential performance of an AD system fed paper recycling waste streams, keeping in mind that different and more efficient designs that apply the same principles, could offer better performance and yield from the waste stream.

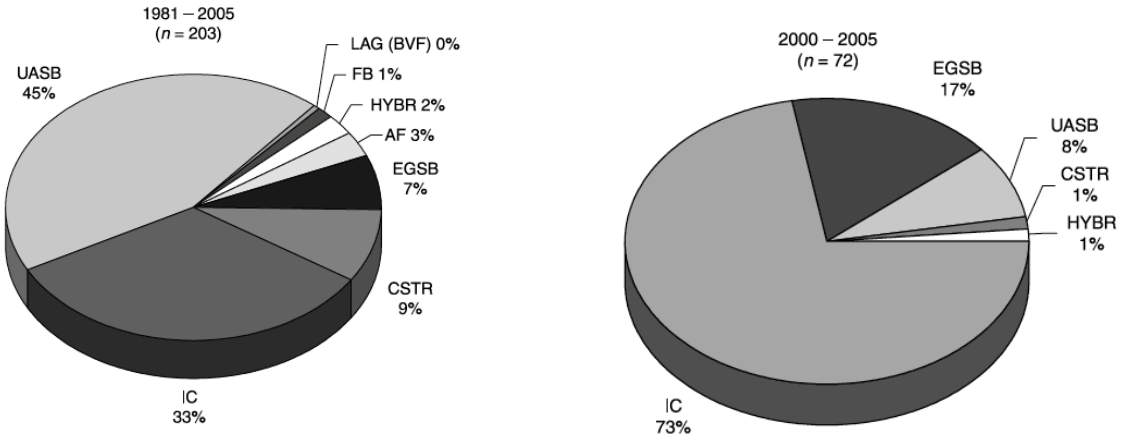


Figure 2.10 Overview of applied anaerobic reactor systems in the pulp and paper industry (Habets & Driessen, 2007).

2.8 Carbon Capture and Biogas Enhancement

A number of researchers have investigated the concept of using anaerobic digestion as a carbon sink, allowing for bioconversion of CO_2 to CH_4. Abedi (2015) showed a 29% increase in methane production, compared to the control, after injecting CO_2 into a pulp and paper Chemi-Thermomechanical Pulping (CTMP) wastewater, adjusted to pH 5.5 and fed into a UASB reactor. Salomoni et al. (2011) observed a 25% methane yield enhancement when bubbling CO_2 into the first stage of their UASB 2-PAD system. Sato and Ochi (1994) increased specific CH_4 yields by 30% when enriching sewage sludge with CO_2. Fernández et al. (2014) injected CO_2 at 0, 0.3, 0.6 and 0.9 M fractions into batch tests treating food waste and sewage sludge, and found daily specific methane production increased 11–16% for food waste and 96–138% for sewage sludge over the first 24 hours. Chun Lee et al. (2012) investigated injecting different mole ratios of H_4 and CO_2 into a single stage fixed bed reactor, with hydrogentrophic methanogens and achieved 100% conversion of CO_2 to CH_4 at the expected stoichiometric rate when the ratio was 1:4 , with a minimum gas retention time of 3.8 h [$4H_2 + 2CO_2 \rightarrow CH_4 + 2H_2O$]. They did not find an increase in acetate. Findings in the research demonstrate overall that there are a number of pathways possible for this conversion, in both the acetogenic and methanogenic phases. The current study focused on the conversion processes of the acetogenic stage, specifically the Wood–Ljungdahl pathway.

The Wood–Ljungdahl pathway is also known as the reductive acetyl-coenzyme-A (acetyl-CoA) pathway. The acetyl-CoA pathway is now recognized as a fundamental component of the global carbon cycle and as essential to the metabolic potentials of many different prokaryotes. This pathway facilitates electrophilic attack or deprotonation (H^+) of carbonyl groups (Strauss, 2010). Acetogenic bacteria use this pathway for the reduction of CO_2 to the acetyl moiety of acetyl-CoA, in this way allowing for conservation of energy and for assimilation into cell carbon (Daniel et al., 2008). In fact with this pathway, acetogenic bacteria are able to use a wide variety of carbon sources, electron donors and electron acceptors such as H_2+ CO_2, CO, formate and methoxylated aromatic compounds, to form acetate (Ragsale & Pierce, 2008). This pathway and its components are illustrated in Figures 2.11 and 2.12. It was expected that the increased concentrations of CO_2 injected into the wastewater would be converted into additional acetate and would consequently result in increased methane production from acetotrophic methanogens. This interaction is illustrated in Figure 2.13.

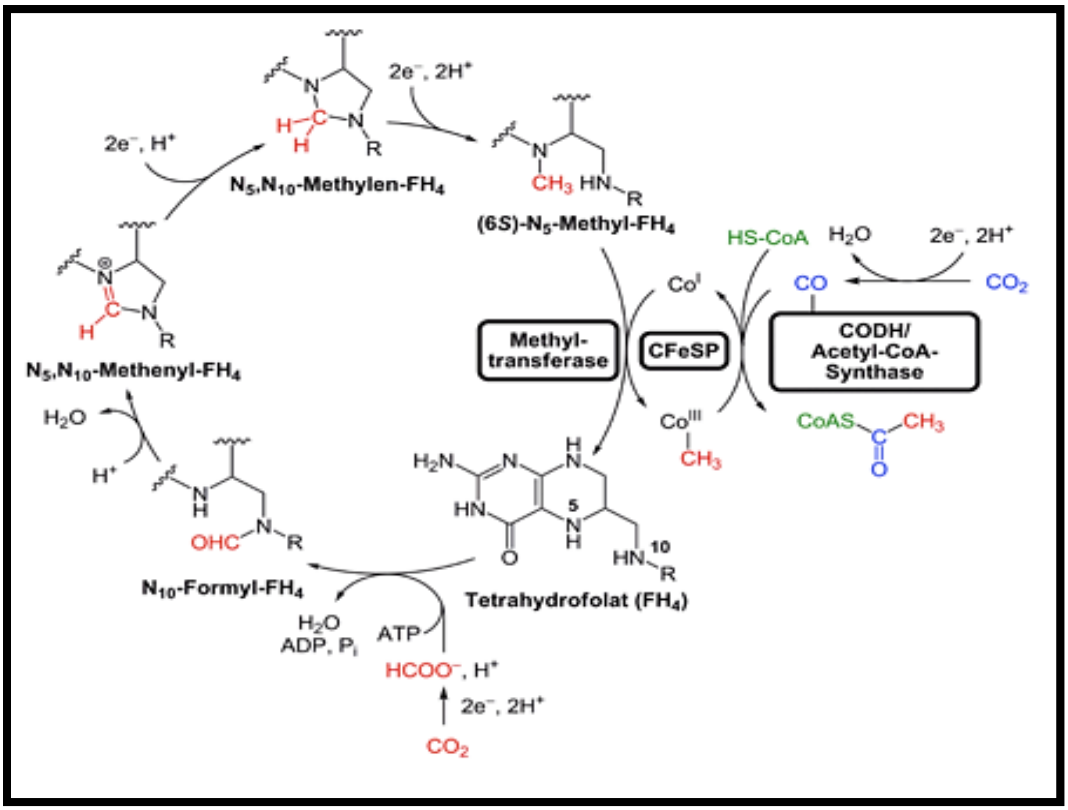


Figure 2.11 The reductive acetyl-CoA pathway (Yikrazuul, 2009).

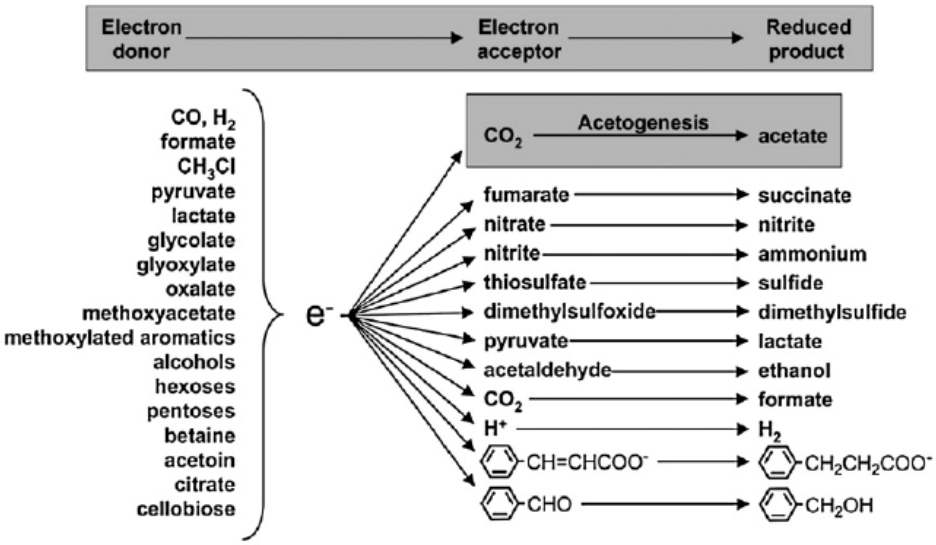


Figure 2.12 Redox processes that are can be used by acetogenic bacteria (Ragsdale & Pierce, 2008).

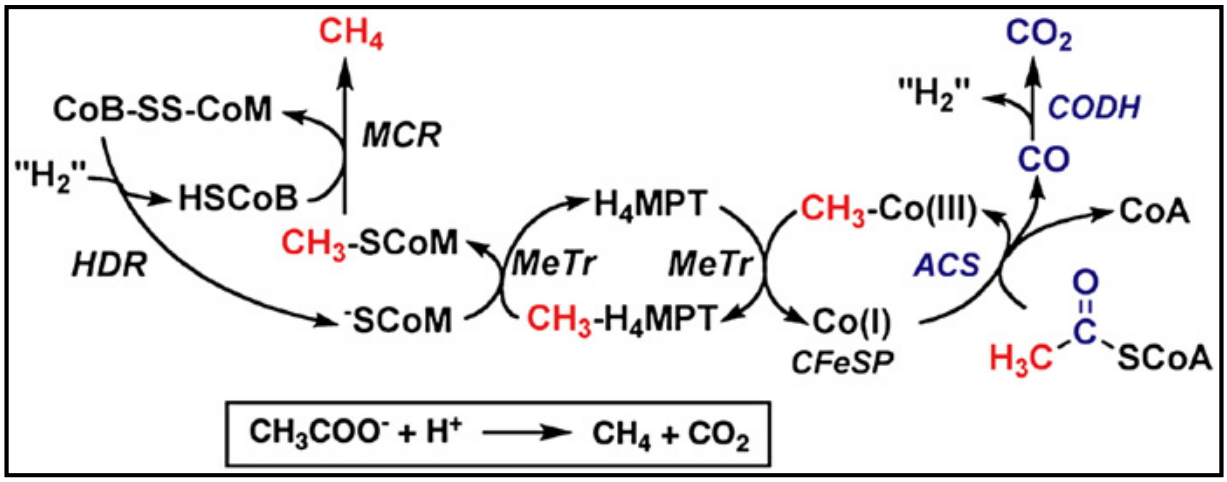


Figure 2.13 Acetoclastic methanogenesis: coupling methanogenesis to the Wood–Ljungdahl pathway (Ragsdale & Pierce, 2008).

Another important parameter of using waste streams as a carbon sink is the effectiveness of dissolution of carbon dioxide in the medium. Dissolution of CO_2 can depend on several factors such as pressure, pH, and temperature. Kazemi et al. (2013) tested the effectiveness of CO_2 solubility in pulp and paper wastewater at different temperatures and flow rates. Results showed that the solubility of CO_2 increased directly with liquid flow rates and inversely with temperature and that CO_2 dissolution was highest at 25 °C. It is

also important to note that CO_2 can only dissolve in liquid. Particulate matter in the water can serve as a nucleus for molecules of CO_2 and thus inhibit full dissolution.

2.9 Toxicity and Inhibition - Deinking Sludge

Toxicity is an important parameter to consider in biological treatment systems; toxicity can lead to inhibition and can lead the AD system to fail. A material may be judged inhibitory when it causes an adverse shift in the microbial population or inhibition of bacterial growth (Chen et al., 2008). Inhibition is usually indicated by a decrease of the steady-state rate of methane gas production and an accumulation of organic acids or other inhibitory substances, such as ammonia (Kroeker et al., 1979). Toxic or inhibitory substances are often found to be the leading cause of anaerobic reactor upset and failure. Akin to the complex anaerobic microbial processes themselves, the causes and effects of toxicity within the AD culture are equally complex. These effects may be manifested on the short term or the long term, upon accumulation. Evaluating toxicity may vary depending on the type of bacteria, acclimatization, the concentration of elements, and the interaction of these elements. Furthermore, mechanisms such as antagonism, synergism, acclimation, and complexing could significantly alter biological functions including the phenomenon of inhibition (Chen et al. 2008).

As mentioned, deinking sludge is an important waste stream and is produced from the recycling process by removing ink from post-consumer fiber. It typically comprises 12% of the total solid waste produced by the Canadian recycling industry (Reid, 1997). Though this can vary widely, the composition consists broadly of about 45–85% organic carbon, such as short cellulose fibers, small amounts of minerals, such as clays and chalks, and chemical additives from the manufacture of paper, printing, and recycling, as well as a range of organic contaminants and heavy metals (NCASI, 1991; Latva-Somppi et al., 1994; Bellamy et al., 1995; CQVB, 1996). Table 2.5 lists some typical concentrations of resin and fatty acids, polycyclic aromatic, halogenated and monoaromatic hydrocarbons. Values indicate means from eight samples over a period of 2 years (Beauchamp et al., 2002).

Table 2.5 Raw Deinking Paper Sludge: Concentrations of resin and fatty acids, polycyclic aromatic, halogenated and monoaromatic hydrocarbons (Beauchamp et al., 2002)

Halogenated hydrocarbons		**Halogenated and monoaromatic hydrocarbons**	
	µg/g		µg/g
Bromomethane	<1.0	Bromodichloromethane	<0.3
Chloroethane	<0.1	Bromoform	<0.3
2-Chlorethyl vinyl ether	<0.1	Chloroform	<0.3
Trichlorofluoromethane	<0.5	Dibromochloromethane	<0.3
Vinyl chloride	<0.5	m+p-Xylene	0:06±0:03a
Dibromoethane	<0.4	o-Xylene	0:05±0:03a
Carbon tetrachloride	<0.3	Benzene	<0.1
Chloromethane	<0.3	Bromochloromethane	<0.1
1,1-Dichloroethane	<0.3	Chlorobenzene	<0.1
1,2-Dichloroethane	<0.3	Chloro-1,2-bromo-propane	<0.1
1,1-Dichloroethylene	<0.3	1,2-Dichlorobenzene	<0.1
trans-1,2-Dichloroethylene	<0.3	1,3-Dichlorobenzene	<0.1
Dichloromethane	<0.3	1,4-Dichlorobenzene	<0.1
1,2-Dichloropropane	<0.3	1,4-Dichlorobutane	<0.1
cis-1,3-Dichloropropene	<0.3	Ethylbenzene	<0.1
trans-1,3-Dichloropropene	<0.3	Mesistylene	<0.1
1,1,2,2-Tetrachloroethane	<0.3	A-Mesistylene	<0.1
Tetrachloroethylene	<0.3	Styrene	<0.1
1,1,1-Trichloroethane	<0.3	Toluene	<0.1
1,1,2-Trichloroethane	<0.3		
Trichloroethylene	<0.3		

Resin and fatty acids		**Polycyclic aromatic hydrocarbon**	
	ug/g		ug/g
Abietic acid	863±884	Benzo[ghi]perylene	0:2±0:1
12-chlorodehydroabietic acid	<30	Fluoranthene	0:4±0:3
14-chlorodehydroabietic acid	<30	Naphtalene	0:2±0:1
Dehydroabietic acid	288±171	Phenantrene	0:6±1:0
12,14-dichlorodehydroabietic acid	<30	Pyrene	0:7±0:1
9,10-dichlorostearic acid	<30		
Isopimaric acid	102±44		
Linoleic acid	73±55		
Linolenic acid	<30		
Neoabietic acid	30±18		
Oleic acid	115±51		
Palmitic acid	197±127		
Palmitoleic acid	<30		
Palustic and levopimaric acids	126±72		
Pimaric acid	53±27		
Sandaracopimaric acid	43±38		
Stearic acid	254±76		

Standard treatment of this deinking waste stream is by composting and agricultural supplement, combustion/incineration or simply landfilling (Brouillette, 1996; Beauchamp et al., 2002; Deviatkin, 2013). The current research proposes to include this waste stream to feed the anaerobic digestion system for an integrated process. The composition of the deinking sludge is a concern due to elements that may have possible toxic effects for anaerobic bacteria. However, Meyer and Edwards (2014) state that co-digestion of different substrates can actually have protective effects for the microbial community. As a consequence of adapting to diverse conditions, the microbial population also becomes more diverse and robust, making it more able to adapt to stress situations or any process disturbances. Researchers recommended focusing on the relationship between wastewater composition, reactor operation and microbial community dynamics during acclimation and start-up to increase chances of success.

In the case of deinking sludge, there are three broad groups of compounds that may potentially be toxic: polycyclic aromatic hydrocarbons (PAHs), resin acids and heavy metals. As seen in Table 2.5, most hydrocarbons are under detection limits. Although concentrations of resin and fatty acids are high, these substances have been shown to successfully degrade in the AD system. PAHs have been shown to fully degrade in anaerobic environments with some research reporting that the degradation can occur with as little as the redox conditions of the acetogenic reactor (Evans, 1988; Grbic-Galic, 1991; Chang et al., 2003; Bonin et al., 2004). Resin acids are a type of carboxylic acids, like the intermediary volatile fatty acids (e.g. butyric, tartaric and propionic acids) produced by acidogenic bacteria. These are considered readily biodegradable and are easily converted to acetate by acetogenic bacteria, which can then serve as food for the acetogtrophic methanogens (Liss et al., 1997). Even at acute and chronic exposure, the resin acid from bleached chemi-thermomechanical wastewater did not substantially affect the performance of a continuous UASB reactor (McCarthy, 1990). Heavy metal concentrations in deinking sludge can be dangerous for anaerobic bacteria if soluble levels surpass a certain threshold. Many metal ions can exert toxicity on biological systems through multiple biochemical pathways simultaneously. The various mechanisms of metal toxicity in microorganisms are: (1) substitutive ligand binding, (2) redox reactions with sulfur groups, (3) Fenton-type reactions, (4) inhibition of membrane-transport processes, and (5) electron siphoning

(Harrison et al., 2007). The susceptibility of microorganisms to toxic metal species has been linked to several metal ion-specific physicochemical parameters, such as the standard reduction potential; electronegativity; the solubility product of the metal–sulfide complex; the Pearson softness index; electron density; and the covalent index (Nies, 2003; Workentine et al., 2008). Given the potential risks, it is best to monitor these concentrations and take preventative or remediative measures.

2.10 System Design

Recycling plant processes rely on the feed source of paper they receive. Since this paper feed source can be quite variable, the resulting characteristics of the plant's wastewater, such as COD, pH and suspended solids, also vary considerably. Thus, a suitable treatment system must be designed to properly accommodate the influent wastewater, in all its variability. Given the sensitivity of methanogenic bacterial populations, it is possible that a 2-stage system offers more stability in its buffering capacity from this variability and accordingly allows for more consistent and stable operations. Furthermore, the relatively high solids content of the initial influent wastewater and deinking sludge, and consequent complications this might pose to sensitive and expensive granular methanogenic sludge, beyond sludge disposal costs, may also favor the 2-stage process. Two phase anaerobic digestion can handle high-solid containing waste, since liquefaction by hydrolysis occurs more effectively in the acetogenic first phase (Derbal et al., 2009; Lozano et al., 2009; Viéitez et al., 2000). Nevertheless, 1-stage systems are often successfully employed in industry, usually after primary clarification of suspended solids, and are perceived to be a simple and less costly option to treat waste streams while creating biogas. This research sought to compare both options and access their feasibility.

Chapter 3 MATERIALS and METHODS

Standard wastewater treatment parameters were used to determine the initial characteristics of the influent waste streams as well as the effectiveness of experimental conditions. These methods are described in detail in this chapter.

3.1 Oxygen Demand

Oxygen demand provides an indication of the amount organic pollution in water of a given substances under specific conditions. It is used to determine appropriate waste loadings and efficiency of water and wastewater treatment (Boyles, 1997). Two methods are primarily measured: chemical and biological oxygen demand. The Biological Oxygen Demand (BOD_5) is a measure of the capacity for naturally occurring microbes in water bodies to reduce organic pollutants upon discharge of wastewater. An adjusted Standard Methods Procedure 5210B was followed for this measurement as described below.

1. Samples were diluted according to the following standard:

Table 3.1 Dilution standards for BOD test

Estimated BOD_5 (mg/L)	Suggested Sample Volumes (mL)	Estimated BOD_5 (mg/L)	Suggested Sample Volumes (mL)
< 5	200, 250, 300	90 - 150	5, 10, 15
< 10	100, 150, 200	150 - 300	3, 5, 10
10 – 30	25, 50, 100	300 - 700	1, 3, 5 ***
30 – 60	15, 25, 50	700 – 1500	0.5, 1, 3 ***
60 – 90	10, 15, 25	1500 - 2500	0.25, 0.5, 1 ***

2. Samples were seeded with 1 mL waste activated aerobic sludge from Cascades bio-selector reactor.
3. Dilution water was prepared immediately before use by leaving tap water running for >3 min. One Nutrient Buffer Pillow (HACH) was added to each 300 mL BOD bottle for aerobic bacterial nutrient requirements. Diluted samples were adjusted to remain between pH 6.5 and 7.5, using 1N sulfuric acid or 1N sodium hydroxide where necessary.

4. Dilution water was added to each bottle until the fill line.
5. A blank bottle of dilution water plus nutrients was included with each BOD test.
6. Dissolved Oxygen (DO) measures were recorded for each bottle using Orion™ Star A223 RDO/Dissolved Oxygen Portable Meter with RDO Optical Sensor from Thermo Scientific.
7. BOD bottles were capped and twisted to seal, then stored in a dark box at room temp (21°C) for 5 days.
8. On the 5th day, DO measurements of each bottle were recorded and BOD was calculated as follows:

$$\boldsymbol{BOD}\ \left(\frac{\boldsymbol{mg}}{\boldsymbol{l}}\right) = [(\boldsymbol{Initial\ DO} - \boldsymbol{DO5}) * \boldsymbol{Dilution\ Factor}] - (\boldsymbol{B1} - \boldsymbol{B2}) \quad \textbf{Equation 3.1}$$

Where:

Dilution Factor = $\frac{\text{Bottle Volume (300 mL)}}{\text{Sample Volume}}$

D_1 = Initial DO of diluted samples (mg/L)
D_2 = DO of diluted sample after 5 d incubation at ~21C (mg/L)
B_1 = DO of seed control before incubation (mg/L)
B_2 = DO of seed control after incubation (mg/L)

Chemical Oxygen Demand (COD) is a measure of the oxygen equivalent to the organic carbon of a sample that is susceptible to oxidation into CO_2 and H_2O by a strong chemical oxidant in acid upon heating. The oxygen demand is determined by measuring the amount of oxidant consumed by way of titrimetric or photometric methods. The COD in this study was measured photometrically using HACH brand kits: TNT 822 (20-1500 (mg/L) High Range) and TNT 823 (250-15000 (mg/L) Ultra High Range) according to the manufacturer's instructions. All TNT kits used here and for additional parameters were heated in a HACH DBR 200 incubator and absorption was measured with a HACH DR 2800 Spectrophotometer. Both total and soluble COD followed this same method with the distinction that soluble COD ($sCOD_)$ samples were initially filtered with a 0.45um syringe filter, while total COD ($tCOD_)$ samples were not filtered. An empirical relationship exists between BOD and COD that must be determined for each sample or type of water, this is called the BOD/COD ratio.

3.2 Organic Fatty Acids

Volatile Fatty Acids (VFAs) are an important indicator of system performance in the anaerobic digestion process. VFAs are a type of carboxylic acid that is a by-product of acetogenic bacterial activity and serve as a feed source for acetoclastic methanogenic bacteria. In a single-phase system, high levels of VFA lower the pH of the reactor and can thus inhibit methanogenic bacteria. However, cultivating elevated levels of VFAs is advantageous in the acetogenic phase of a 2-phase system, as they then become a concentrated feed source for the second phase. VFAs were measured in 2 ways, TNT HACH kits and High Pressure Liquid Chromatography (HPLC). HACH kit TNT 872- Volatile Acids (50-2500 (mg/L) Range) was used to measure VFAs with reagents and spectrophotometry by the following method. Volatile acids react with diols in an acidic environment to form fatty acid esters. The extent to which these esters are reduced by iron (III) salts to form red complexes was measured by spectrophotometry at a wavelength of 497 nm. High Pressure Liquid Chromatography (HPLC) was also used to measure concentrations of individual concentrations of organic acids in the samples and influent wastewaters. In the HPLC method, the mobile phase with the sample was pumped through a column filled with under high pressure. Each component in the sample reacts differently with the column material and so elutes with specific characteristics (retention time and peak area) that can be used to quantify concentrations. The HPLC has the advantage of identifying individual acids. A System Gold 168 detector by Beckman Coulter was used with an RSpak KC-811 Shodex packed column at room temperature. The method was operated with the parameters listed in Table 3.2. Samples were filtered with a 0.45um syringe filter. Peak values were calculated by 32 Karat 8.0 software generated standard curves of acetic, propionic, tartaric, and butyric. Standard curves can be found in Appendix A.

Table 3.2 Operating parameters for HPLC VFA method (Shodex Operation Manual)

Mobile phase	0.1% phosphoric acid
Temperature °C	23 (room temp)
Run time (min)	36
Flow rate (mL/min)	0.5
Operating pressure (kPa)	3.5
Detector wavelength (nm)	210

3.3 Soluble Nutrients and Dissolved Metals

Unless otherwise specified, additional elemental and nutrient concentrations of wastewater and samples were measured with the range-appropriate HACH TNT kits (listed below) and were used in accordance with the manufacturer's instructions.

Total Nitrogen TNT 827 (5-40 mg/L - High Range)
TNT 828 (20-100 mg/L - Ultra High Range)
Phosphorus TNT 844 (1.5-15.0 mg/L - High Range)
TNT 845 (6-60 mg/L) -PO_4 Ultra High Range)
Ammonia TNT 832 (2-47 mg/L Range)
Sulphate TNT 865 (150-900 mg/L High Range)
Nickel TNT 856 (0.1-6.0 mg/L Range)
Aluminum TNT 848 (0.02-0.50 mg/L Range)
Chromium TNT 854 (0.03-1.00 mg/ Range)

3.4 Solids Analysis

Solids analysis is important in the control of biological and physical wastewater treatment processes and for assessing compliance with regulatory effluent limitations. Suspended solids refer to matter suspended in wastewater or the mixed liquor of the reactor. Total and volatile solids indicate the concentration of mass in sludge, mud or otherwise solid samples. In accordance with the Standard Methods Procedure 2540 to measure Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) was as follows:

1. For testing volatile suspended solids (VSS) and total suspended solids (TSS), Whatman Brand GF/A pore size 1.6 µm, 42.5 mm-diameter glass microfiber filters were placed on aluminum plates and pre-ignited in a muffle furnace at 550°C for 20 minutes.
2. Filters were transferred to a desiccator to cool for at least 30 minutes, and then measured for initial weight (Weight A).
3. Using a vacuum filter gasket and an Erlenmeyer flask, a specific volume of sample

was filtered over paper. Sample volumes were selected to result in a final weight of at least 2 mg.

4. Filters were then put into the oven, preheated to 105°C, to dry for 1 hour. Dried filters were transferred to the desiccator to cool for at least 30 minutes and then weighed (Weight B).
5. Filters were then ignited in pre-heated muffle furnace at 550°C for 15-40 min until a stable weight was obtained. Ignited filters were transferred to the desiccator to cool for at least 30 minutes and then weighed (Weight C).
6. Equations 3.2 and 3.3 were applied to determine the concentrations of TSS and VSS.

$$\textbf{Total suspended solids}\ \left(\frac{mg}{l}\right) = \frac{(B-A)*100}{\textbf{sample volume}\ (ml)} \qquad \textbf{Equation 3.2}$$

$$\textbf{Volatile suspended solids}\ \left(\frac{mg}{l}\right) = (B-C)*\frac{100}{\textbf{sample volume}\ (ml)} \qquad \textbf{Equation 3.3}$$

Total solids (TS) and volatile solids (VS) for solid samples were determined by the same method with the exclusion of sample filtration, substituted for ceramic dish or crucible as follows:

A- Dish weight after ignition and desiccating
B- Total solids were determined by heating in the oven at 105°C overnight
C- Volatile solids were determined by ignition at 550°C for 30 minutes or until a stable weight was reached.

3.5 Sludge and Waste streams

Granular sludge was obtained from a single-phase, completely mixed anaerobic treatment facility for Lassonde Juice in Rougemont, Quebec and had a VSS of 6.0 g/L. Liquid sludge was obtained from Sainte Hyacinthe municipal anaerobic wastewater treatment system and had a VSS of 9.0 g/L. The wastewater samples were obtained from the direct influent, before primary treatment. Samples were sent by the environmental supervisor at

the Cascades plant in 20-liter drums by Purolator delivery. Shipments averaged every 4 months though this varied depending on the experimental conditions being run. Samples were refrigerated upon arrival and stored in temperatures ranging from 4 – 10 °C. These samples were characterized and analysed by methods described here.

3.6 Nutrient Supplementation

Anaerobic bacteria require a minimum proportion of vital nutrients, nitrogen and phosphorus, to maintain healthy functioning (Ammary, 2004). As is often the case with industrial wastewater, supplementation of these nutrients was necessary to meet the ideal ratio. For these experiments, this ratio was defined by the Environment and Process Technologist at the Lassonde Treatment Plant as: COD > 6000 mg/L: 500:5:1 (wt) , COD < 6000 mg/L: 300: 5:1 (wt). NH_4Cl and K_2PO_4 (Fisher Brand) were used to meet the minimum phosphorus and nitrogen requirements for anaerobic bacteria.

3.7 Waste stream Characterization

Table 3.3 Characterization of Waste streams from Cascades Plant at Kingsey Falls, Qc.

Parameter	Waste streams		
	Paper Recycling Influent wastewater		De-inking Sludge
Flow (m^3/d)	6500		165
Temperature (°C)	38 (winter) 45 (summer)		40
BOD (kg/m^3)	1.10 - 2.00		2.54
COD (kg/m^3)	3.00 - 6.70		3.28
sCOD (kg/m^3)	1.96 – 4.45		2.16
pH	4.80 - 7.00		8.30
VFA (kg/m^3)	0.25 - 1.50		1.79
TSS (kg/m^3)	0.90 - 1.80		20.66
VSS (kg/m^3)	0.80 - 1.80		11.42
TN (mg/L)	11 – 20		1050 (dry solids - mg/kg)
Phosphate (mg/L)	0.64 - 4.11		0.63
Sulfate (mg/L)	230 –250		0.2% (Abubakr et al., 1995)
Sodium (mg/kg)	n/a		97483
Nickel	1.27 (mg/L)		38.69 (mg/kg)
Aluminum	0.62 – 0.73 (mg/L)		24874 (mg/kg)
Chromium	0.77 – 1.50 (mg/L)		91.21 (mg/kg)

Table 3.4 Concentrations of relevant light, heavy metals and calcium in raw de-inking sludge for dry solids at flow ratio concentrations (0.025 v/v) [4% solids].

	Ca	Cr	Mn	Ni	Cu	Zn	As	Cd	Co	Pb	Al
mg/kg	$1.57x10^4$	2.28	21.82	0.97	10.81	79.17	0.26	0.04	0.36	1.45	622.0

The average relation of soluble and total COD (sCOD /tCOD) ranged between 0.50-0.90 for influent samples. For these experiments the BOD/COD ratio was found to vary between 0.25 and 0.35. The COD/VSS ratio was measured as 3.5, while the TSS/VSS ratio was 0.95.

3.8 Experimental Apparatus

3.8.1 Batch Test Procedure

Figure 3.1 1000 mL bottle with septum cap and gas collection bag used for batch tests.

Initial experiments were done by batch tests in septum capped glass 1000 mL bottles. pH was adjusted in batch bottle with pure hydrochloric acid (37% from Fisher Scientific) after 300 mL wastewater had been added to approximately 200 g granular anaerobic bacteria. Carbon dioxide was then injected into the bottle at atmospheric pressure at a constant and low flow, until a stable pH was reached. Bottles were then

purged with pure nitrogen gas to ensure anaerobic conditions. Batch bottles were kept in an incubator at 35 °C. The batch set-up is shown in Figure 3.1.

3.8.2 Bench Top Reactors

Continuous experiments were done using an Armfield Bench top apparatus that consisted of two 5.0 L upflow anaerobic sludge blanket (UASB) reactors. Reactors contained 2.25 L of anaerobic bacteria, leaving 2.25L for hydraulic flow of wastewaters and were fitted with digitally controlled heating blankets to maintain internal temperate in accordance with the experimental condition (25, 35, 37, 38 or 45 °C). The feed was delivered by peristaltic pump at the desired flow. Biogas flowed from bioreactor into water displacement reactor to indicate volume of gas. In single-phase experiments, flow passed only through one reactor, while in 2-phase conditions, the first reactor served as the acetogenic phase while the second served as the methanogenic phase. Figure 3.2 illustrates these reactors and their flows. Details about the reactor can be found in Appendix B.

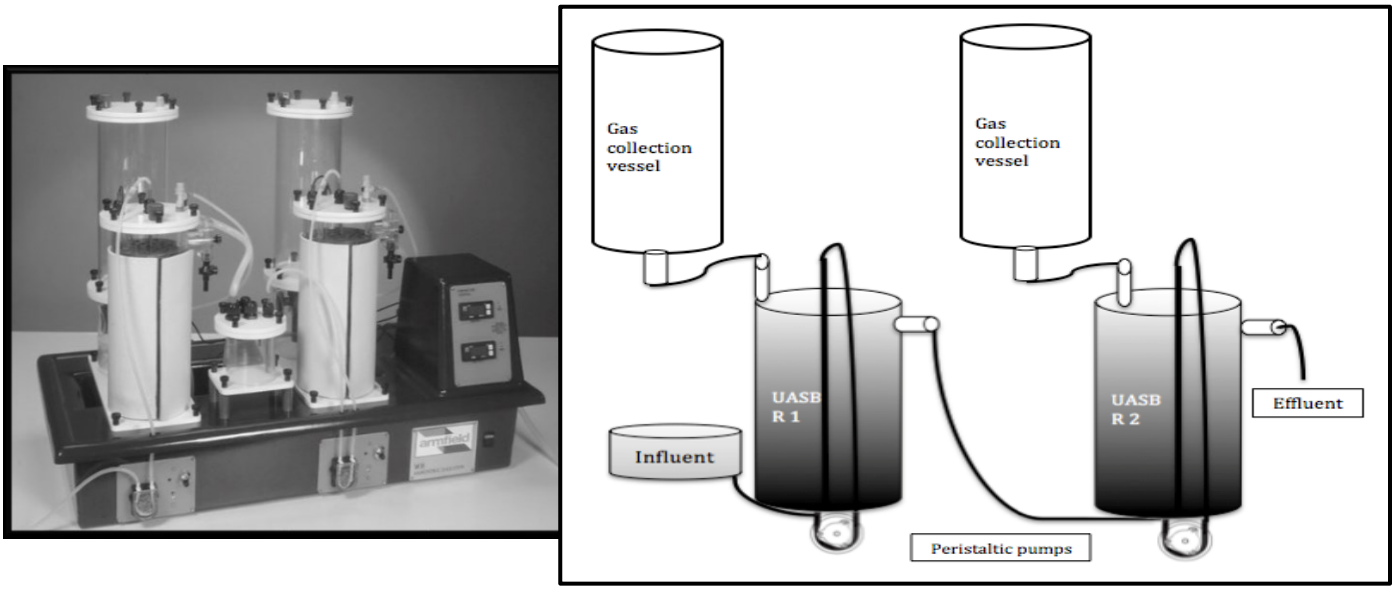


Figure 3.2 Bench top UASB reactors. The left shows a photograph while the right shows a schematic

3.9 Continuous Experimental Design

Both the granular and liquid sludge used in experiments were taken from treatment plants operating in single-phase systems in which methanogenic and acetogenic bacteria existed together. Phase separation was carried out through kinetic control by applying operating parameters specified by Ghosh et al. (1975) to create optimal conditions for acetogenic bacteria. This method was applied for acetogenic conversion of both granular and liquid sludge. Anaerobic bacteria were initially inoculated with a feed of nutrient supplemented 10,000-mg/L-glucose solution at an organic loading rate of 8.0 g COD/L/d for 2-3 days; this was then reduced to a glucose solution with comparable COD to the wastewater at 5 g/L and a loading rate of 3.6 g/L/d. These conditions were maintained until pH and VFA concentrations reached stable levels with pH between 4.3- 4.7 and total VFA at an average of 1626 mg/L, from initial measures of 7.5 and of 92 mg/L respectively. Increased VFA levels indicated higher activity and dominance of acetogenic bacteria within the reactor. Once these conditions were attained, trials proceeded with Cascade waste streams. Operating temperatures were 25, 38 or 45°C.

Conditions for methanogenic bacteria were determined in line with previous research findings (Amani et al., 2010; Abedi et al., 2015) and experimental results and then later adjusted according to plant operations. Longer acclimation periods ensured more stable and efficient system performance. Reaching a short retention time involved several weeks of gradual increase in loading rate so as not to shock and acidify the system, each loading condition was held between 5-7 days or until stable, increased at a rate of between 0.4 and 1.2 kg/m^3-d .

Table 3.5 Summary of Operational Parameters for the Continuous System

	Acetogenesis	Methanogenesis
Temperature (°C)	25-45	35-45
Q (L/day)	1.25 – 5.0	0.42-2.25
Θ RT (Day)	0.5 – 3	1 – 5
OLR (kg tCOD/m^3/day)	2 - 7	0.5 - 1.9

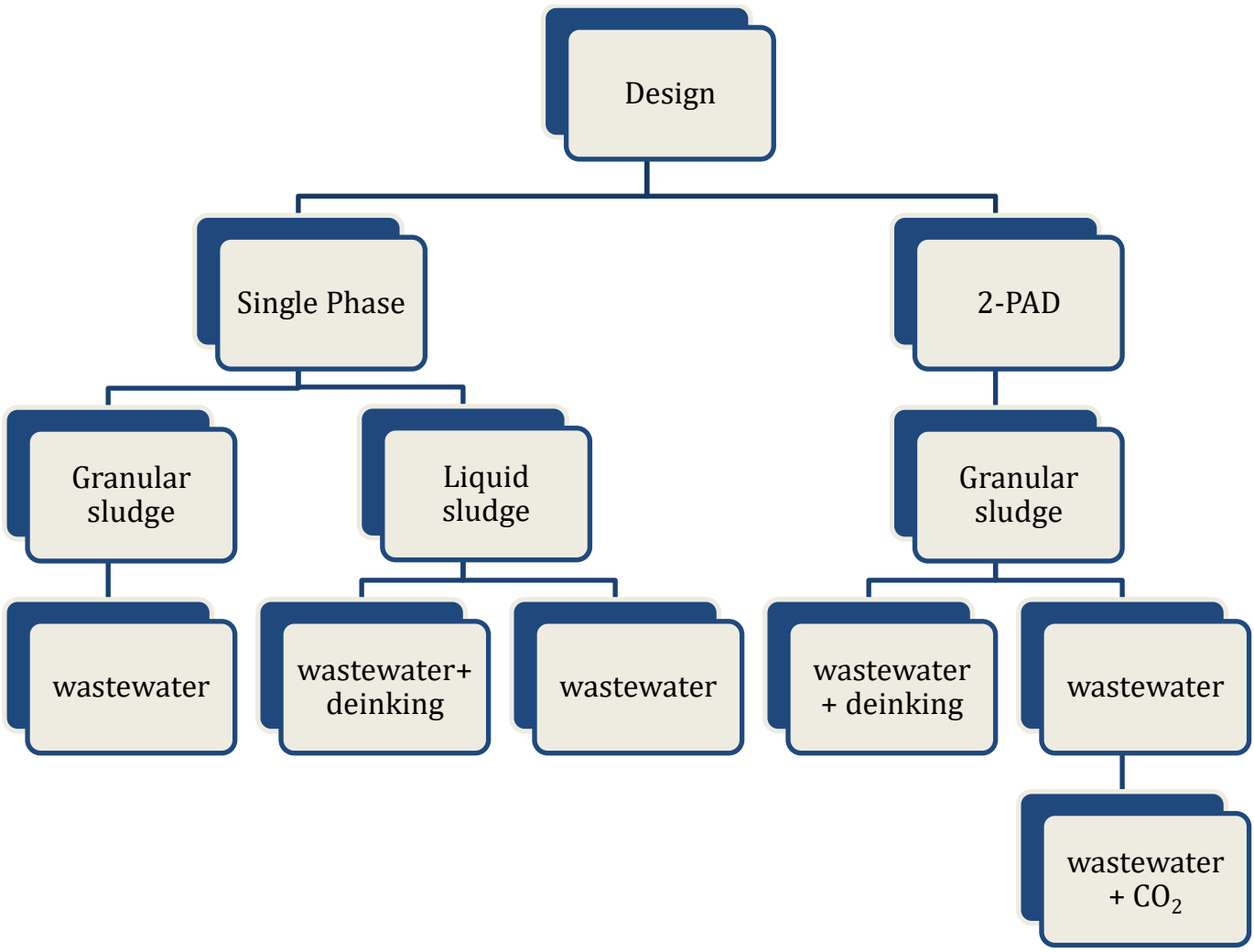


Figure 3.3 Schematic chart of operational parameters and conditions for continuous system experiments

3.10 Operational Equations

3.10.1 Hydraulic Retention Time and Organic Loading Rate

Hydraulic retention time is a very important parameter in the design and operation of the system as whole. In addition to regulating the flow rate/d to the reactor, HRT is also used to determine the reactor volume required for the system design. In the case of the UASB reactor, the required volume is usually twice as large as the volume required for the hydraulics, to account for the permanent volume of the sludge bed in the reactor. Organic loading rates (OLR) are used to determine the feed rate- reactor volume of the system.

Hydraulic retention times were calculated by Equation 3.4. Organic loading rates can be calculated with both Equations 3.5 and 3.6.

$$\boldsymbol{Hydraulic\ Retention\ TIme\ (HRT)(d)} = \frac{\boldsymbol{Reactor\ capacity\ volume\ (m^3)}}{\boldsymbol{Flow\ of\ subsrate\ added\ daily\ \left(\frac{m^3}{d}\right)}} \qquad \textbf{Equation 3.4}$$

$$\boldsymbol{Organic\ Loading\ Rate\ (OLR)}\ \frac{kg}{m^3 * d} = \frac{\boldsymbol{Organic\ mass\ added\ daily\ \left(\frac{kg}{d}\right)}}{\boldsymbol{Reactor\ capacity\ volume\ m^3}} \qquad \textbf{Equation 3.5}$$

$$\boldsymbol{V = \frac{Q*C}{OLR}}$$

V = volume of the reactor m^3
Q = influent flow rate, m^3/d
C = influent COD, kg COD/m^3
OLR = acceptable organic loading rate kg/(m^{3*}d)

Equation 3.6

3.10.2 Solids Retention Time

In completely mixed systems, the solids retention time (SRT) is equal to the HRT, while in systems with inbuilt sludge retention such as the UASB system; the SRT is higher than the HRT. In these cases SRT is calculated according to Equation 3.7. This equation is applied for wastewater with a high concentration of suspended solids, and for systems that are not hydraulically limited. In this study, Equation 3.7 was also used to calculate sludge wasting from the partially mixed acetogenic reactor.

$$\boldsymbol{HRT = \frac{COD_{SSin}}{\chi} * R * (1 - H) * SRT}$$

Equation 3.7

COD-SS_{in} = COD of suspended solids in the influent (g/L)
χ = sludge concentration in the reactor (g VSS/L)
where (1 g VSS=1.4 g COD)
R = fraction of the COD_SS removed
SRT = solids retention time
H = fraction of the removed COD_SS , which is hydrolyzed at the imposed SRT

3.11 Biogas analysis

3.11.1 Gas Chromatography (GC)

The percentages of each compound in the biogas were determined with a VARIAN CP 3800 gas chromatography instrument, using the thermal conductivity detector (TCD) and GS-CARBONPLOT capillary column from Agilent Technologies (30m, 0.53nm Inner Diameter, and 3.0-µm film thickness). The method included the parameters in Table 3.6.

Table 3.6 Gas chromatographic method to measure CH_4 and CO_2 concentrations in biogas

Carrier gas	Helium
Injector temperature °C	220
Column oven temperature °C	35
Run time (min)	3.00
Injection flow rate (mL/min)	7.0
Filament temperature °C	290

To create the standard curve for this method, pure gases of methane and carbon dioxide (Praxair and Air Liquide suppliers at 99.99% purity) were collected in pre-vacuumed gas 1L foil SCV Sigma Aldrich gas sampling bags. A 10 µl gastight Hamilton syringe (model 1701) was used to withdraw through the fitted septum, ratios of methane to carbon dioxide in concentrations of 10, 20, 30, 40, 60, 80 and 90% vol/vol. Calibration curves are illustrated in Appendix C. Biogas samples were withdrawn daily from the gas sampling port in the reactor using a 50 mL gas-tight syringe. This sample was then transferred to a pre-vacuumed gas-sampling bag using a 0.45-micron syringe filter. The 10 µl gastight syringe was used to withdraw samples from the bag through the septum port. The volumes of methane and carbon dioxide were calculated by multiplying the total volume of biogas, measured by water displacement, by the corresponding percentage of CH_4 or CO_2, indicated by the GC.

3.11.2 Methane Yield

An important parameter for measuring anaerobic digestion system performance is the proportion of methane produced for each unit of COD that has been removed. With this calculation, it is possible to find the amount of biomethane, and by extension caloric energy, that will be produced as from a given COD input into the system. This is calculated with Equation 3.8 as follows.

$$\frac{\boldsymbol{CH_4\ L}}{(\boldsymbol{COD\ initial - COD\ \ final})\left(\frac{\boldsymbol{mg}}{\boldsymbol{L}}\right)} = \frac{\boldsymbol{L\ CH_4}}{\boldsymbol{mg\ COD_{removed}}}$$

Equation 3.8

3.12 Alkalinity and pH

Alkalinity is a measure of water's capacity to neutralize acids. It can be determined based on the stoichiometric relationships between pH and dissolved species HCO_3^-, CO_3^{2-}, OH^- (Table 3.7) in water. As can be seen in Figure 3.4, the extent of dissolution varies with pH. pH is an indication of the concentration of hydrogen proton ions in solution. It was measured with an Oaklon 10 series pH and temperature meter with combination potentiometric probe. pH was served as an indication of system performance, and a method to indicate dissolved species in samples. Continuous operation was also monitored by pH indicator strips (BDH, 0-14.0 range by VWR) to ensure consistency of results.

$$\boldsymbol{Alkalinity\ as\ CaCO_3}\ \left(\frac{\boldsymbol{mg}}{\boldsymbol{l}}\right) = \frac{\boldsymbol{volume\ acid\ mL}}{\boldsymbol{volume\ sample\ mL}} * \frac{\boldsymbol{normality}}{\boldsymbol{CaCO_3}\left(\frac{\boldsymbol{mg}}{\boldsymbol{eqv}}\right)} * \frac{\boldsymbol{1000\ ml}}{\boldsymbol{L}}$$

Equation 3.9

Carbonate alkalinity is defined as the point in which all the existing bicarbonate in the solution is converted to dissolved carbon dioxide or carbonic acid through reactions with hydrogen of the acid that had been added to shift the solution to a pH of 4.5. Assuming carbonate alkalinity as an endpoint, a pH meter was used to titrate 0.02N sulphuric acid

(H_2SO_4) into the sample until reaching a pH of 4.5. The volume of acid required to reach pH 4.5 is used to calculate the alkalinity of the sample as calcium carbonate ($CaCO_3$) according to Equation 3.9.

Table 3.7 Stoichiometric relationships between pH and dissolved carbonate species

pH	Chemical Equation
4.5-8.3	$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$
> 9.5	$CO_2 + OH^- \rightleftharpoons HCO_3 \rightleftharpoons H^+ + CO_3^{2-}$

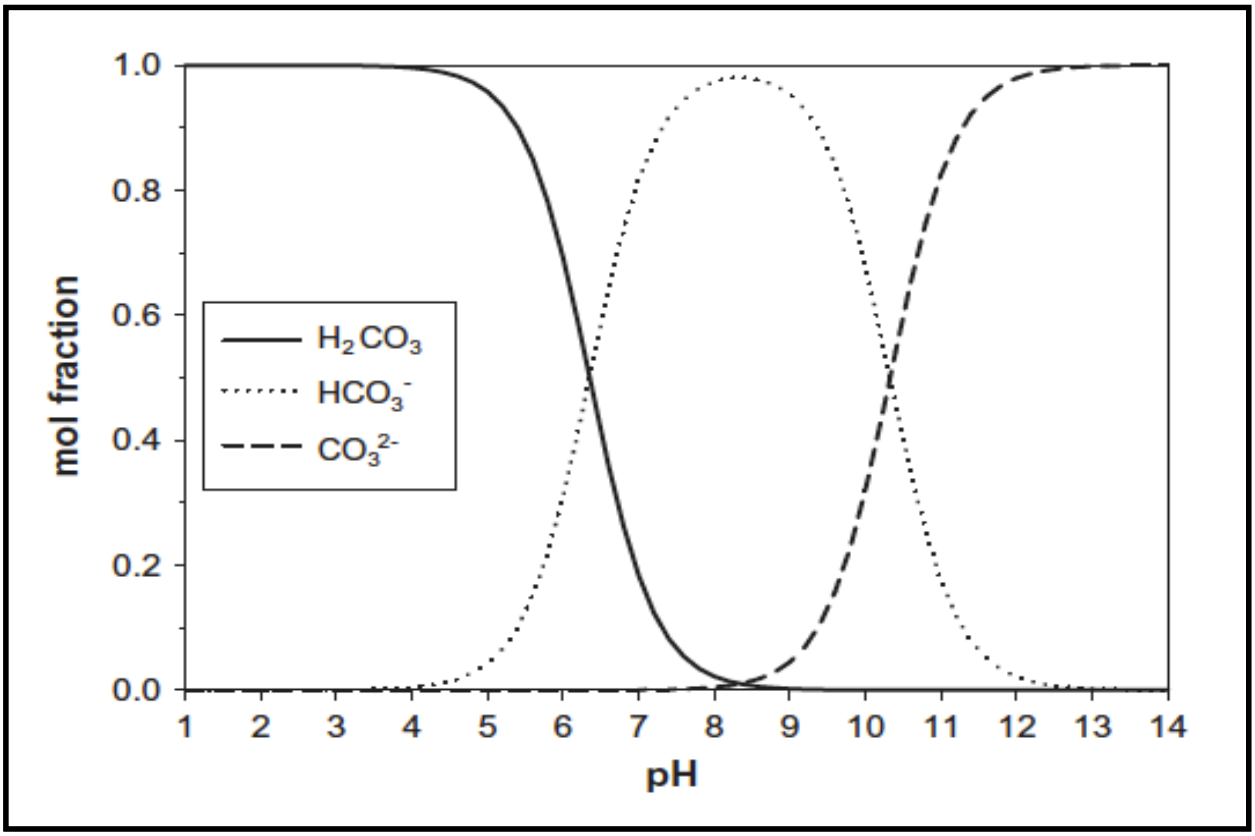


Figure 3.4 Equilibrium distribution of H_2CO_3, HCO_3^-, and CO_3^{2-} species in solution (Steel et al., 2013).

3.13 Carbon Dioxide Bioconversion

The procedure for testing bioconversion of carbon dioxide was as follows. Maintaining the parameters in Table 3.8, pure CO_2 gas, was passed through a flow and pressure gauge

before being injected into influent wastewater until a stable pH was reached. CO_2 was measured and converted to mg/d for each stage in the digestion system (Table 3.9).

Table 3.8 Carbon dioxide injection parameters for bioconversion conditions

Pressure	40	kPa
Flow	100	mL/min
Time	30-45	Min
Temperature	7-10	^oC

Table 3.9 Assumed state of CO_2 in each stage of the 2-PAD system and bioconversion process

Stage	Initial Influent	Acetogenic	Acetogenic	Acetogenic Effluent	Methanogenic	Methanogenic Effluent
State of CO_2	Dissolved	Gas	Acetate	Dissolved	Gas	Dissolved

To determine the net effect of CO_2 injection and gain better indications of bioconversion, the CO_2 condition measurements of each stage, were subtracted from values of the regular, or control, conditions to remove the effect of naturally dissolved CO_2 in the liquid. Dissolved CO_2 concentrations were determined by titration with 0.5N NaOH solution and calculated by Equation 3.10. The NaOH titration method is substantiated by the relation between pH, CO_2 and hydroxide alkalinity, illustrated in Figure 3.5 and Table 3.7.

$$mg\frac{CO_2}{L} = \frac{mL\ NaOH}{mL\ sample} * \frac{\frac{N}{mol}NaOH}{L} * \frac{mol\ CO_2}{mol\ NaOH} * \frac{44g\ CO_2}{mol} * \frac{1000mg}{g}$$

Equation 3.10

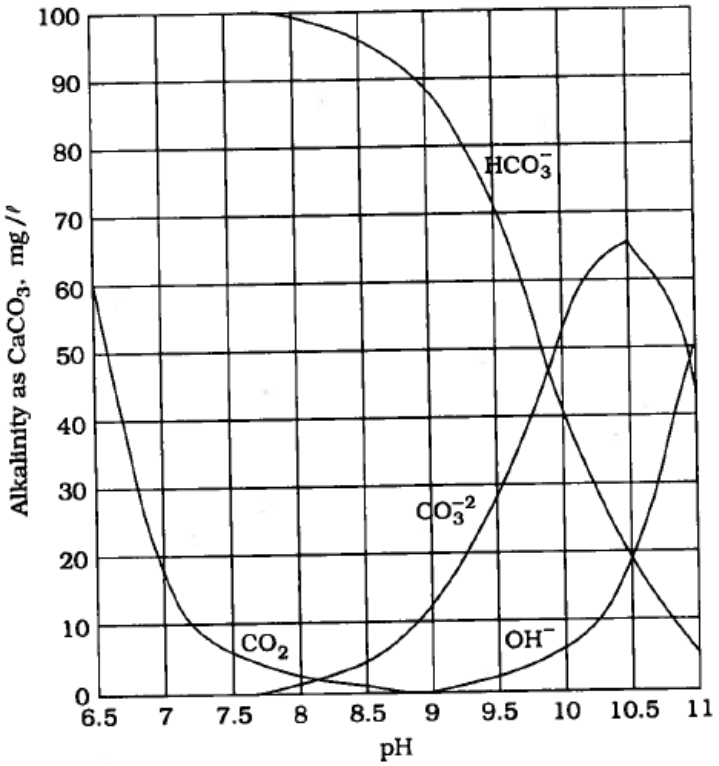


Figure 3.5 Equilibrium distribution of CO_2 with three forms of alkalinity, calculated for total alkalinity of 100 mg/L (Sawyer et al., 1994).

Gas measurements, first calculated by volume from the gas displacement reactor, were then converted from milliliters to milligrams using the ideal gas law (Equation 3.11). The net value of experimental from regular conditions was calculated.

$$\boldsymbol{PV = nRT} \qquad \textbf{Equation 3.11}$$

P = Partial pressure kPa
V = Volume Litres
n = mol
T = temperature Kelvin
R = Avogadro's Number 8.314 kPa*L/k*mol

In addition to calculating gaseous CO_2 emissions, each additional milligram of gaseous CH_4 produced in the experimental CO_2 condition was also considered as a milligram of converted CO_2 in line with the stoichiometric Equation 3.12.

$$CO_2\,(g) + 4H_2\,(g) \rightarrow CH_4\,(g) + 2H_2O\,(L)$$ **Equation 3.12**

In the same vein, each additional milligram of volatile fatty acid, measured as acetate (CH_3COOH), was considered 2 mg of converted CO_2 in line with the following stoichiometric Equation 3.13. An example of this calculation can be found in Appendix D.

$$8H + 2CO_2 \rightarrow CH_3COOH + 2H_2O$$
$$1\ CH_3COOH: 2\ CO_2$$

Equation 3.13

3.14 Metal and Salt Analysis of Sludge

Since the ratio of deinking sludge is at 0.025 v/v ($165m^3/6500\ m^3$) to that of influent wastewater, experimental conditions that included deinking sludge in the feed were always maintained this ratio. In addition to standard measures of performance, the effect of deinking sludge on the performance of the digester was investigated by measuring the amount of accumulation of heavy metals, light metals and salts in the anaerobic bacteria to monitor for relevant changes in system performance and/or toxicity. Mixed liquor samples of sludge were taken before experiments and after one month of operation. These samples, as well as raw deinking sludge, were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS by Agilent 1260 Infinity Model) after acid digestion method MA 200- Met 1.2 (CEAEQ, 2014). Extractible metals in sludge, sediment and vegetation tissue. Sludge samples were dried overnight in an oven at 105 °C. After thoroughly mixing and grinding the sample with a mortar and pestle for homogeneity, 0.5 g of each condition were weighed and reserved for digestion and then transferred to digestion flask. One control condition held 5mL-deionized water. The digestion procedure was as follows:

- Add 10mL 50% nitric acid (V/V) to each flask, mix and cover with a watch glass. Place flasks on a heating plate and heat with reflux just below boiling for 10-15

minutes.

- After cooling, add 5 mL nitric acid and heat was resumed with reflux below boiling for 30 minutes or until oxidation with nitric acid is complete.
- After cooling, rinse watch glass with water and heat flasks to below boiling, allowing for evaporation until total volume in flask reaches 5mL (approximately 2 hours).
- Allow flask to cool and add 2mL deionized water and 3mL 30% hydrogen peroxide.
- Replace flasks on the heating plate and heat until effervescence subsides.
- Continue to add 30% hydrogen peroxide in 1mL aliquots for 10mL.
- Let cool then add 5mL hydrochloric acid and 10mL water. Cover with a watch glass and heat with reflux blow boiling for 15 minutes.
- Allow flask to cool and rinse watch glass with water.
- Filter through Whatman No. 41 filter paper (or equivalent) and dilute filtrate to a volume of 100 mL
- After cooling completely, transfer for storage in a plastic bottle.

3.15 Cost Calculations

Current operating costs were calculated for the Cascades WAS system using the following parameters:

- Energy consumption of equipment (flotation, pumps, aeration pressing, drying)
    - Hydroelectric Power (0.0426$/kWh)
    - Natural gas heating of building (71.50$/day or 2160 kWh/d)
- Consumable materials
    - Nutrients (0.11$/$m^3$)
    - Flocculent (0.08$/$m^3$)
- Solids Disposal Costs (primary, WAS and deinking sludge)
    - 25$/humid ton (65% solid)

Proposed operating conditions were calculated for the Cascades AD system using the previous (current) as well as the following parameters:

- Consumable materials
    - Nutrients (0.17 $/m^3)
    - Caustic (0.12$/m^3)
    - Biofilter materials (14 $/day)
- Waste Activated Sludge
    - Food/microbe ratio: (0.25)
    - Sludge production factor: (0.45 metric ton BOD: metric ton sludge)
- Estimates of additional operating costs for AD system (conservative estimate) (0.12$/m^3)

Information was obtained in collaboration with Cascades' Environmental Supervisor.

Estimates of construction costs are not available at this time.

Details on cost calculations can be found in Appendices F-H.

Biogas caloric value was calculated by the following:

Specific energy for CH_4	55	MJ/kg
Ideal gas law	35	MJ/m^3

Chapter 4 RESULTS and DISCUSSION

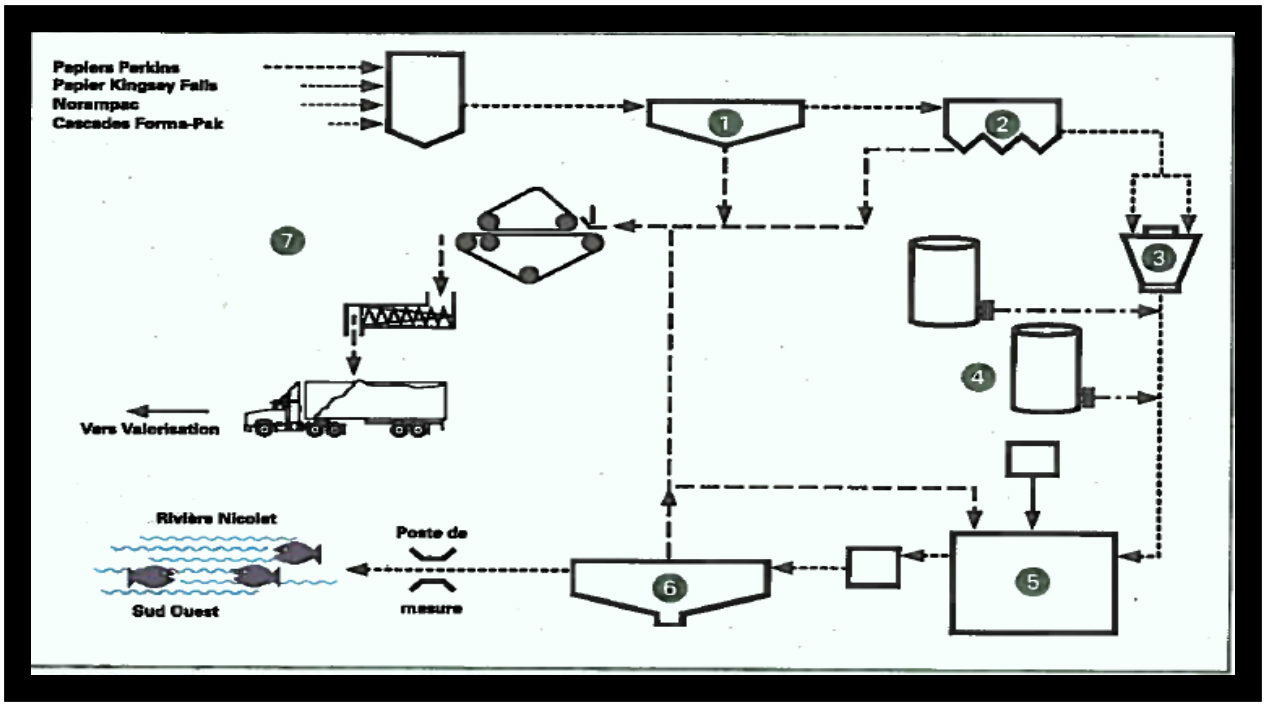
4.1 Current Wastewater Treatment

The Cascades Recycling Plant in Kinsey Falls, Quebec currently treats its wastewater by: a primary clarifier, biological selector reactor and waste activated aerobic sludge treatment (WAS). This flow sequence is illustrated in Figure 4.1. Although this system is very effective, and reduces 99% of the wastewater's biological oxygen demand, its disadvantages include high growth rates of bacterial sludge and high oxygenation requirements. This leads to high costs related to aeration, as well as the cost of pressing, drying and disposal of wasted sludge solids. The recycling process produces another waste stream in the form of deinking sludge, not shown in the Figure 4.1 flow chart. The deinking sludge also requires drying and disposal.

4.2 Batch Test Results

As noted in the introduction, previous work in the research group conducted batch tests on Cascades wastewater samples, the principle aim was to investigate the role of pH in the bioconversion of CO_2 into CH_4 by anaerobic bacteria. The wastewater samples for previous tests were withdrawn between units 4 and 5, but before the bioselector unit, which is not shown i.e. after clarification, flotation and addition of nutrients. Characteristics of wastewater from that sampling point are listed in Table 4.1.

As a verification of methods, a replication of the batch experiment using post-primary treated wastewaters was run at the most successful pH in the conversion process (pH 5.5) (Abedi et al., 2015). As can be seen in Figure 4.2, there is a very small difference in COD reduction over 2 weeks between the regular wastewater and the wastewater with dissolved CO_2, both showing approximately 60% reduction. Figure 4.3 shows a slightly greater volume of total biogas production of the CO_2 condition compared to the regular wastewater condition, though this difference is not significant. The GC was not operational during these tests and so exact methane yields could not be determined.



#	Unit
1	Primary clarifier
2	Flotation reactor
3	Cooling tower
4	Nutrient addition
(not shown)	Biological selector reactor
5	Waste activated sludge reactor
6	Secondary clarifier
7	Pressing, dehydration and disposal

Figure 4.1 Current flow chart of Cascades wastewater treatment plant at Kingsey Falls, QC. Biological selector (between units 4 and 5) is not shown, as it was a later addition to the plant. Deinking sludge collection and treatment is not shown.

Table 4.1 Characteristics of wastewater after primary treatment

Parameter	Value	Unit
COD	2050	mg/L
BOD	600	mg/L
TN	23	mg/L
PO_4^{3-}	14	mg/L

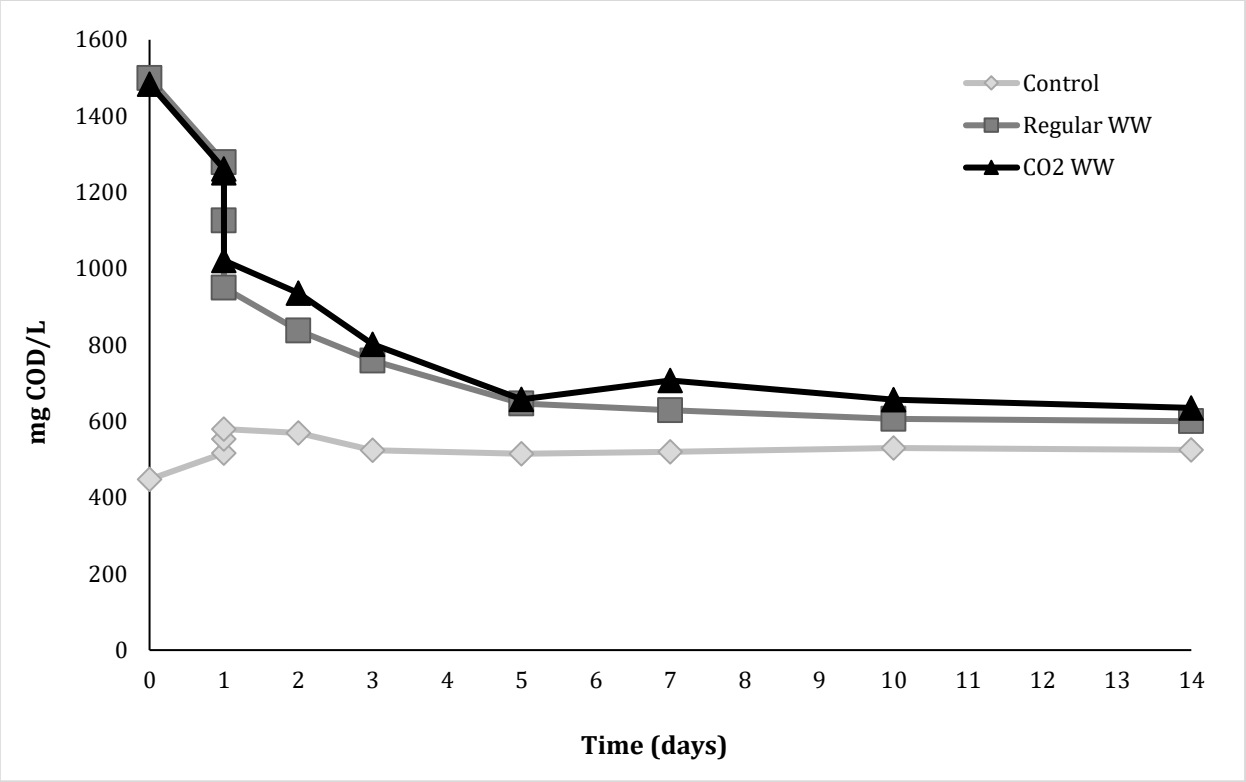


Figure 4.2 Total COD concentration of 3 conditions with respect to time for Cascades wastewater after primary treatment at pH of 5.5 in batch test.

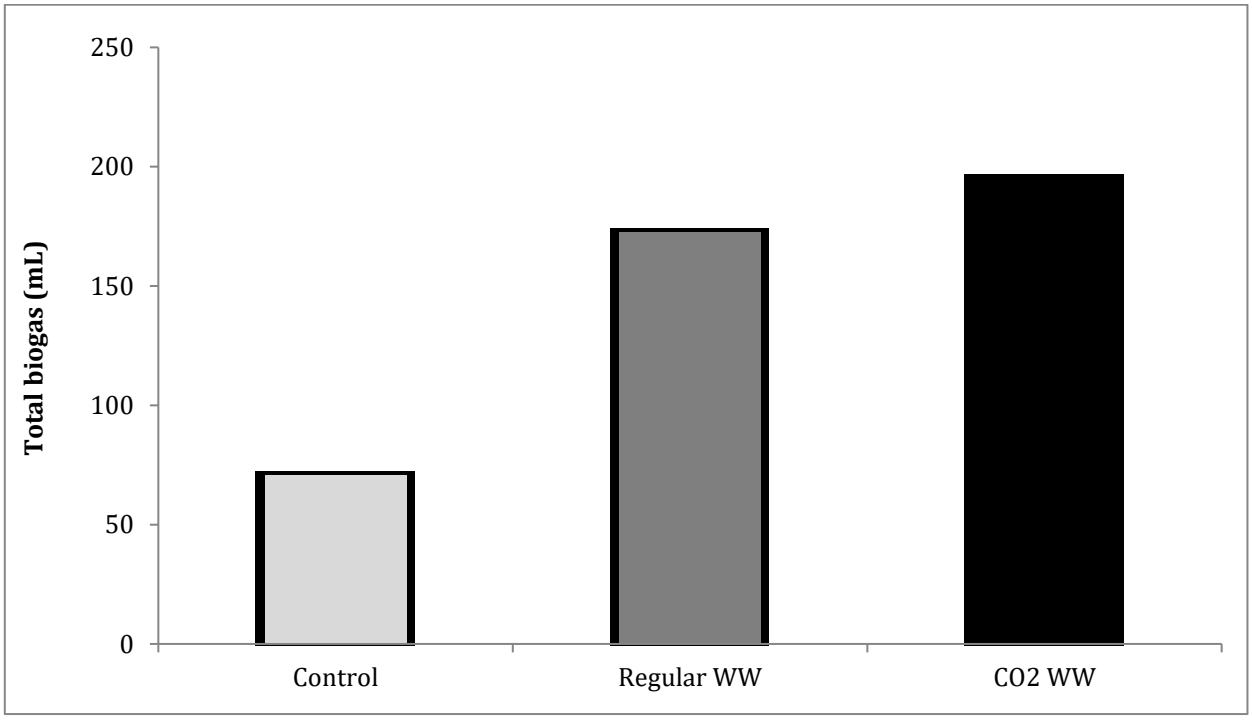


Figure 4.3 Total biogas production for 3 conditions of batch tests with Cascades wastewater after primary treatment at pH 5.5 over 14 days.

These tests differed in that the wastewater samples were taken from the point prior to any treatment, i.e. direct influent into the treatment system before unit 1 in the flow chart (Figure 4.1). Characteristics of the wastewater are listed in Table 4.2. The variability of the influent wastewater's COD and nutrients are illustrated in Figure 4.4. Since the COD is higher, it was believed that this water would yield more biogas, thereby providing a more feasible option for the AD system. All subsequent batch and continuous experiments were conducted using this untreated raw influent wastewater. The following batch tests were also fixed at a pH of 5.5. Figure 4.5 shows a small initial difference between regular wastewater and the CO_2 condition, which became null by day 3 of 14. Both conditions show approximately 80% reduction of COD overall. Figure 4.6 shows the volume of biogas production of the CO_2 condition to be slightly greater than that of the regular wastewater condition.

Table 4.2 Characteristics of influent wastewater

Parameter	Value	Unit
COD	5500	mg/L
BOD	1500	mg/L
VSS	1553	mg/L
TN	15	mg/L
PO_4^{3-}	1	mg/L

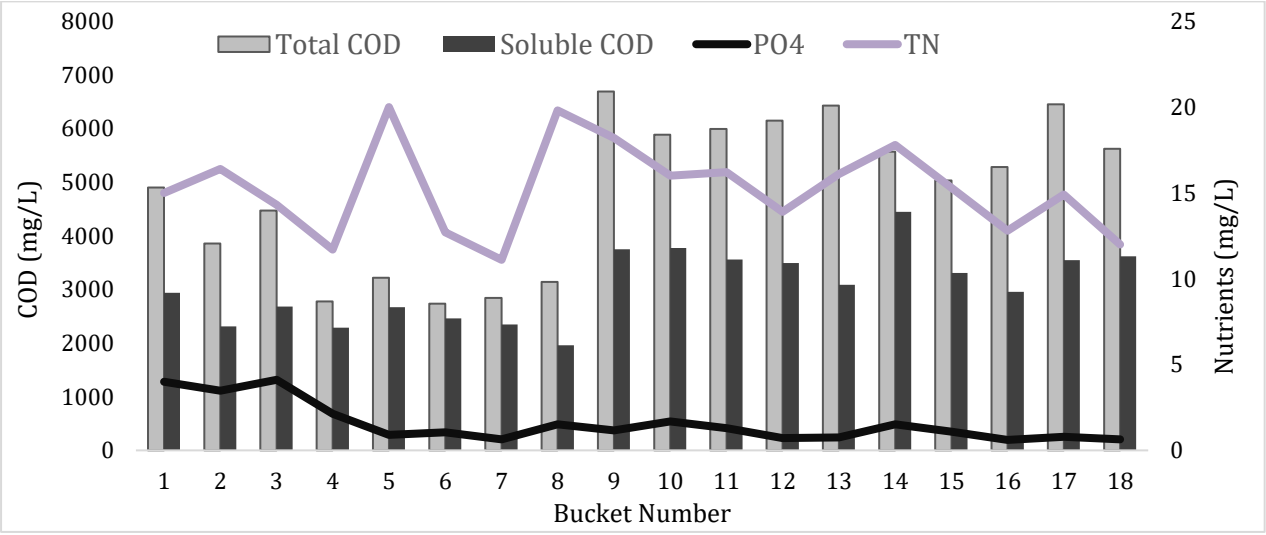


Figure 4.4 Variation of influent wastewater by bucket received. Parameters listed: total and soluble chemical oxygen demand (COD), total nitrogen (TN) and phosphate (PO_4^{3-}), all in mg/L.

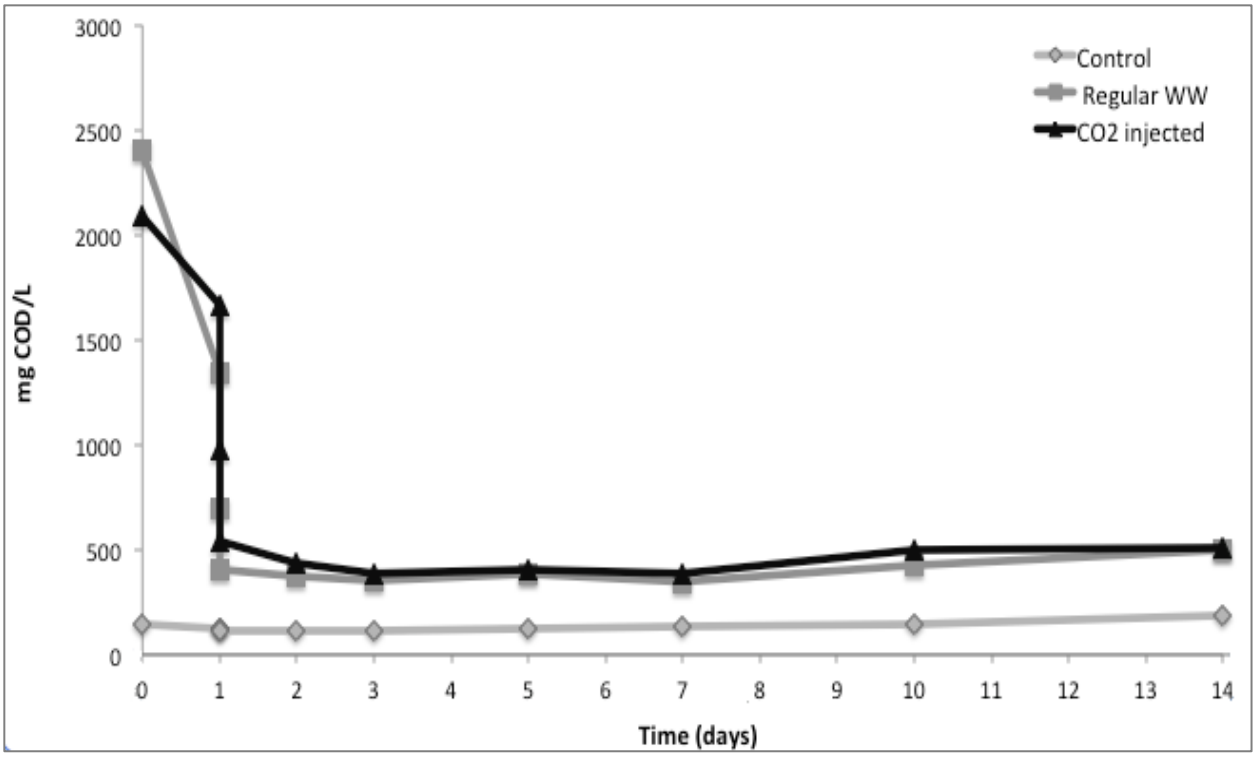


Figure 4.5 Total COD concentration of 3 conditions with respect to time for influent wastewater at pH 5.5 over a period of 14 days in a batch test.

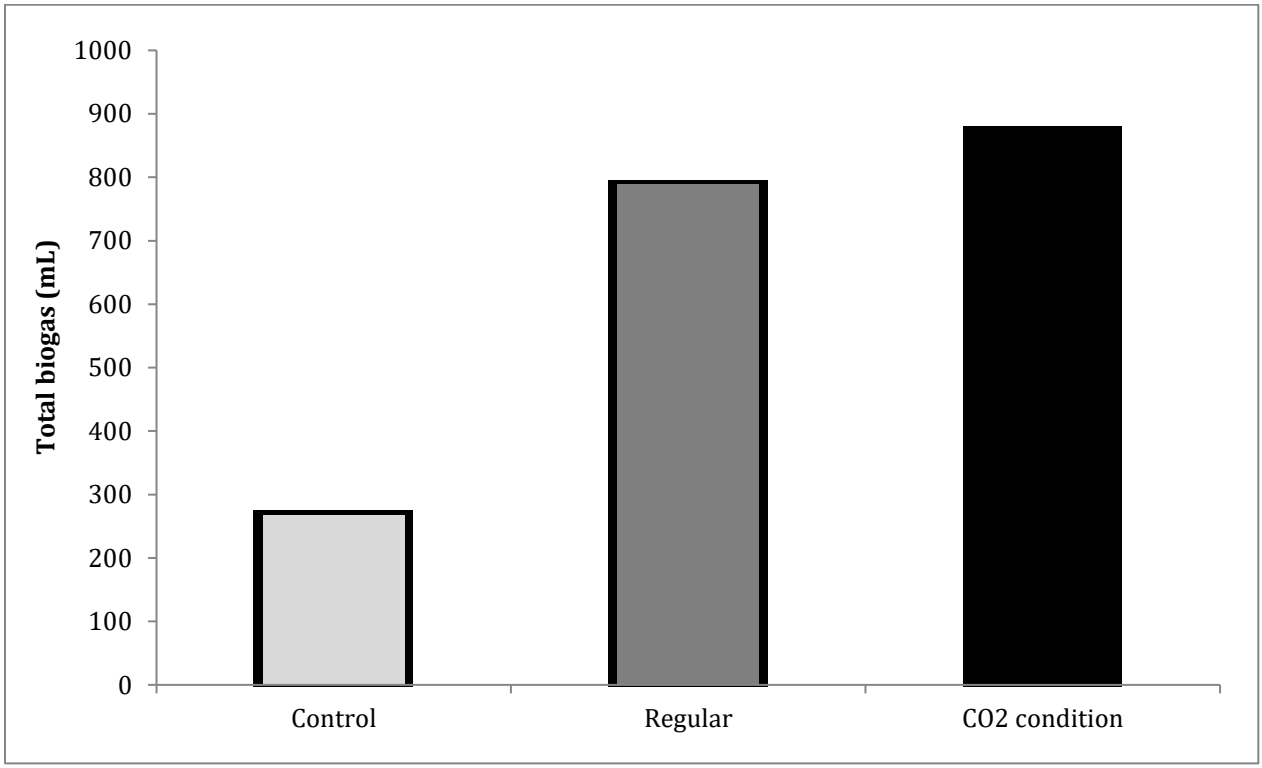


Figure 4.6 Total biogas production for 3 conditions of batch tests with Cascades influent wastewater at pH 5.5 over 14 days in a batch test.

4.3 Continuous Experiments

Given the positive results of the batch tests for both regular and CO_2 conditions using this influent wastewater, it was determined worthwhile to proceed with continuous reactor experiments. However, continuous experiments did not include external adjustment of pH level.

4.3.1 Single Phase Continuous Experiments

The most simple and presumably least-costly production design was conducted using granular sludge in a single-phase condition. The retention time was set at 5 days for reliable reduction in COD and methane production. These parameters were tested at two temperatures corresponding to operating temperatures at the Cascades treatment plant: 38°C during the winter and 45°C during the summer. The total COD of the influent in these trials averaged 6.0 kg tCOD/m^3, while the soluble COD averaged 3.6 kg sCOD/m^3. Figures 4.7 and 4.8 show results for total COD. Figure 4.7 shows continuous operation at 38°C with an average organic loading rate of 1.25 kg tCOD/m^3-d. Reduction of total COD was stable at an average of 94% while methane yield ranged between 0.13 and 0.20 m^3 CH_4/ kg $tCOD_{rmv}$. Figure 4.8 shows the results of continuous operation at 45°C with an average organic loading at 1.05 kg $tCOD_l/m^3$-d. Total COD was consistently reduced at an average of 91% while methane yield ranged between 0.20 and 0.25 m^3 CH_4/kg $tCOD_{rmv}$. Figures 4.9 and 4.10 show results for soluble COD. At 38°C, the average OLR was 0.60 kg sCOD/m^3-d and produced an average methane yield of 0.35 m^3 CH_4/ kg $sCOD_{rmv}$ with a soluble COD reduction of 87% (Figure 4.9). At 45°C, the average OLR was 0.78 kg sCOD/m^3-d and produced an average methane yield of 0.32 m^3 CH_4/ kg $sCOD_{rmv}$ with a soluble COD reduction of 88% (Figure 4.10). Methane yields calculated for soluble COD were up to 2 times larger than yields for total COD.

Future tests under these conditions would monitor performance on a longer term. It would be informative to measure the performance at lower retention times, gradually reducing HRT to 3 days and for a prolonged period to monitor stability, degradation of solids and potential changes in efficiency. Although methane yields at 45°C were shown to be high, subsequent consultation with industry developers of AD plants recommended that

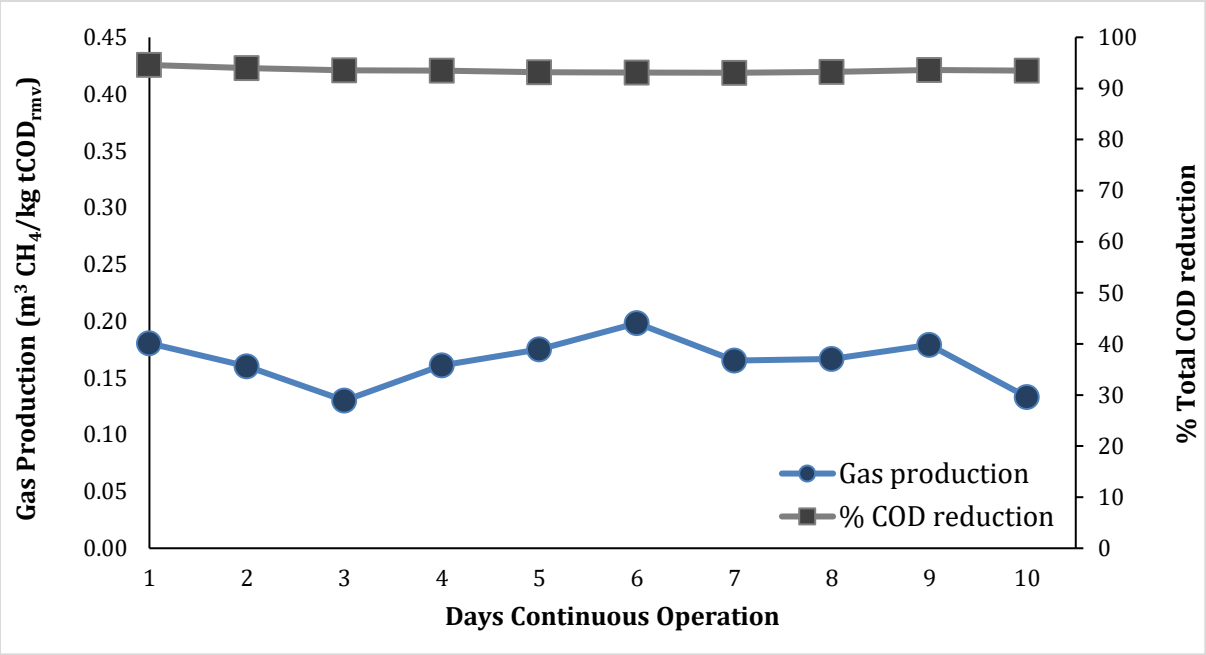


Figure 4.7 Continuous operation of a single stage reactor at 38°C with granular sludge. OLR = 1.25 kg tCOD/m³-d. HRT= 5 d. Percentage of reduction and methane yield for total COD are illustrated.

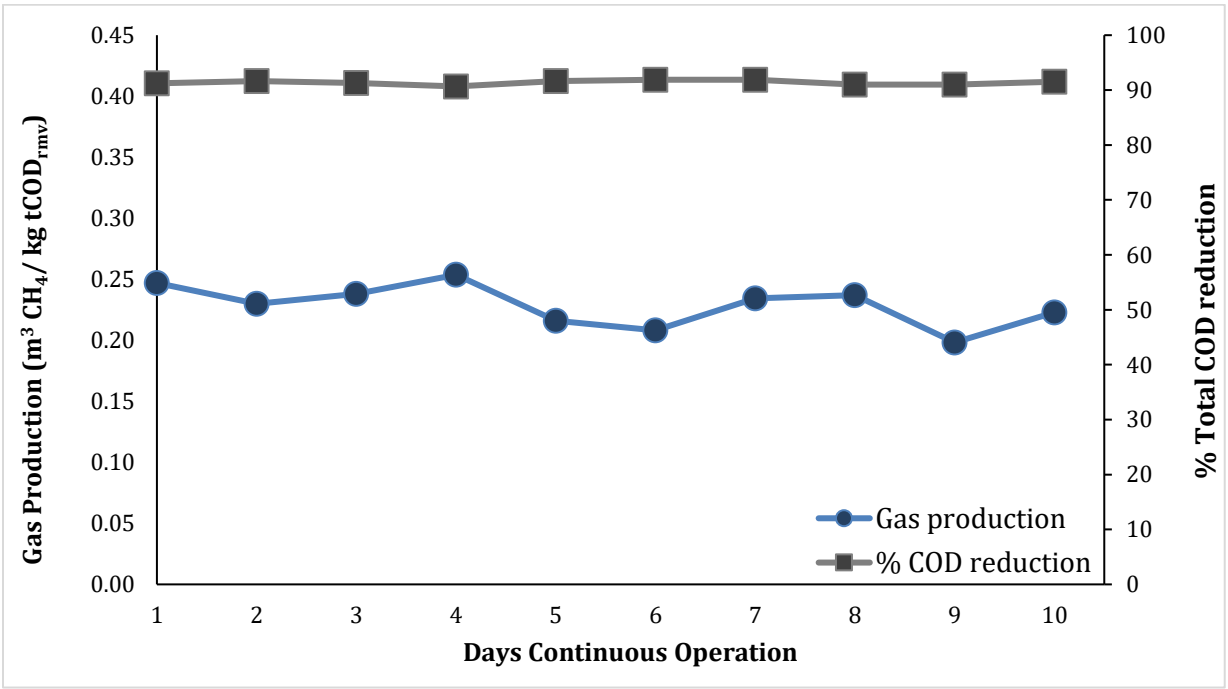


Figure 4.8 Continuous operation of a single stage reactor at 45°C with granular sludge. HRT = 5 days. OLR = 1.05 kg tCOD/m³-d. Percentage of reduction and methane yield for total COD are illustrated.

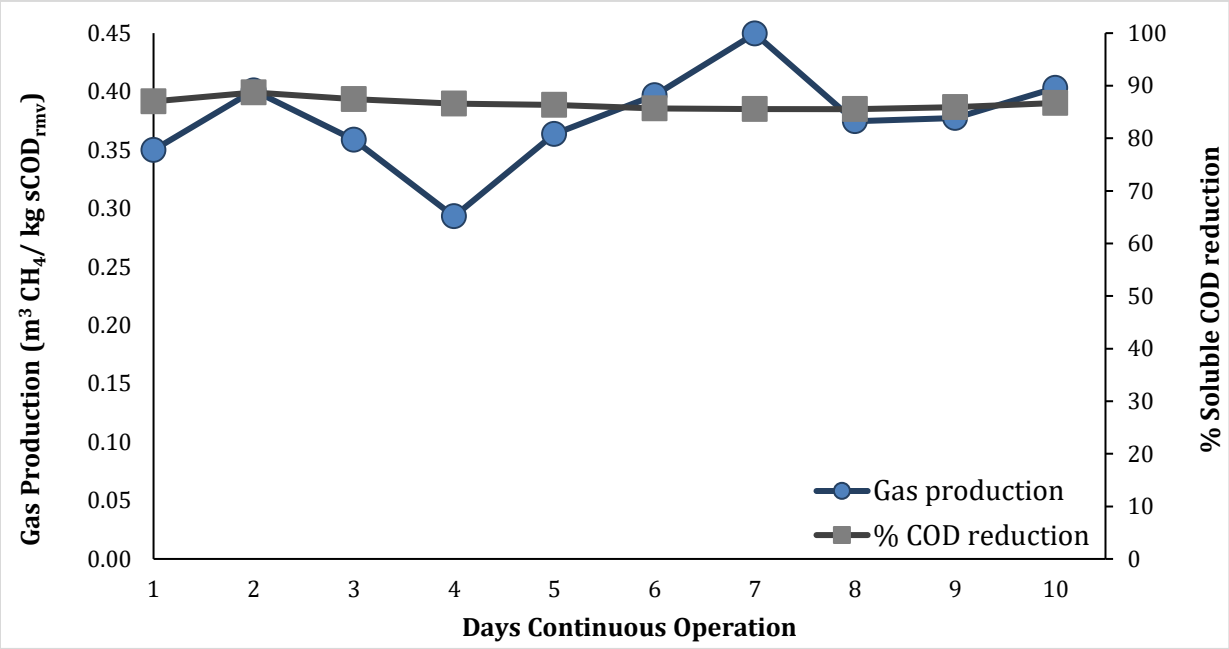


Figure 4.9 Continuous operation of a single stage reactor at 38°C with granular sludge. OLR = 0.60 kg sCOD/m³-d. HRT= 5 d. Percentage reduction and methane yield for soluble COD are illustrated.

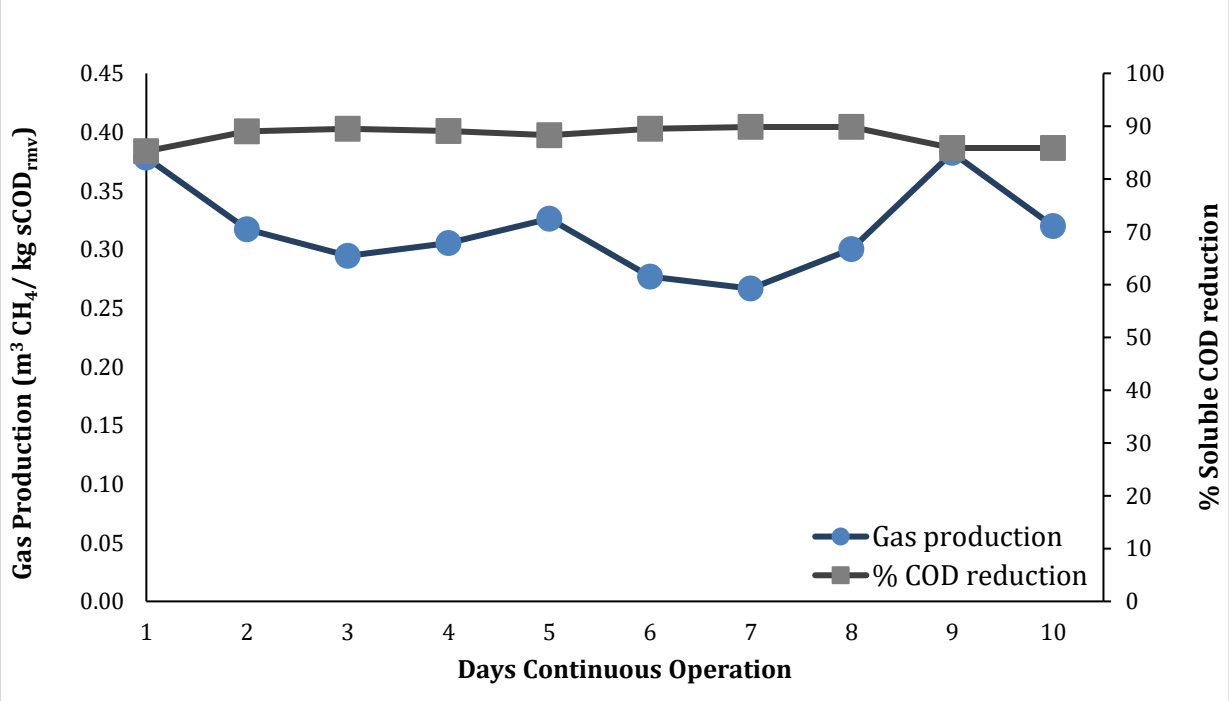


Figure 4.10 Continuous operation of a single stage reactor at 45°C with granular sludge. OLR = 0.78 kg sCOD/m³-d. HRT= 5 d. Percentage reduction and methane yield for soluble COD are illustrated.

temperatures be maintained at a maximum of 38°C for methanogenic health and long-term performance. Furthermore, since the treatment plant already has an installation of a cooling tower designed to cool influent wastewater during the summer months to a temperature of 38°C, ensuing experiments focused only on operating between temperatures between 35-38°C.

4.3.1.1 Cascades Operating Parameters - Liquid Sludge

Having established the performance of a single-phase reactor using granular sludge, trials were run using liquid anaerobic sludge. Although liquid sludge has been consistently shown to be less effective at reducing COD and producing methane than granular sludge, the purpose of these tests was to obtain an estimate of performance upon start-up, time to granulation if applicable, and effectiveness of sludge mixing with solids content. Initial use of liquid sludge would be a more cost-effective option since it can be obtained for little or no cost from municipal treatment centers and is run under the assumption that granular sludge would form with continuous operation in a UASB system.

Trials for the single-stage liquid sludge reactors were run at a temperature of 38°C and a retention time of 4.5 days. The total COD of the influent in these trials averaged 5.4 kg tCOD/m^3, while the soluble COD averaged 3.4 kg sCOD/m^3. Figures 4.11 and 4.12 show the results of trials using only influent wastewater as the feedstock. Organic loading rate was an average of 1.20 kg tCOD/m^3-d and 0.72 kg sCOD/m^3-d over a period of 18 days. Total COD reduction was stable at around 94% while methane yield ranged between 0.08 - 0.13 m^3 CH_4/kg tCOD. Soluble COD removal was stable around 90% while methane yield averaged 0.18 m^3 CH_4 /kg sCOD. Figures 4.13 and 4.14 show the results of trials using both wastewater and deinking sludge as a feedstock at the same proportion produced in wastestreams at the Cascades recycling plant, i.e. 0.025 m^3 deinking sludge/m^3 influent wastewater. Integrating deinking sludge has advantages but also comes with some risks. The high volume of deinking sludge produced each day creates a cost of approximately 1 million CAD/year in disposal and treatment costs. Preliminary batch experiments (results not shown) indicated that the deinking sludge is highly degradable by anaerobic digestion. Furthermore, the sludge contains nutrients and minerals essential to the AD process that

could supplement the current requirement and cost of nutrient addition. However, the disadvantages are that it contains a small, but possibly cumulative mixture of heavy metals and PAHs. The condition integrating deinking sludge ran for 28 days and maintained an organic loading rate of 1.35 kg tCOD/m^3-d and 0.87 kg sCOD/m^3-d. Total COD reduction was stable at 93% while methane yield ranged between 0.12 - 0.18 m^3 CH_4/kg tCOD (Figure 4.13). Soluble COD reduction was stable around 90% with a methane yield averaging 0.23 m^3 CH_4/kg sCOD (Figure 4.14).

Results of all 4 single-phase conditions are summarized in Table 4.3. Operating conditions for the single phase reactors were all similar having HRTs between 4-5 day, OLRs between 1.06-1.33 kg tCOD/m^3-d and 0.60-0.87 kg sCOD/m^3-d. Conditions differed by sludge type, operating temperature and feed stream. Overall, methane yields calculated for soluble COD were substantially higher than those of total COD. Methane yield for total COD removed was highest for granular sludge at 45 °C, showing 0.22 m^3 CH_4/kg $tCOD_{rmv.}$ While the highest methane yield for soluble COD removed were highest for granular sludge at 38 °C , showing the theoretical yield of 0.35 m^3 CH_4/kg $sCOD_{rmv}$. COD and BOD removal was consistent between 80-90% for all experiments. Although the condition that integrated deinking sludge produced a lower methane yield than the granular sludge, it was higher than the equivalent condition using liquid sludge without deinking feed for both total and soluble COD removed. There was no evidence of toxicity from the deinking sludge during this time, as demonstrated by stable methane yields and COD reduction. Concentrations in solids were also assessed by ICP-MS metal analysis and will be discussed in section 4.6. No build up of solids was observed during the experiment which suggests the successful degradation of the majority of these solid particles. After close to 2 months of trials with liquid sludge, some changes were observed in the sludge, but not enough to qualify as granulation. Future testing would run continuous trials reducing retention times to a minimum and monitoring for build-up of toxic elements from the deinking sludge that may affect methanogenic bacteria. Sludge degradation would also be monitored regularly.

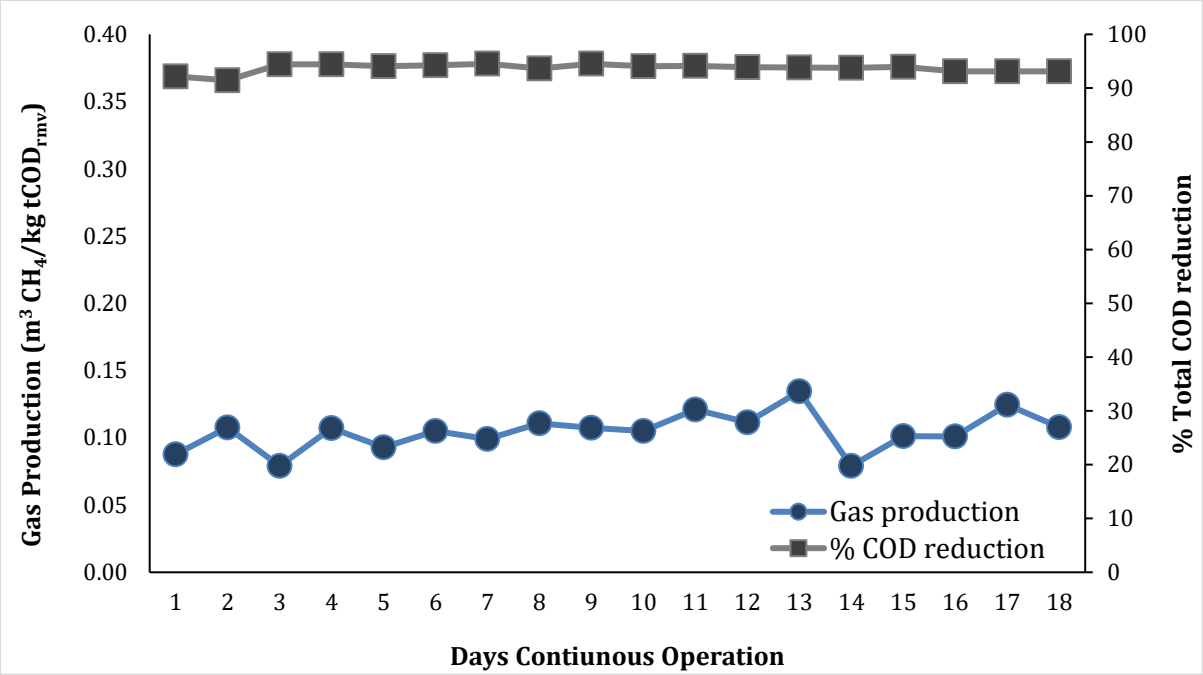


Figure 4.11 Continuous operation of a single stage reactor at 38°C with liquid sludge fed only wastewater at an OLR of 1.20 kg tCOD/m³-d. HRT = 4.3 days. Percentage of reduction and methane yield for total COD are illustrated.

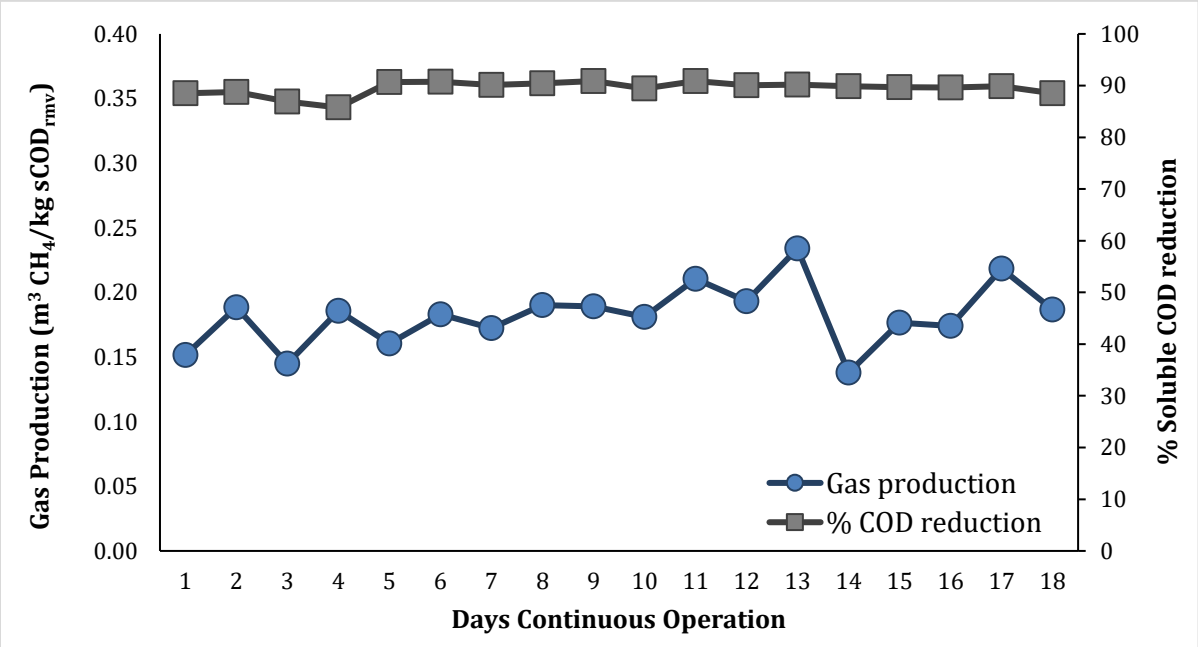


Figure 4.12 Continuous operation of a single stage reactor at 38°C with liquid sludge fed only wastewater at an OLR of 0.72 kg sCOD/m³-d. HRT = 4.3 days. Percentage of reduction and methane yield for soluble COD are illustrated.

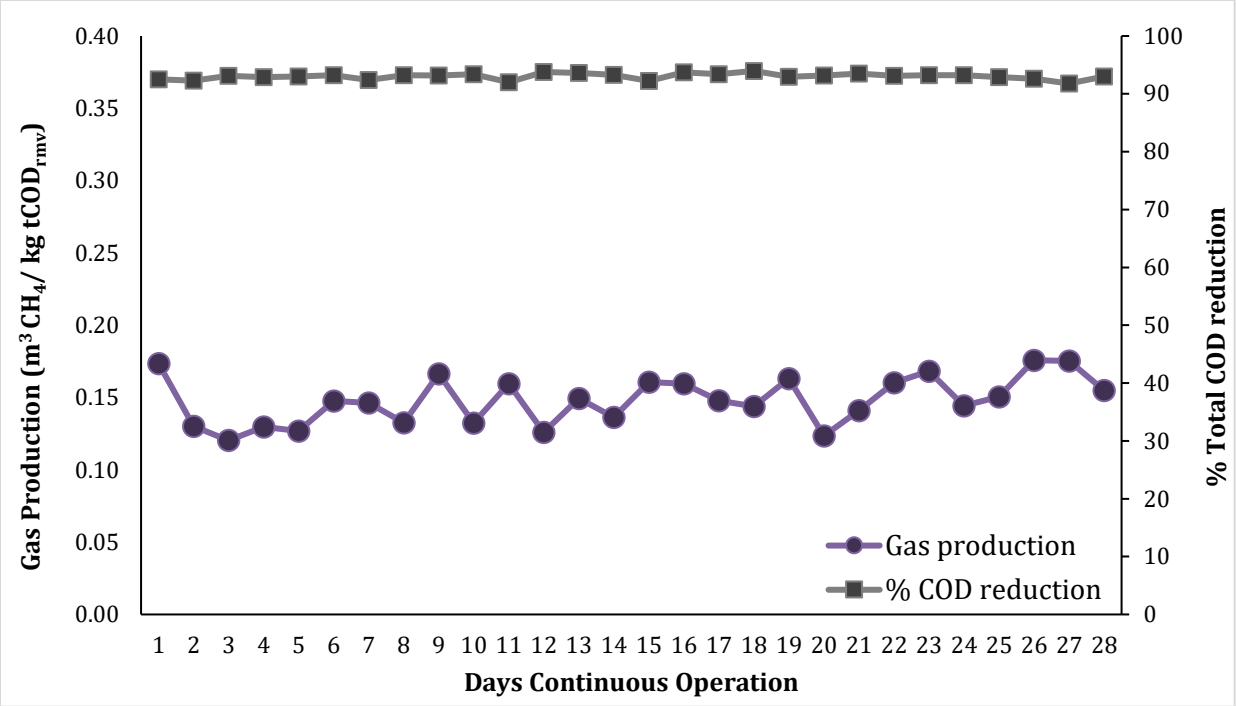


Figure 4.13 Continuous operation of a single stage reactor including deinking sludge in the feed stream at 38°C with liquid sludge with an OLR of 1.35 kg tCOD/m³-d. HRT = 4.5 days. Percentage of reduction and methane yield for total COD are illustrated.

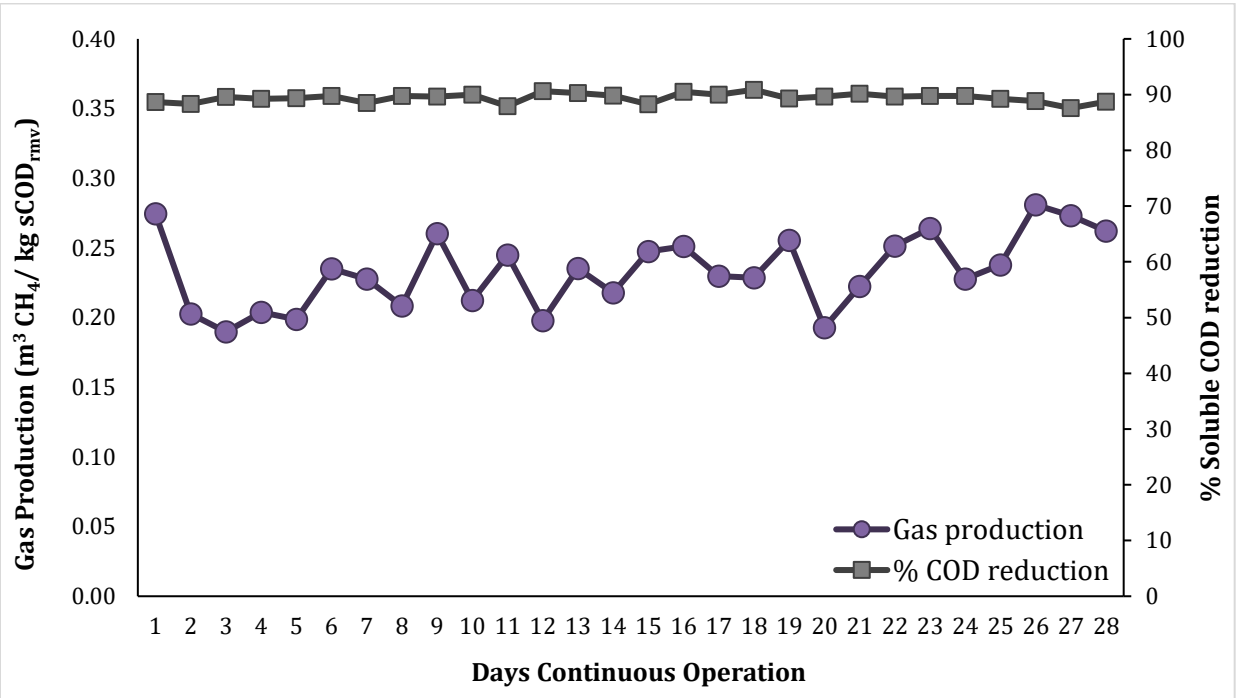


Figure 4.14 Continuous operation of a single stage reactor including deinking sludge in feed stream at 38°C with liquid sludge with an OLR of 0.87 kg sCOD/m³-d. HRT = 4.5 days. Percentage of reduction and methane yield for soluble COD are illustrated.

Table 4.3 Summary of parameters for single-phase conditions and effluent results

Cascades Operating Conditions

Parameter					Units
Sludge type	Granular	Granular	Liquid	Liquid	
Feedstock	wastewater	wastewater	wastewater	Wastewater + deinking sludge	
Length of test	10	10	19	29	Days
Temperature	38	45	38	38	^{o}C
HRT	5.1	5.2	4.3	4.5	Days
Methane yield Total	0.16	0.22	0.10	0.15	m^3 CH_4/ kg $tCOD_{rmv}$
Methane yield Soluble	0.35	0.32	0.18	0.23	m^3 CH_4/ kg $sCOD_{rmv}$
OLR $_{total\ COD}$	1.24	1.06	1.20	1.33	kg tCOD/m^3-d
OLR $_{soluble\ COD}$	0.60	0.78	0.72	0.87	kg sCOD/m^3-d
COD effluent	0.41	0.47	0.33	0.39	kg/m^3
tCOD reduction	94	92	94	93	%
sCOD reduction	87	88	90	90	%
BOD effluent	0.14	0.12	0.12	0.14	kg/m^3
BOD reduction	92	94	89	81	%
VSS effluent	0.03	0.07	0.05	0.06	kg/m^3
VSS reduction	99	98	96	97	%
Alkalinity	770	870	760	840	mg/L $CaCO_3$
pH	7.74	7.65	7.60	7.83	
TN	163	123	69.2	67.8	mg/L
PO_4^{3-}	47.8	23.7	15	14	mg/L
SO_4^{2-}	72.2	72	100	71	mg/L
Al (soluble)				0.042	mg/L
Cr (soluble)				0.083	mg/L

4.3.2 Two-Phase Anaerobic Digestion (2-PAD) Continuous Experiments

The next series of experiments focused on a 2-phase system. This 2-PAD condition was expected to hold additional benefits such as increased stability (given the variability of influent wastewater), increased degradation of solids and reduced overall reactor size.

4.3.2.1 Cascades Operating Parameters (2-PAD)

This condition was designed to maximize methane production and cost savings during the start-up period while simulating realistic operating conditions at the Cascades plant such as an operating temperature of 38 °C and including deinking sludge at an operating ratio of 0.025 v/v with influent wastewater. The acetogenic phase was inoculated with liquid anaerobic sludge that had been cultivated to contain predominantly acetogenic bacteria, as per Method 3.9. The use of liquid sludge in this condition facilitated the complete mixing in the bench-top reactor to aide in the accurate measurement of sludge growth and degradation, although the additional sludge proved to be a confounding factor as well. Typically, the bacterial population in the acetogenic reactor will be maintained by virtue of appropriate residence time and naturally occurring bacteria within the solids and wastewater and so the wasting from this reactor does not present a loss in valuable sludge. Mixing was done with an internal paddle operated daily upon feeding. Since this initial stage would be loaded with 99% of the solid content of the waste streams, it was expected that the high acidity and hydrolysis would aid in the degradation of these solids, the disposal of which is one of the major costs for the Cascades plant. Additional justifications for employing a 2-phase system in this case are supported by the robustness of acetogenic bacteria to toxicity from heavy metal build-up. These factors will be discussed in greater detail in section 4.6 upon analysis of toxicity accumulation after 1 month of operation with deinking sludge feedstock. The second, methanogenic, phase contained granular sludge, as would be employed in an efficient and advanced UASB system. Nutrient concentrations were monitored in this experimental series in the interest that additional nutrients contained in the deinking sludge could supplement nutrient requirements of the anaerobic bacteria, thereby providing a source of cost reduction. The following results were calculated by measuring gas emissions and effluent from the methanogenic reactor of a 2-

phase system. All reports of retention time pertain only to the methanogenic reactor; the acetogenic reactor is taken to be constant at a retention time of 1 day for the purpose of these analyses. A more detailed analysis of the results of different experimental conditions for acetogenic operation is discussed section 4.4.

This condition ran for 21 days at 37°C and maintained an organic loading rate of 1.50 kg tCOD/m^3-d and 1.05 kg sCOD/m^3-d. Total COD reduction was stable at 94% while methane yield ranged between 0.08 - 0.15 m^3 CH_4/kg $tCOD_{rmv}$ (Figure 4.15). Soluble COD reduction was stable around 91% with a methane yield averaging 0.20 m^3 CH_4/kg $sCOD_{rmv}$ (Figure 4.16). Methane yields from soluble COD are almost twice those of total COD.

Table 4.4 summarizes the operating parameters and results from this condition. Nutrient concentrations were expected to remain constant throughout the treatment system, as is standard with anaerobic digestion. Using the ratio 500:5:1 (wt:wt:wt) for COD influent > 5000 mg/L, nutrients levels were adjusted to: 5500 mg COD: 55 mg TN: 11 mg PO_4^{3-}. Results showed nutrient concentrations to be consistent through the trials although concentrations were slightly higher than estimated. Acetogenic effluent showed average concentrations as 76 mg/L TN and 33 mg/L PO_4^{3-} while average nutrient concentrations of methanogenic effluent were as indicated in Table 4.4. This suggests that solubilization of nutrients from deinking sludge may have been greater than anticipated. This method could benefit from further refining to determine optimal dosing in the future and minimize costs of additional nutrient supplementation.

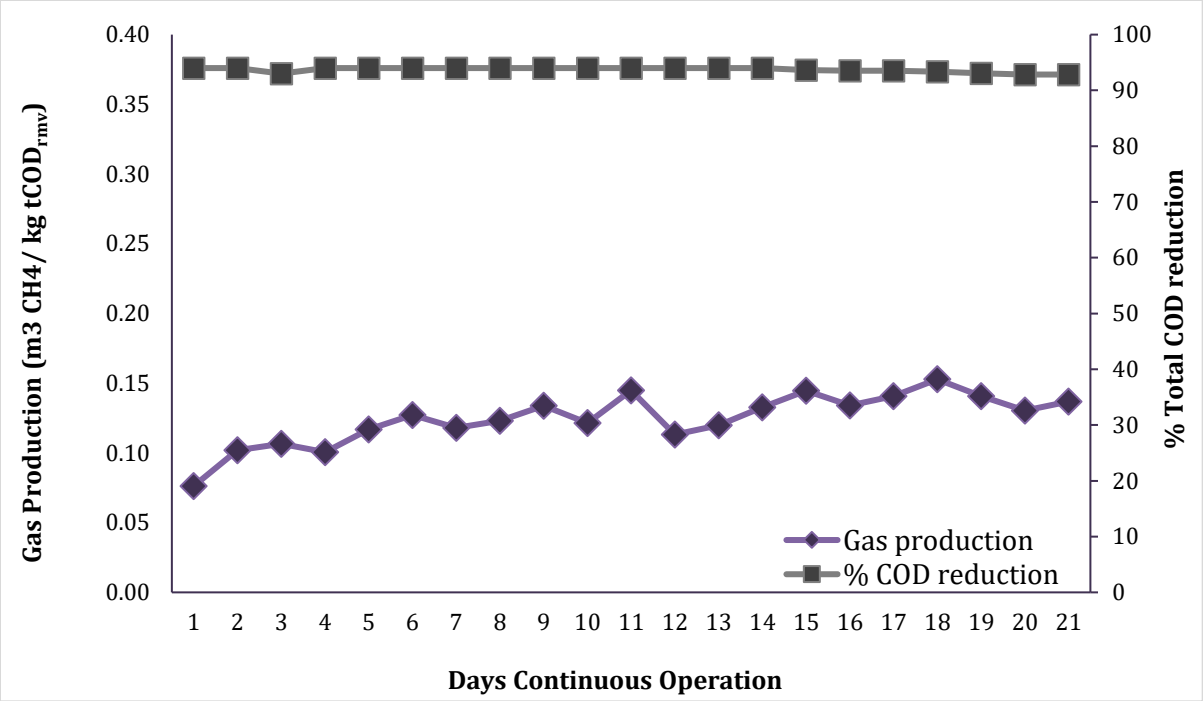


Figure 4.15 Continuous operation of a 2-PAD. The acetogenic phase was operated at 38°C with liquid sludge and a while granular methanogenic reactor was operated at 37°C with an OLR of 1.50 kg tCOD/m³-d, including deinking sludge . Percentage of reduction and methane yields for total COD from methanogenic reactor are illustrated.

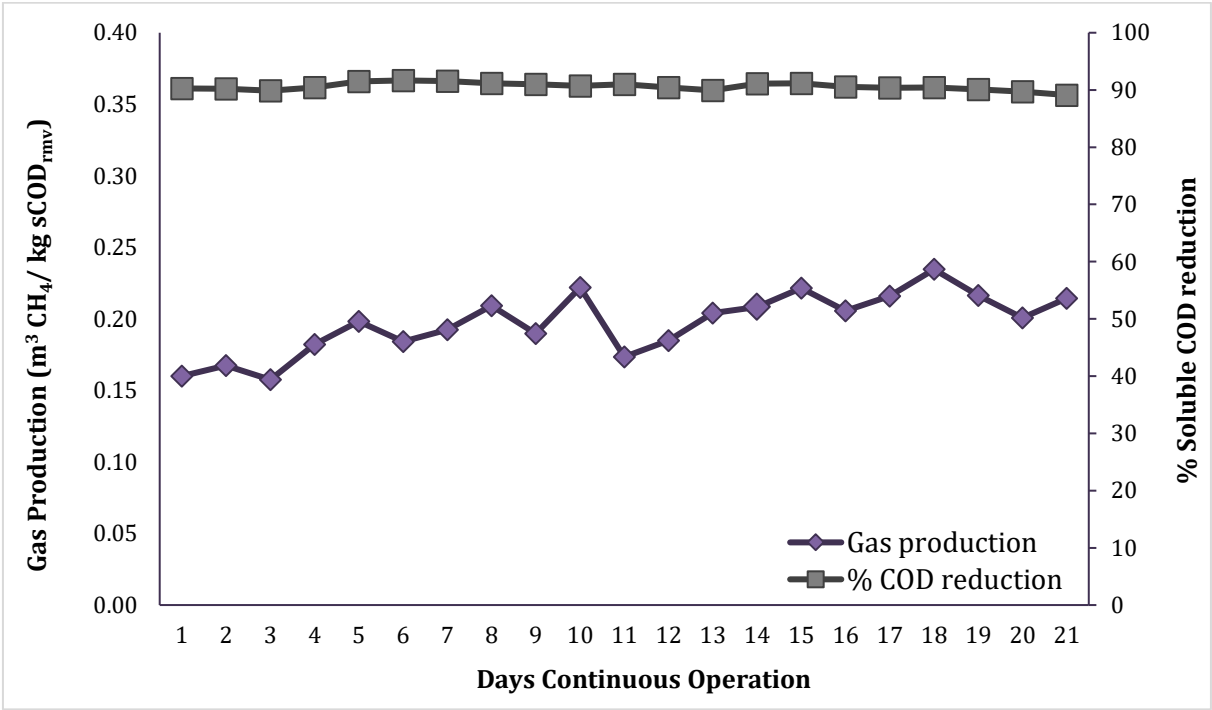


Figure 4.16 Continuous operation of a 2-PAD. The acetogenic phase was operated at 38°C with liquid sludge and a while granular methanogenic reactor was operated at 37°C with an OLR of 1.05 kg sCOD/m³-d, including deinking sludge. Percentage of reduction and methane yields for soluble COD from methanogenic reactor are illustrated.

Table 4.4 Summary of parameters and effluent concentrations for a 2-phase condition – Cascades Operating Conditions Simulation

Parameter	Value		Units
Length of test	21		days
Feedstock	wastewater + deinking sludge		
Temperatures	38 (aceto) – 37 (methano)		^{o}C
HRT	3-4		days
Methane yield Total	0.12		$m^3\ CH_4$/ kg $tCOD_{rmv}$
Methane yield Soluble	0.20		$m^3\ CH_4$/ kg $sCOD_{rmv}$
OLR $_{total\ COD}$	1.4-1.8		kg $tCOD/m^3$-d
OLR $_{soluble\ COD}$	0.91-1.22		kg $sCOD/m^3$-d
COD effluent	0.34		kg/m^3
tCOD reduction	94		%
sCOD reduction	91		%
BOD effluent	0.10		kg/m^3
BOD reduction	92		%
VSS effluent	0.02		kg/m^3
VSS reduction	99		%
Alkalinity	922		mg/L $CaCO_3$
pH	7.75		
TN	98		mg/L
PO_4^{3-}	28		mg/L
SO_4^{2-}	77		mg/L
Al (soluble)	1.094(aceto)	0.025 (methano)	mg/L
Cr (soluble)	0.826 (aceto)	0.054 (methano)	mg/L
Fe (soluble)		0.409 (methano)	mg/L

4.3.2.2 Range of Organic Loading Rates (2-PAD)

Two-phase tests were also conducted using granular sludge for both acetogenic and methanogenic phases. This set of experiments was conducted using wastewater influent only with no deinking sludge. All acetogenic trials in this set of experiments were operated at 25°C except for 1.5 weeks at 38°C and 45°C. The methanogenic reactor was operated at 35°C for all trials. Results from the methanogenic reactor are summarized in Figure 4.17. Five retention times were tested between 1.0 - 4.5 days. Variable influent COD led to an occasionally non-linear relation between retention time and the organic loading rate. However, this effect became smaller with increased number of trials. OLR for total COD ranged between 5.10 - 0.64 kg tCOD/m^3-d while OLR for soluble COD ranged between 3.0 – 0.52 kg sCOD/m^3-d. Gas yields were highest for total COD, at 0.21 m^3 CH_4/ kg $tCOD_{rmv}$ and for soluble COD at 0.25 m^3CH_4/kg $sCOD_{rmv}$, at a retention time of 4.5 days (Figure 4.17). Shorter retention times between 1.0 - 4.0 days averaged gas production of 0.14 m^3 CH_4/ kg $tCOD_{rmv}$ for total COD and 0.23 m^3 CH_4/kg $sCOD_{rmv}$ for soluble COD. Figure 4.18 illustrates the total COD, soluble COD and BOD removal efficiency measured for each retention time. Removal is stable and consistent around 90% for all conditions with the exception of an average removal efficiency of 78% for soluble COD at the shortest retention time, 1 day. Although effluent COD was highest, in this shortest retention time, it is worth noting that the methane yield for soluble COD is equal to that of other conditions at 0.23 m^3 CH_4/kg $sCOD_{rmv}$. Though slightly higher than expected, nutrient concentrations remained consistent and roughly in line with nutrient supplementation at ideal ratios depending on the influent COD (500:5:1 (wt), or 300:5:1 (wt)) throughout the system. Table 4.5 presents a summary of these results and additional parameters.

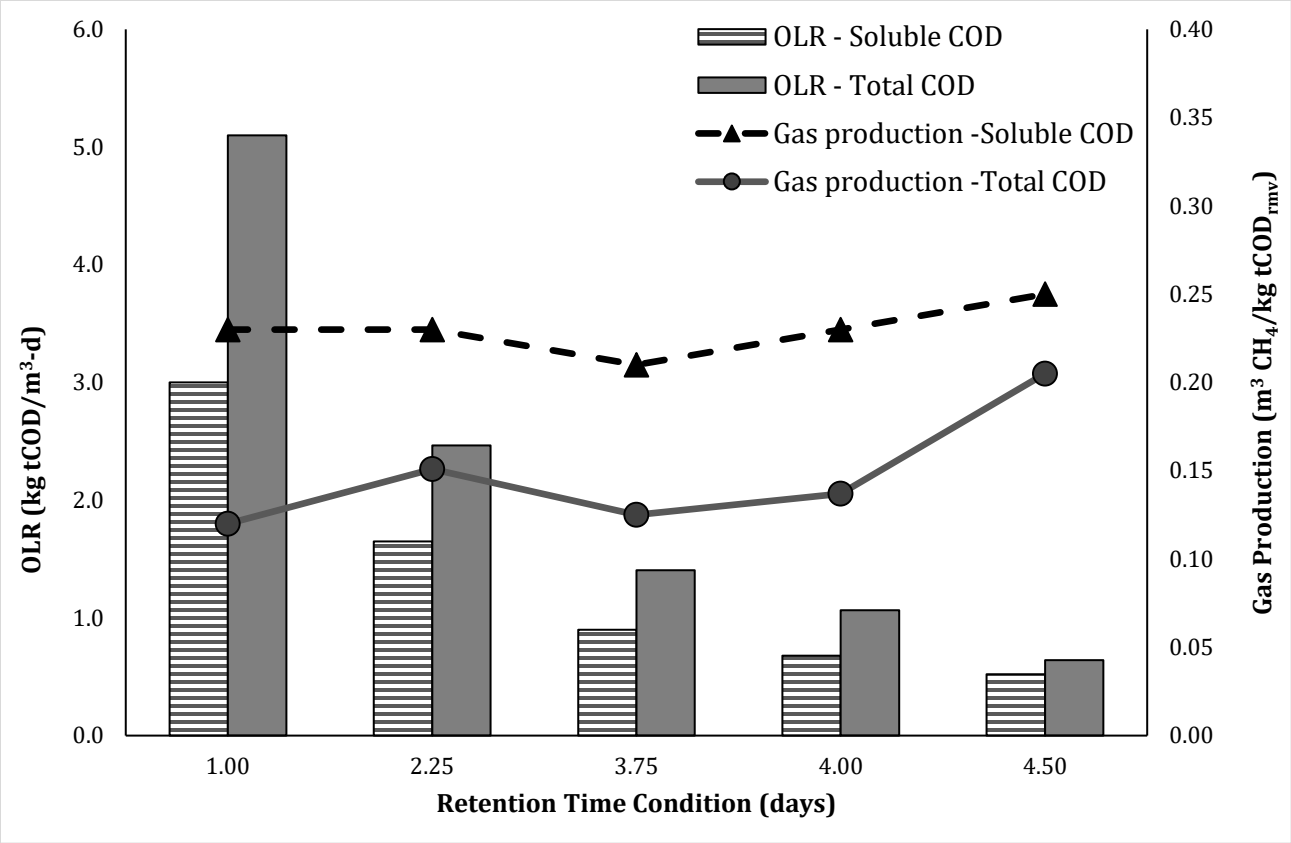


Figure 4.17 Average values of organic loading rates (kg COD/m³-d) are presented with corresponding gas production (m³ CH_4/ kg) for total and soluble COD for this series of experiments. These variables are plotted by retention time (days).

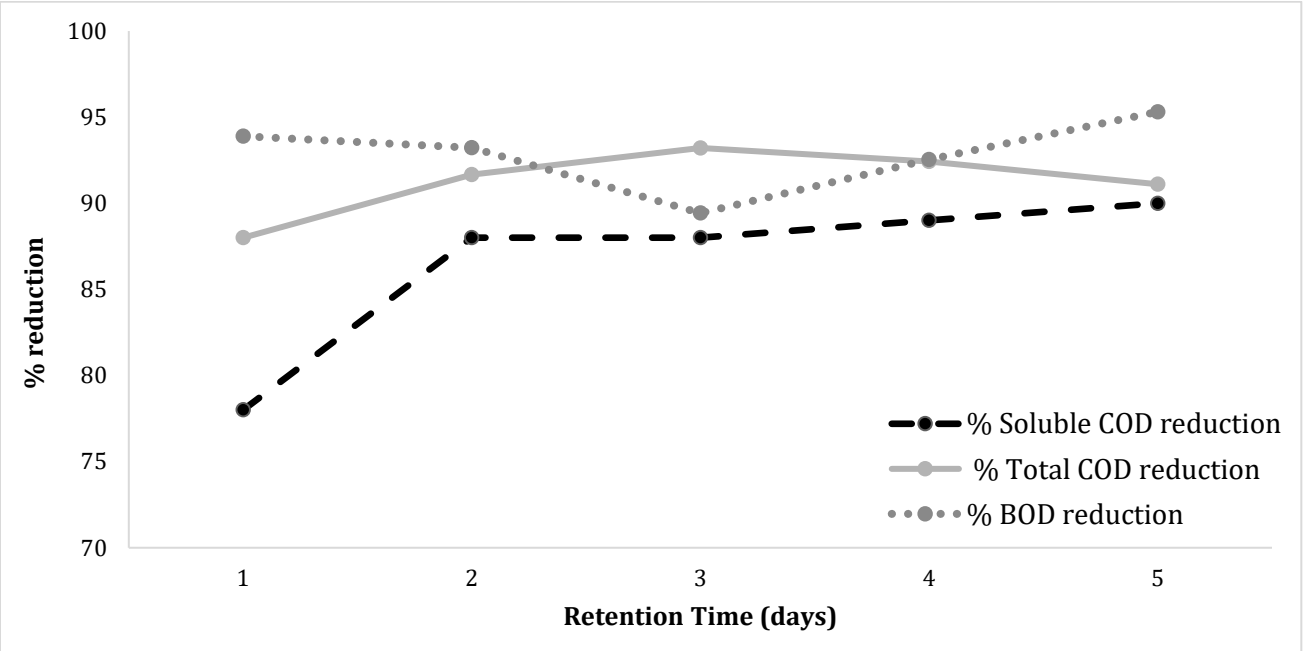


Figure 4.18 Average removal efficiencies of total COD, soluble COD and BOD from influent to methanogenic effluent, for each retention time.

Table 4.5 Summary of parameters and effluent concentrations for 2-phase condition

Parameter	Values					Units
Length of test	11	20	50	25	48	days
Feedstock	wastewater					
Temperature	25 (aceto) – 35 (methano)					^{o}C
HRT	1	2.25	3.75	4	4.5	days
Methane yield Total	0.12	0.15	0.13	0.14	0.21	$m^3\ CH_4/kg\ tCOD_{rmv}$
Methane yield Soluble	0.23	0.23	0.21	0.23	0.25	$m^3\ CH_4/kg\ sCOD_{rmv}$
OLR $_{\text{total COD}}$	5.1	2.46	1.40	1.07	0.64	kg tCOD/ m^3-d
OLR $_{\text{soluble COD}}$	3.0	1.65	0.90	0.68	0.52	kg sCOD/ m^3-d
COD effluent	0.70	0.47	0.35	0.32	0.28	kg /m^3
tCOD reduction	88	92	93	93	91	%
sCOD reduction	78	88	88	89	90	%
BOD effluent	0.12	0.12	0.14	0.12	0.12	kg /m^3
BOD reduction	94	93	89	93	95	%
VSS effluent	53.3	27	10	25	20	kg /m^3
VSS reduction	98	98	99	98	96	%
Alkalinity	630	725	750-910	620-720	790	mg/L CaCO3
pH	7.30	7.20	7.45	7.10	7.35	
TN	73-100					mg/L
PO_4^{3-}	10-18					mg/L
SO_4^{2-}	64- 92					mg/L
Al (soluble)			0.127 (aceto) - 0.015 (methano)			mg/L
Cr (soluble)			0.775 (aceto) - 0.045 (methano)			mg/L

4.3.3 Summary of all conditions

The following analysis compares the performance at all conditions to this point. These can be compared because of the roughly consistent trend of results from varying waste streams, acetogenic conditions and temperatures, illustrated in Figure 4.19.

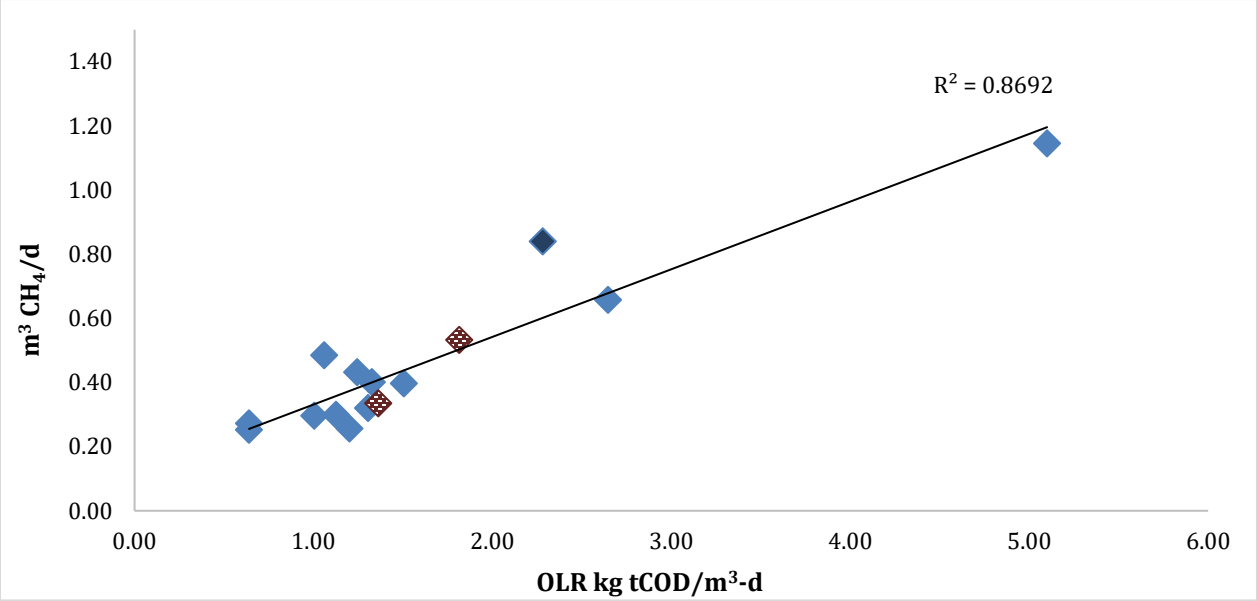


Figure 4.19 Scatterplot of all conditions tested by continuous reactor (OLR) by methane yield for total COD.

Analysis of the summary of results indicates that single-phase reactors produced a greater yield of methane in 3 of 4 cases. This may be due to the fact that single-phase reactors were fed influent, including solids, directly, which the bacteria were able to degrade and convert immediately. Another possible explanation is that the sludge samples were taken from treatment facilities operating single-phase reactors, which means that the bacterial colonies were already adapted to these conditions and were thus more efficient. Although no analysis of bacterial populations was done, it can be inferred that acetogenic populations existed along with methanogens in the methanogenic reactor since increased levels of propionic and butyric fatty acids were also converted and consumed through the methanogenic HRT. The relatively short trials of the current experiments may not have been sufficient to optimize methane production for the 2-PAD or other conditions. Nevertheless, even if a single-phase condition was shown to consistently show higher

methane yields, the additional advantages of the 2-PAD system such as decreased overall retention time and volume of reactor required, enhanced solids degradation and better integration of deinking sludge make it a valuable option.

In general, methane yield appears to increase with retention time, suggesting that the longest retention time may produce the highest methane yield. However, there are some notable inconsistencies where shorter retention times show almost equivalent methane yields, such as for the 2.25 day HRT and 4 day HRT. The advantage of a higher yield from a longer retention time and larger volume must be weighed against the proportional construction cost of the reactor size to determine the most feasible option. This discussion is continued in Chapter 5 with the cost analysis. Overall, initial results of the experiments including deinking sludge (indicated by the hatched symbols in Figure 4.19), fall in line with the other conditions and demonstrate that integrating the deinking sludge does not negatively affect AD operation. Experiments that used a liquid sludge blanket in the reactor were also in line with other conditions, although the methane yield for the single phase condition using liquid sludge was lowest overall. Further analysis on certain conditions of these results will be discussed in the CO_2 bioconversion section.

4.3.3.1 Biogas Composition

It is important to consider that this experiment was limited by reactor size. Thus it is not meaningful to make comparison of biogas volumes between conditions having different retention times. At an operational scale, the flow would be constant and only reactor volume would differ, with longer retention times requiring larger, more costly reactors. This highlights the importance of methane yield and percentage methane in the reported results. The actual methane producing potential of conditions and the costs of these alternatives at scale is discussed in the economic analysis section. A summary of average compositions of biogas in each of the conditions is illustrated in Figure 4.22. All conditions showed a similar composition of biogas. Methane percentage was consistently between 55 and 65% of total biogas. Most experimental values of methane yield for total COD were within the range of 0.12-0.15 m^3 CH_4/kg $COD_{rmv,}$ while methane yield values for soluble COD were mostly around 0.23 m^3 CH_4/kg COD_{rmv}, with single phase reactors reaching the theoretical yield of 0.35 m^3 CH_4/kg COD_{rmv} at 38 °C and an HRT of 5 days.

Though it is not uncommon for actual methane yield to be below the theoretical, this is perhaps indicative of the bench-top reactor's operational limit of efficiency, which may not be the case in a more efficient reactor design such an expanded bed reactor. This may also be related to the biodegradation rate of solids that was not sufficient in the first phase of the 2-PAD conditions, this will be discussed in more detail in section 4.3.3.2. Finally it is notable that the best methane yield was achieved for the condition that most closely resembled the operating parameters of sludge's reactor of origin.

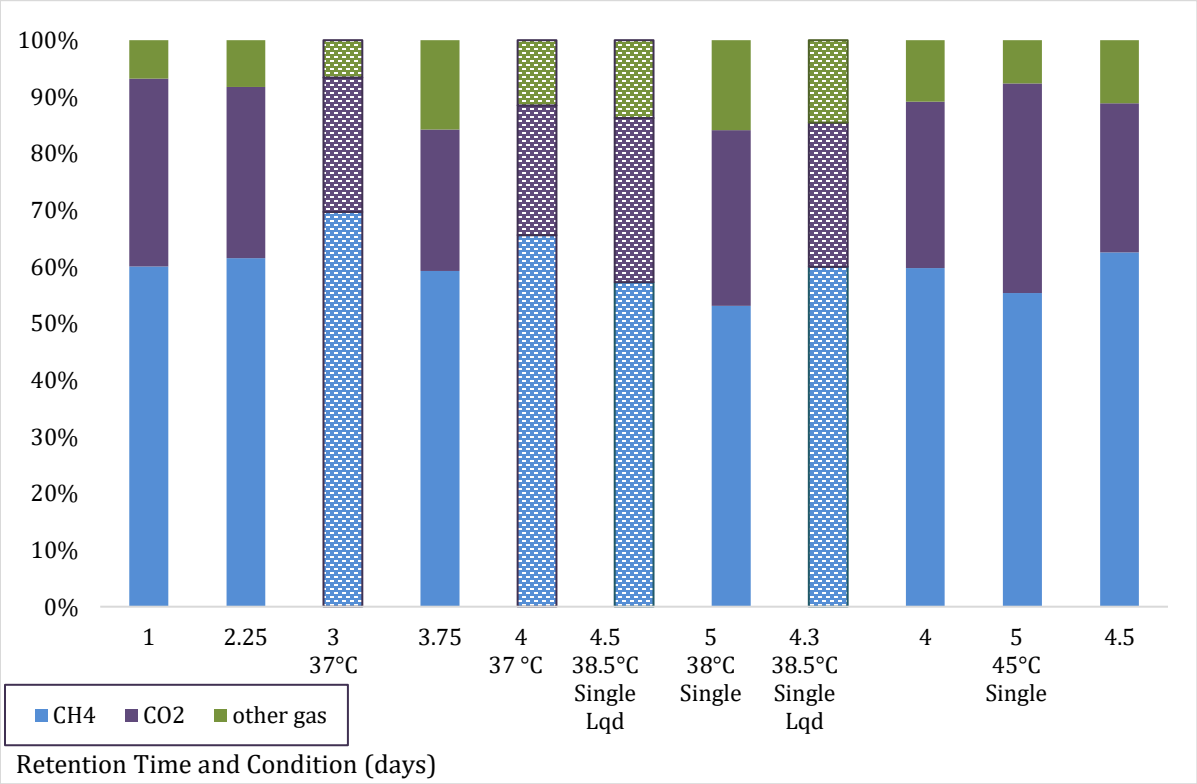


Figure 4.20 Percentage composition of biogas produced from all conditions for continuous experiments. Average values of daily gas production are plotted by condition of retention time, phase, temperature and sludge type. Hatched columns included deinking sludge. Temperatures were at 35 °C, sludge was granular and condition was 2-PAD unless otherwise indicated.

4.3.3.2 Solids Biodegradation

As seen in Tables 4.3 - 4.5 and in Figures 4.07 and 4.17, there is a substantial difference between the methane yields of total and soluble COD. The reason for this difference is likely due to the component of particulate matter in the influent COD. It was assumed that the majority of the particulate matter would be biodegradable and so would

translate into COD that is converted by anaerobic processes. However, the discrepancy between the total and soluble COD methane yields indicates that this solubilization did not occur as expected. While it is possible that the biodegradable particulate was actually a smaller component, the lack of solid build up suggests that solubilization was taking place though there were perhaps additional factors involved. One possibility is that the retention time in the acetogenic reactor was not sufficient to allow complete degradation. Evidence for this possibility is also found in the higher methane yields observed in single phase reactors that were able to directly make the influent particulates at a lengthened retention time. It is worthwhile to note also that the concentrations of soluble influent COD and the effluent from the acetogenic reactor differed only between 0- 200 mg/L of each other (results not shown). This further supports the possibility that the retention time in the acetogenic reactor was not sufficient to allow for full degradation of the solid particulates as the COD did not increase in accordance with this solubilization. Calculating methane yield from the influent into the second phase (soluble COD or acetogenic effluent in this case) allows a more accurate calculation of the methane yield potential of the reactor. Eastman and Ferguson (1981) demonstrated that additional solubilization is possible at longer detention times though the rate of solubilization is reduced. They further concluded that hydrolysis of particulates is the rate-limiting step in the acid phase of anaerobic digestion. They also suggested that high concentrations of hydrolysis products may inhibit the hydrolytic enzymes, a factor which may have come into play in the current study given the high loading rate. Finally, an important factor is that this high loading rate to the acetogenic reactor resulted in a much lower than recommended pH which would have inhibited cellular function and capacity to optimally hydrolyse particulate matter and solids, in addition to other effects (Eastman & Ferguson, 1981; Baronofsky et al., 1984). This aspect will be discussed further in sections 4.4 and 4.5.3. A more successful balance could be maintained with proper flow rate and adequate retention time. Future tests would verify this potential and evaluate more precisely the biodegradable component of the particulate and solid matter of the waste streams.

4.4 Acetogenic Conditions

Another advantage of the 2-phase system is the ability to substantially increase VFA concentrations. Literature and experimental research report that methane yields typically increase proportionally with VFA concentrations in the influent as they are consumed by a principle methane producing bacterial population, acetotrophic methanogens (Demirel & Scherer, 2008). Different conditions of OLR, RT, temperature and sludge type were tested in the acetogenic reactor with the aim of maximizing VFA formation. These experiments were further affected by the variability of influent wastewater characteristics and initial VFA concentrations.

Average values of all conditions are reported in Figure 4.21, varying according to temperature (25, 38, and 45°C), and type of sludge. The percentage increase in VFA concentrations appeared to inversely correspond to initial VFA concentrations as expressed by the organic loading rate, i.e. high initial VFA concentrations do not undergo the same proportion of increase upon treatment in the acetogenic reactor. This is consistent with research which has shown that the end product of fermentation can limit growth by acting as an uncoupling agent. Below a tolerable pH, as might be reached due to excessive loading and acetogenesis, the cytoplasm of the cells becomes acidified. Since acetic acid can diffuse passively across the cytoplasmic membrane, this results in a decreased internal pH, which inhibits additional pH-sensitive cellular reactions such as growth and additional fermentation (Baronosfky et al., 1984). pH levels were monitored daily with indication strips and found to lie consistently between a pH of 4 and 5. Detailed pH values, measured by potentiometric probe, were recorded for each of the bioconversion conditions and are reported in Table 4.8. The potential effects of the observed acetogenic pH levels are discussed further in section 4.5.1.

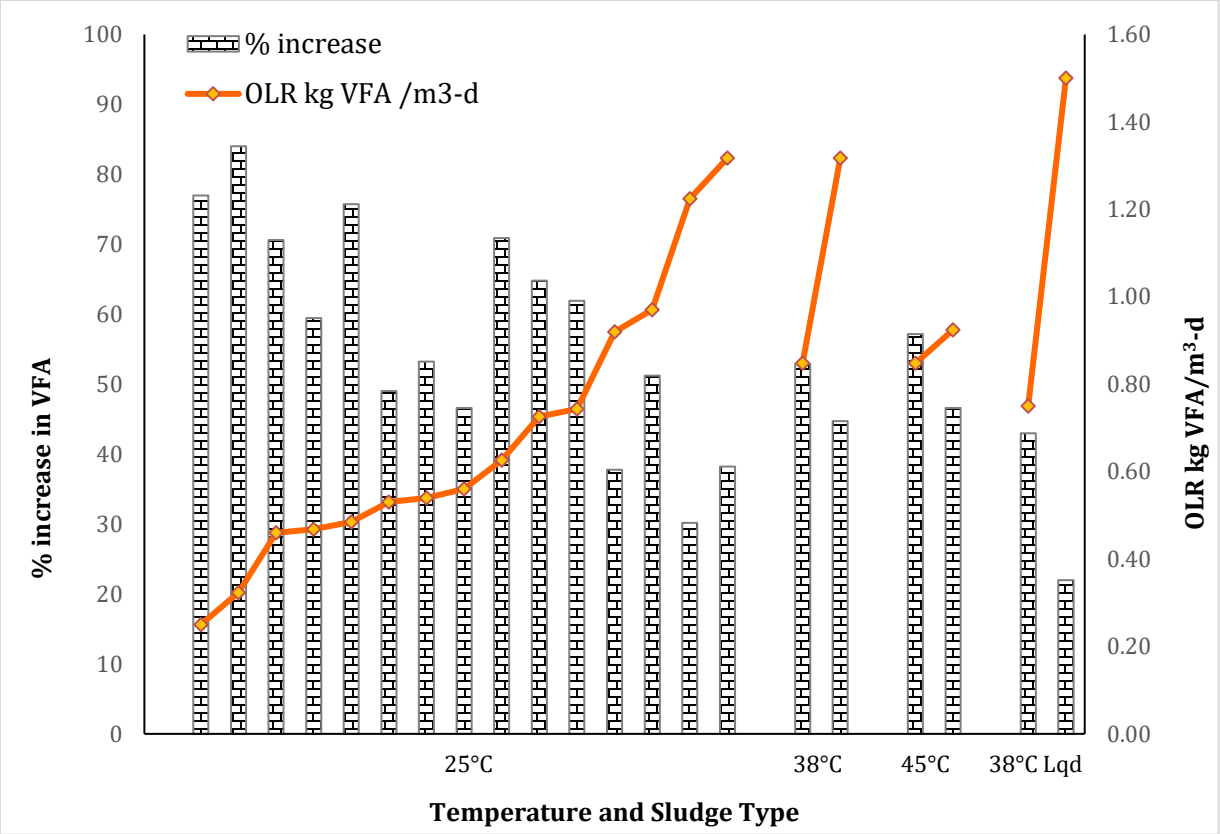


Figure 4.21 All conditions for acetogenic reactor. Results are grouped by operating temperature and sludge type. Sludge type was granular unless otherwise indicated. Percentage increase of VFA is plotted with corresponding OLR of VFA in the influent wastewater.

Figure 4.22 shows the composition of 3 principle volatile fatty acids produced by acetogenic bacteria (acetic, propionic and butyric) as measured by HPLC. Composition varies slightly with temperature, sludge type and loading rate, but most notably by feed source with tests that included deinking sludge having a distinctly greater proportion of propionic acid. It is important to monitor concentrations of specific acids as an indication of bacterial activity and health; build-up of specific acids is often a helpful indicator of disruption in the microbial balance (Pullammanappallil et al., 2001; Wang et al., 2009; Sivagurunathan et al., 2014). In general, risk of toxicity from fatty acids (VFAs) increases with the length of the carbon chain due to the fact that the acid becomes proportionally difficult to degrade (Chen et al., 2014). Acetic acid has the lowest lipid number at C2 :0, propionic is next at C3 :0 and butyric has the highest lipid number of C4 :0 (Wikipaedia, 2018). Research has shown that when the propionic acid concentration was increased to 900 mg /L, significant inhibition occurred, methanogenic bacterial concentration decreased

from by 90% and their activity did not recover (Wang et al., 2009). However, other research showed no inhibition for propionic concentrations as high as 2750 mg/L and suggested that the increase in propionic acid was not the cause but a consequence of a functional disruption (Pullammanappallil et al., 2001). The increase in propionic acid, seen in Figure 4.22 was likely a result of the increased resin acids and acidic degradation of aromatic hydrocarbons. As it did not cause a disruption in the system, it is not deemed to be problematic though it warrants additional monitoring.

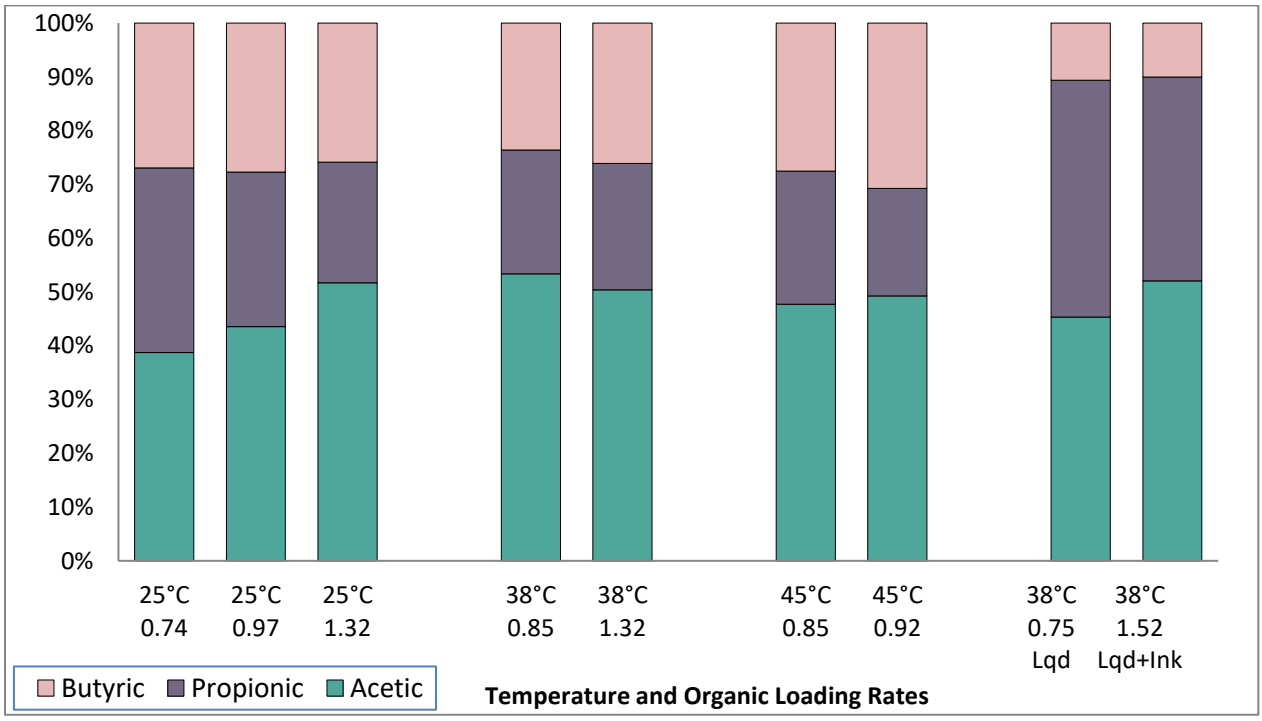


Figure 4.22 Composition of dominant organic acids (acetic, propionic and butyric) in acetogenic effluent is presented by operating temperature and VFA organic loading rate (kg VFA/m^3-d) as well as by feed stream and sludge type. All sludge was granular and feed waste was wastewater unless otherwise indicated.

4.5 Carbon Dioxide Bioconversion

As discussed in the introduction, a component of this research project investigated the possibility of using Cascades wastewater as a carbon sink for CO_2 emissions, in an intermediary step to allow anaerobic bacteria to bioconvert this CO_2 into CH_4. This topic has been investigated by other researchers with positive results (Alimahmoodi & Mulligan, 2008; Chun Lee et al., 2012; Fernández et al., 2014; Abedi, 2015). Previous work in the

research group focused on bioconversion within the methanogenic phase, presumably through the reduction of CO_2 with H_2 by hydrogentrophic methanogens. However, this series of experiments focused on the effectiveness of bioconversion within the acetogenic phase. It was theorized that the acetogenic bacteria would make use of the Wood–Ljungdahl pathway to convert CO_2 into acetate (CH_3COO^-), which would, in turn, increases methane yield, since methane yield is expected to increase proportionally with VFA concentration. Support for this theory was found in scientific literature. Working at a temperature of 25 °C, Salomoni et al. (2011) injected 0.49 m^3 CO_2/d into the fermentation phase of a pilot 2-PAD system. Their system is illustrated in Figure 4.23. They found 40% absorption of injected CO_2, which amounted to an average of 229 L CO_2/d. In parallel, their system showed substantially increased concentrations of VFA when compared to similar 2-PAD systems. They stipulated this to be the result of the Wood–Ljungdahl pathway (Muller, 2000; Ragsdale & Pierce, 2008). They found methane production rates to be at least 2 times higher than without CO_2 injection.

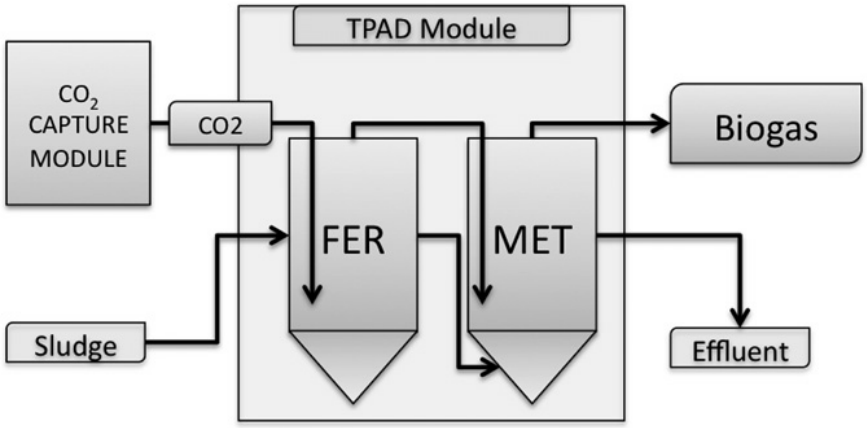


Figure 4.23 Two Phase Anaerobic Digestion model with CO_2 injection for bioconversion (Salmoni et al., 2011)

In the current study, several conditions were tested. Organic loading rates of both acetogenic and methanogenic reactors were varied. Acetogenic loading rates were distinguished by loading rates of VFA concentration in the initial influent of: 0.46, 0.56, 0.735 and 0.95 kg VFA/m^3 –d, and methanogenic loading rates were distinguished by organic loading rates of COD which ranged between 0.64-2.6 kg COD/m^3-d. Previous

results from the research group showed CO_2 to be maximally dissolved at 25°C (Kazemi et al., 2013). Since this was consistent with favourable operating conditions for the acetogenic reactor and previous literature (Salomoni et al., 2011), the temperature was maintained at 25°C for the duration of these experiments. The methanogenic temperature was maintained at 35°C. All conditions used granular sludge in both reactor phases. CO_2 was dissolved into the wastewater prior to feeding and measured as described in the methodology section. The average influent concentrations of dissolved carbon dioxide in the influent wastewater for the control condition were 200 mg/L while the concentrations of dissolved CO_2 in the experimental condition were 1000 mg/L. A mass distribution of CO_2 is shown in Figures 4.35 and 4.36. As will be discussed in section 4.5.2, analysis of ultimate methane yields indicated overall favourable, though limited conversion for methane yields from soluble COD.

4.5.1 CO_2 Conversion to VFAs and Acetate – Potential Mechanisms

The Wood-Ljungdahl pathway proposes that excess CO_2 injected into wastewater would be converted directly into acetate (acetic acid). However, results to this end were not in line with expectations. Figure 4.24 illustrates the effect of injecting carbon dioxide on the concentration and type of VFAs in the effluent of the acetogenic reactor based on different loading conditions with their corresponding pH levels. In all OLR groups except 0.735 kg VFA/m^3-d, concentrations of acetic acid are higher for CO_2 conditions than for the control, though this difference is not substantial for OLR group 0.95 kg VFA/m^3-d. There appears to be a general trend towards a lower OLR allowing for more successful conversion of CO_2 into VFAs, specifically acetic acid. Possible reasons for this observation are discussed further in this section. Another observable trend is the interaction of pH level and VFA increase. The degree of change in pH between the control and CO_2 conditions corresponds to the increase in VFA concentrations. A 28% increase in VFA levels (1% increase in acetate) in the CO_2 condition for the 0.95 kg VFA/m^3-d group corresponds to a 12% decrease in pH. A 7% decrease in VFA levels (16% decrease in acetate) in the CO_2 condition for the 0.735 kg VFA/m^3-d corresponds to a 21% increase in pH. A 2% increase in VFA concentrations (7% increase in acetate) of the CO_2 condition for the 0.56 kg

VFA/m^3-d group corresponds to a 2% decrease in pH. Finally, a 12% increase in VFA concentrations (8% increase in acetate) of the CO_2 condition for the 0.46 kg VFA/m^3-d group correspond to an 11% decrease in pH. OL

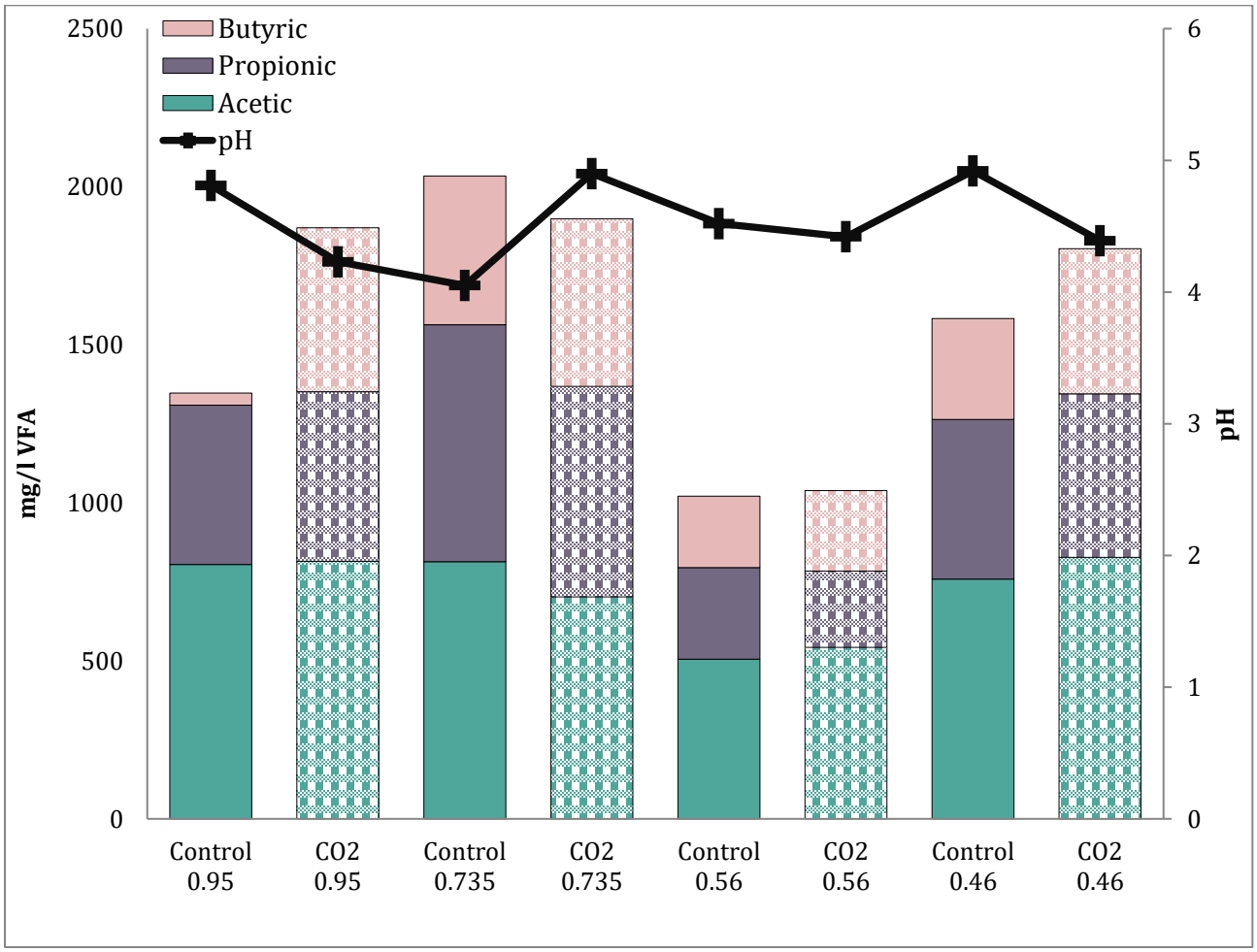


Figure 4.24 Comparison of VFA concentrations, composition and pH between regular and CO_2-injected conditions for all 4 organic loading rates (0.95, 0.735, 0.56 and 0.46 kg VFA/m^3-d). CO_2 injected conditions are represented by textured columns, regular conditions by solid columns. pH levels of each condition are illustrated by the + marker.

It is interesting to note that the difference in VFA concentrations between control and CO_2 conditions for OLR groups of 0.46 and 0.95 kg VFA/m^3-d condition were composed primarily of butyric acid and not acetic acid as the Wood-Ljungdahl pathway would propose. There are a number of possible explanations for this finding. For instance, the excessive loading rate and acid production in the reactor for the 0.95 kg VFA/m^3-d group

may have resulted in a cessation of cellular activity due to an intolerably low pH level (Baronosfky et al., 1984). The possible effects of pH in the acetogenic reactor are discussed in more detail in section 4.5.3. In this context of extreme conditions, the complexity of the bacterial reactions may have come into play, allowing facultative microbes and mechanisms to become active. For example, it is possible that the excess of acetate was metabolised with methanol into butyric acid by the Marburg strain, a mesophilic bacterium that performs homoacetic, homobutyric, or heteroacidic fermentations (Zeikus et al., 1980). As seen in Table 4.6, fed an energy source of methanol (CH_3OH) and acetate, the Marburg bacteria produced only butyric acid and allowed the pH to rise, both of which would be favourable in an over-loaded and over-acidified context such as the 0.95 kg VFA/m^3-d condition.

Table 4.6 Relationship between growth and acid production by the Marburg strain (Zeikus et al., 1980)

Energy source	Growth (ΔA_{540})	Acid products (ΔmM)		Final pH
		Acetic	Butyric	
Glucose	1.20	31	8	5.1
CH_3OH/acetate	1.10	ND	30	6.7
H_2/CO_2	0.19	19	0	5.5
CH_3OH	0.31	3	6	7.2
None	0.06	1	2	7.5

The increased butyric acid observed in the lower OLR group of 0.46 kg VFA/m^3-d might be explained by the results of researchers, Kuratomi and Stadtman (1966), who monitored radioactive isotopes of carbon in an acetogenic environment and found that the isotopes were taken up mostly by formate, pyruvate, and acetate, with small amounts in propionate, butyrate, and malate acids. Assuming there was an excess of CO_2 and sufficient concentrations of acetate, butyric acid may have formed to prevent the pH from decreasing below the tolerable threshold. Table 4.7 lists additional examples of acetogenic species that convert H_2 and CO_2 into butyrate which may have played a role in the increased concentrations of butyric acid.

Table 4.7 Substrates, products and characteristics of some identified acetogenic bacteria (Amari et al., 2010)

Substrate	Product	Typical species	Temperature, °C	Gram reaction	Shape
Butyrate	Acetate	*Syntrophobacter wolinii*	35–40	–	rod
	H_2/CO_2, Formate	*S. fumaroxidans*	35–40	–	rod
Propionate	H_2/CO_2, Formate	*Syntrophomonas wolfei*	35–40	–	rod
		Pelotomaculum thermopropionicum	50–60†	+	rod
		P. schinkii	32–37	+	rod
	Butyrate, acetate	*Smithella propionica*	35–40	–	rod
H_2, CO_2	Acetate	*Clostridium aceticum*	30–37	–	rod

4.5.2 Biogas Yields and Volumes

Results of each condition show comparisons of biogases (CH_4 and CO_2) produced by volume and also by methane yield for soluble COD. Since CO_2 can only dissolve in liquid, and would only agglomerate around any particles present in the wastewater (rendering it inaccessible for microbial conversion), the conversion efficiency of CO_2 in soluble COD is presumed to have more practical relevance for bioconversion analysis. As in previous sections, all yields reported in this section were calculated for COD removed.

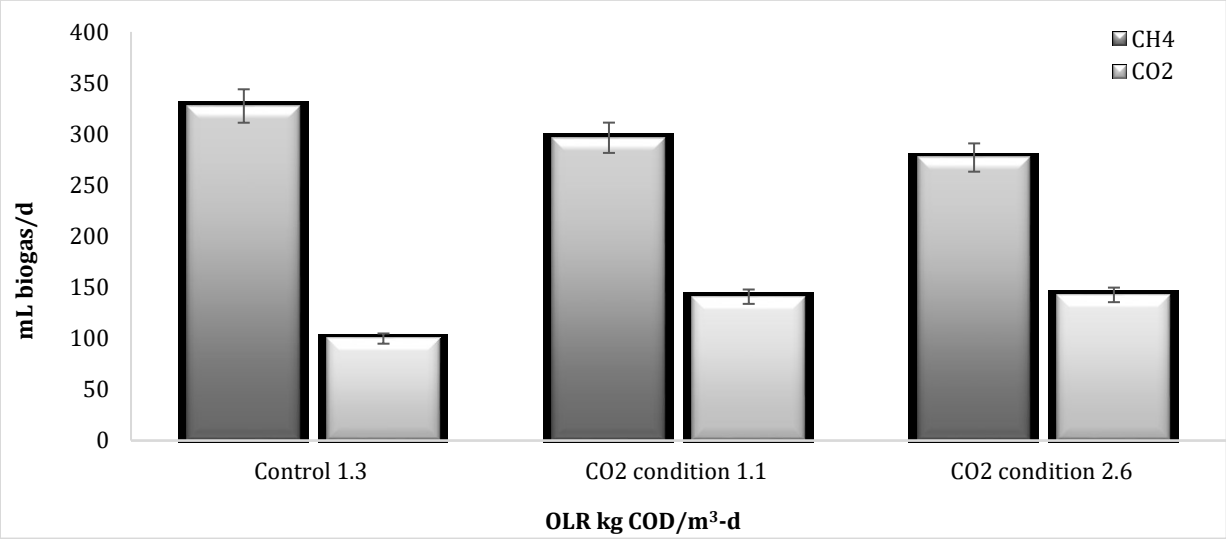


Figure 4.25 Biogas volume (mL/d) from methanogenic reactor fed acetogenic effluent from 0.95kg VFA/m^3-d condition. OLR for methanogenic reactor was held at 1.3 kg COD/m^3-d for regular trials and 1.1 and 2.6 kg COD/m^3-d for CO_2 conditions.

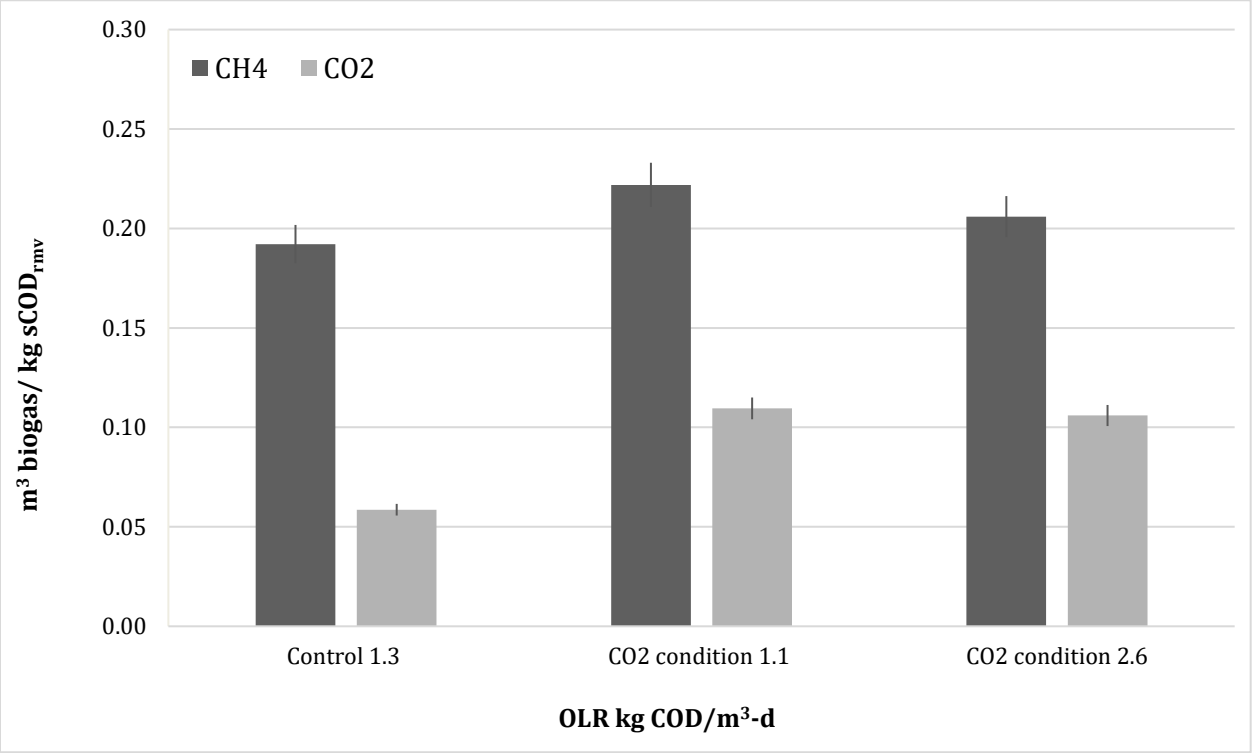


Figure 4.26 Biogas yield (m^3/kg sCOD) is reported for OLR 0.95kg VFA/m^3-d acetogenic feed condition. Regular and CO_2 trials with are presented.

Figure 4.25 illustrates that despite the increased VFA concentrations seen in Figure 4.24, this did not translate to an increase in the volume of methane produced for the 0.95 kg VFA/m^3-d condition for either of the two methanogenic loading rates, although there was a marked increase in CO_2 emissions. However, the methane yields for soluble COD at this highest OLR did show an increased yield for the CO_2 condition relative to the control condition at the OLR of 1.1 kg COD/m^3-d; in this case, methane yields were 14% greater while carbon dioxide yields were 45% greater than the control (Figure 4.26).

As seen in Figure 4.24, the condition of OLR 0.735 kg VFA/m^3-d did not result in increased VFA concentrations, thus no increased methane production was expected and Figure 4.27 indicates that there was also no corresponding increase in absolute methane volume for this condition. Although surprisingly, the methane yield for soluble COD was slightly (5%) greater for the CO_2 condition than the control at the OLR of 1.5 kg COD/m^3-d, while the carbon dioxide yield was 9% greater (Figure 4.28).

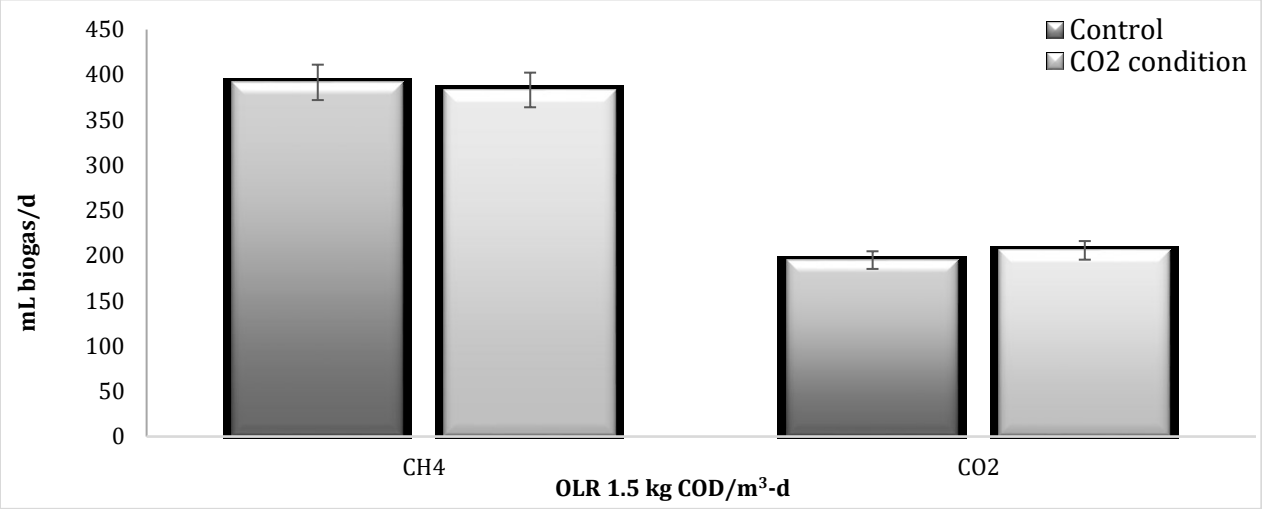


Figure 4.27 Biogas volume (mL/d) from methanogenic reactor fed acetogenic effluent from 0.735kg VFA/m³-d condition. OLR for the methanogenic reactor was held at 1.5 kg/m³-d.

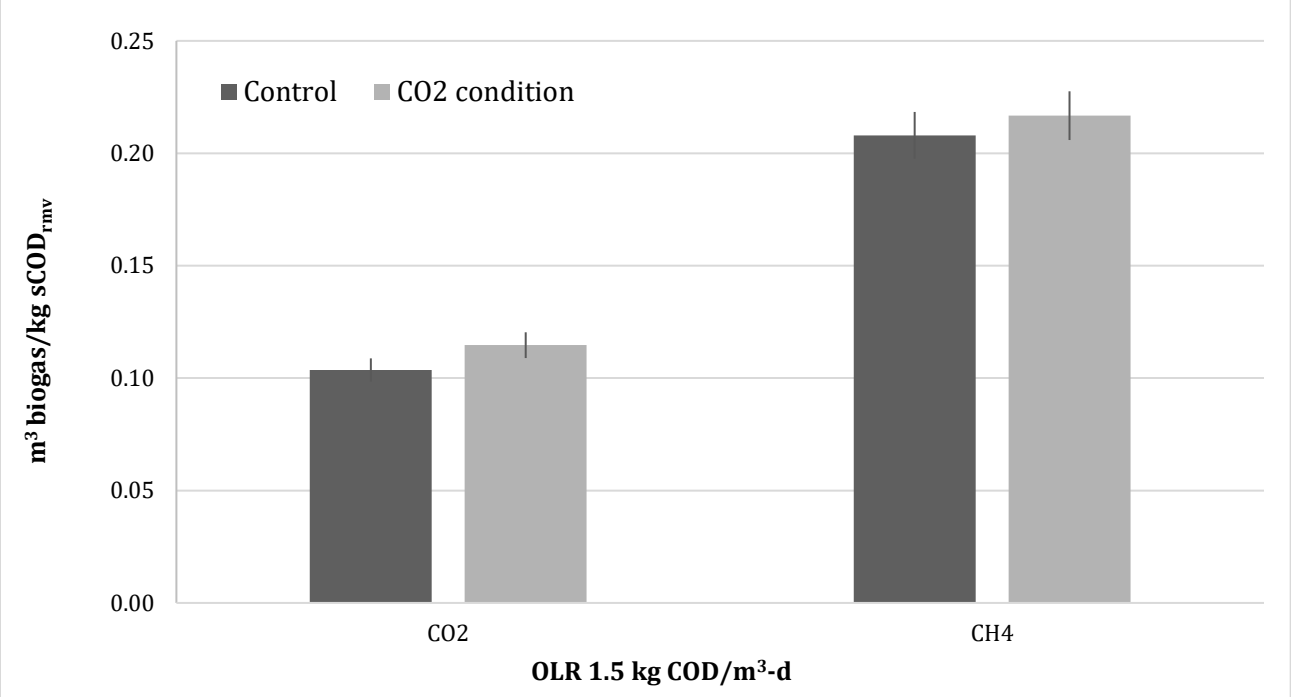


Figure 4.28 Biogas production (m³/kg sCOD) is reported for 0.735 kg VFA/m³-d acetogenic feed condition. Regular and CO_2 trials with are presented for methanogenic reactor OLR of 1.5 kg COD/m³-d

The condition of 0.56 kg VFA/m³-d is illustrated in Figures 4.29-4.32. There was a small increase in the volume of methane produced in the CO_2 condition for the methanogenic OLR of 0.64 kg COD/m³-d (Figure 4.29). This effect was much more pronounced for the soluble COD methane yield (12% greater) along with a 50% increase in

carbon dioxide yield (Figure 4.30). At the methanogenic OLR of 1.0 kg COD/m^3-d, the absolute volume of methane produced was also slightly higher than the control (Figure 4.31). The methane yield of soluble COD showed the inverse results, with CO_2 condition having a greater yield of methane (21%) and a substantially increased carbon dioxide yield (50%) (Figure 4.32).

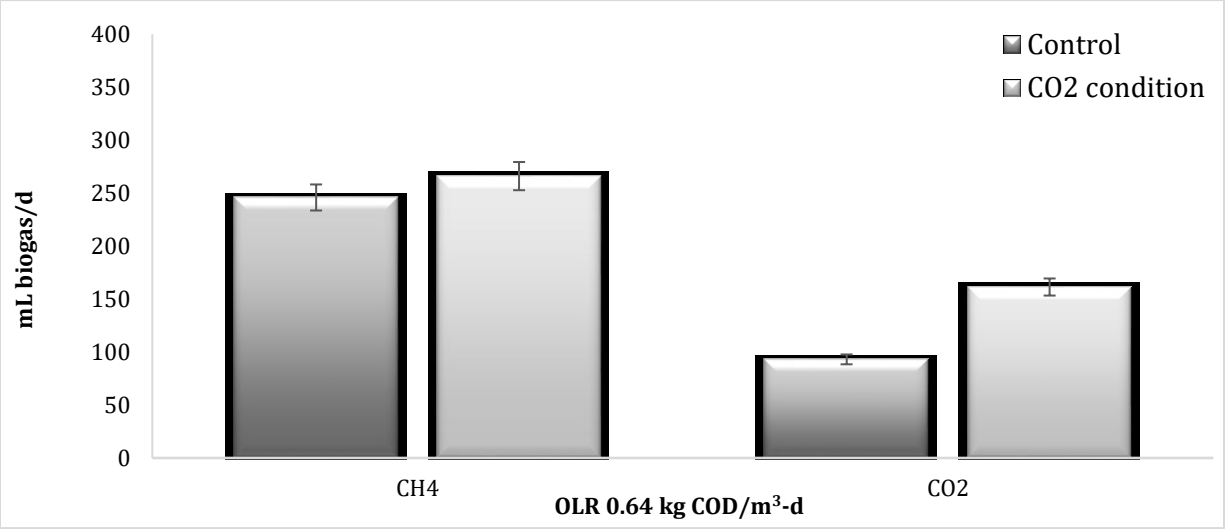


Figure 4.29 Biogas volume (mL/d) from methanogenic reactor fed acetogenic effluent from 0.56 kg VFA/m^3-d condition. Methanogenic reactor loading rates were held at 0.64 kg/m^3-d.

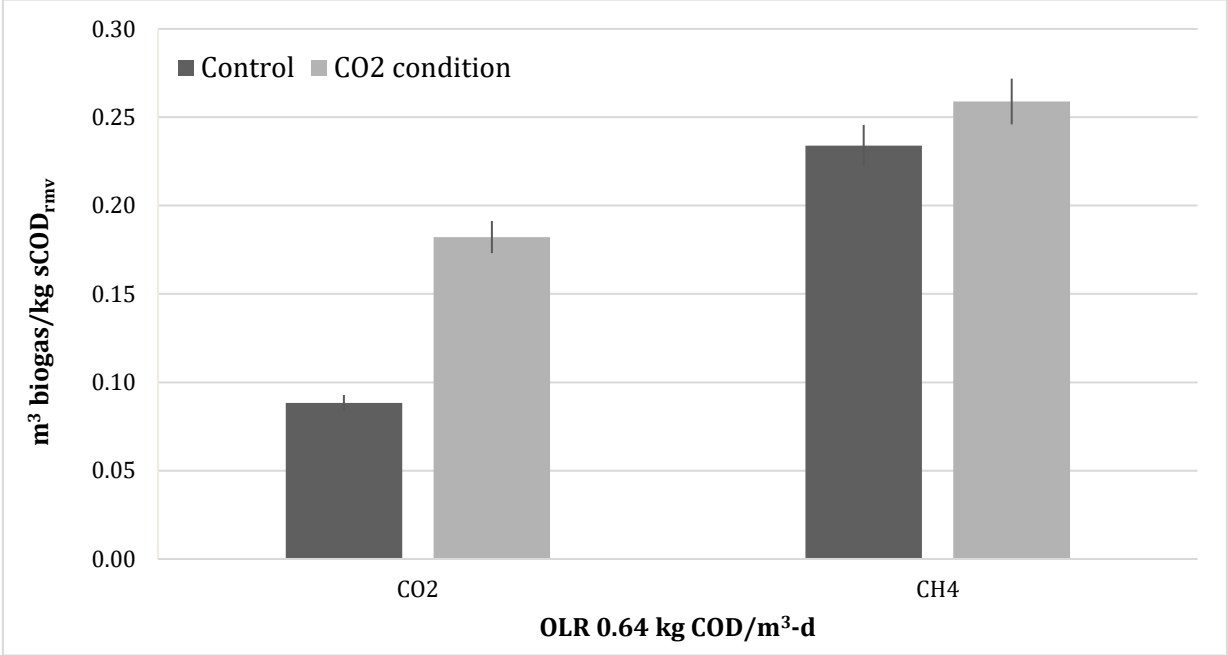


Figure 4.30 Biogas production (m^3/kg sCOD) is reported for 0.56 kg VFA/m^3-d acetogenic feed condition. Regular and CO_2 trials with are presented for methanogenic OLR of t 0.64 kg COD/m^3-d.

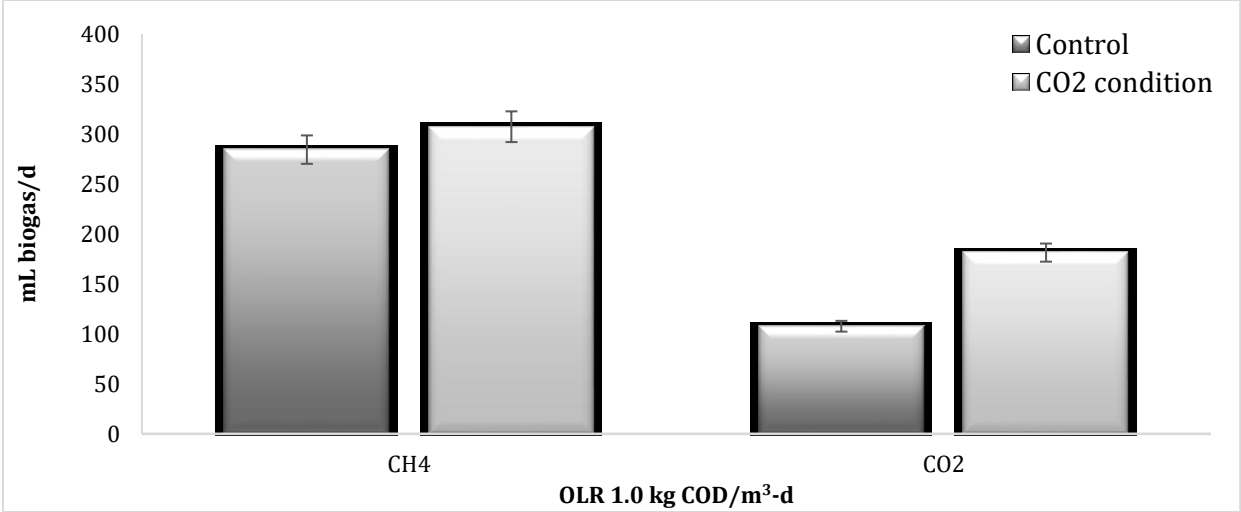


Figure 4.31 Biogas volume (mL/d) from methanogenic reactor fed acetogenic effluent from 0.56 kg VFA/m³-d condition. Methanogenic reactor loading rates were held at 1.0 kg/m³-d.

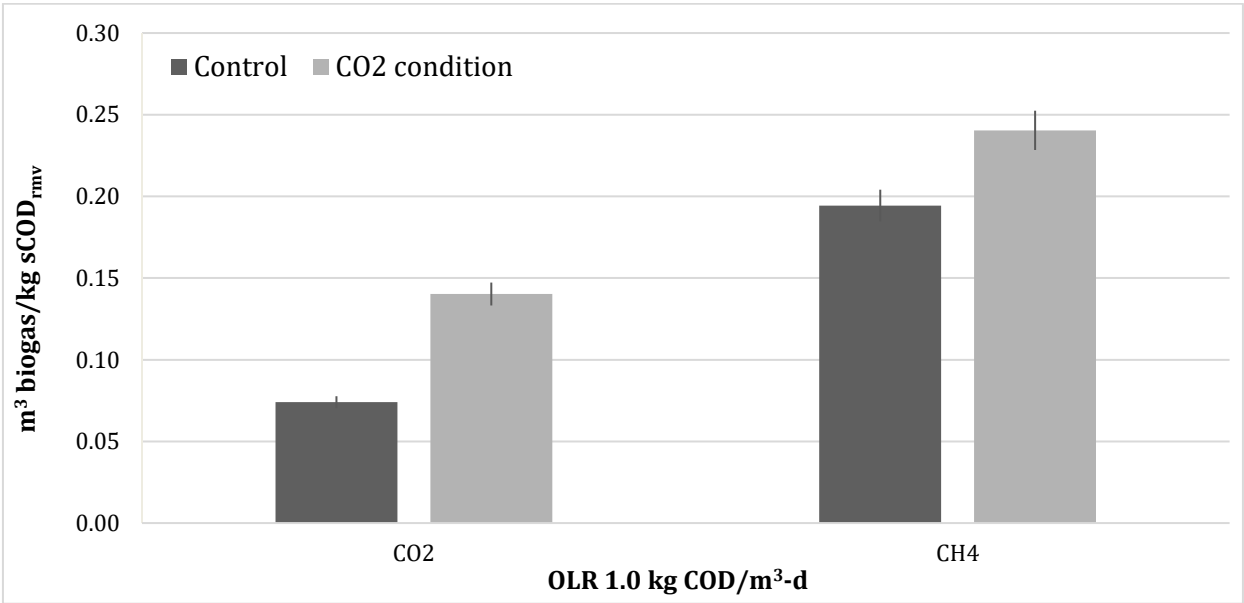


Figure 4.32 Biogas production (m³/kg sCOD) is reported for 0.56 kg VFA/m³-d acetogenic feed condition. Regular and CO_2 trials with are presented for methanogenic reactor OLR of 1.0 kg COD/m³-d.

Results from the final condition of 0.466 kg VFA/m³-d also suggest potential bioconversion. Carbon dioxide injected trials showed elevated VFA concentrations as illustrated in Figure 4.24. In the methanogenic reactor, the CO_2 injected trials also showed a slightly increased production of methane volume relative to the control (Figure 4.33). The methane yields from soluble COD show a17% increase, while the magnitude of the carbon dioxide yields was 50% greater (Figure 4.34). It is necessary to note that the organic

loading rates of the methanogenic reactor in this condition differed (1.3- regular and 2.6 CO_2 kg COD/m^3-d) and so it is possible that the differences observed could be attributed to the different organic loading rates that were operated. Obstacles in operation lead to the discrepancy in loading rates. Future tests would need to be repeated at this condition for reliability. In summary, all conditions tested showed some indication of bioconversion as defined by an increase in the methane yield from soluble COD.

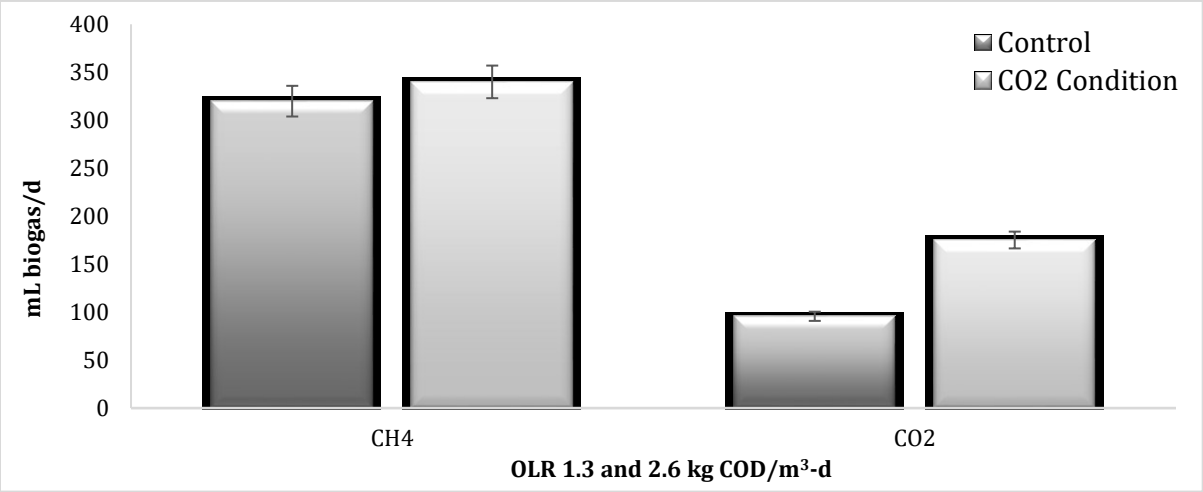


Figure 4.33 Biogas volume (mL/d) from methanogenic reactor fed acetogenic effluent from 0.466 kg VFA/m^3-d condition. Methanogenic loading rates were held at 1.3 for regular and 2.6 for CO_2 kg/m^3-d.

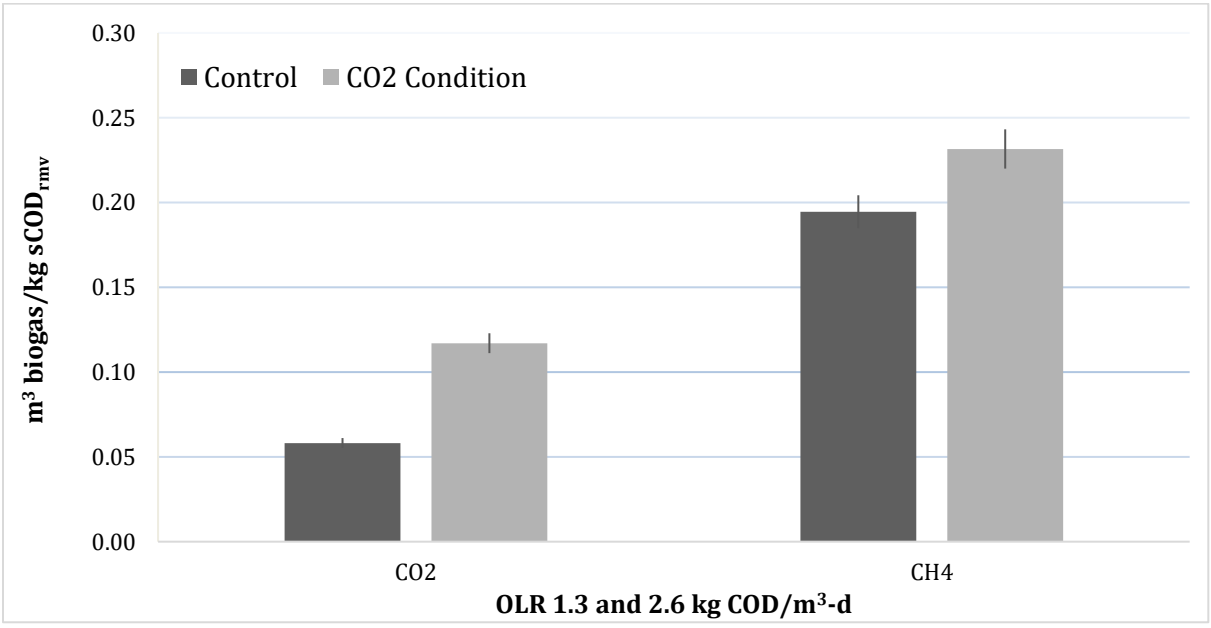


Figure 4.34 Biogas production (m^3/kg sCOD) is reported for 0.466 kg VFA/m^3-d acetogenic feed condition. Regular and CO_2 trials are presented for methanogenic OLR of 1.3 for regular and 2.6 for CO_2 kg/m^3-d.

4.5.3 CO_2 Mass Distribution

Conditions were classified by VFA OLR from the acetogenic reactor and COD OLR to the methanogenic reactor (kg/m^3-d), these conditions are listed in Table 4.8. The mass distribution of CO_2 throughout the entire system is calculated as a net value, i.e. the difference between CO_2 injected condition and the equivalent regular condition. Values of CO_2 in emissions reflect the sum of both CH_4 and CO_2 emissions, as shown in Equation 3.12. The results of these calculations are reported as mass values in Figures 4.35 and 4.36.

Table 4.8 pH levels of each stage in 2PAD system during bioconversion experiments

OLR Group		Condition	Wastewater Influent	Acetogenic Effluent	Methanogenic Effluent
kg VFA/ m^3-d	**kg COD/ m^3-d**				
0.950	1.3	Control	5.21	4.81	7.72
		CO_2	4.55	4.23	7.21
0.735	1.5	Control	5.26	4.05	7.47
		CO_2	4.57	4.90	6.99
0.560	0.64	Control	5.01	4.52	7.48
		CO_2	4.76	4.42	7.17
0.560	1.0	Control	5.07	4.42	7.20
		CO_2	4.74	4.45	6.94
0.466	1.3, 2.6	Control	5.12	4.92	7.62
		CO_2	4.97	4.39	7.01

Higher VFA loading rate conditions are illustrated in Figure 4.35. Conditions of 0.95 kg VFA/m^3-d had similar patterns for the acetogenic phase, with the highest CO_2 concentrations released from the acetogenic reactor, dissolved in acetogenic effluent and measured as VFA. In the methanogenic phase, higher COD loading rates of 2.6 kg/m^3-d had higher gas emissions (the majority of which were CO_2 and not CH_4,) while the lower COD loading rate of 1.3 kg/m^3-d maintained a higher concentration of dissolved CO_2 in the final effluent. The condition of 0.735 kg VFA/m^3-d interestingly showed lower concentrations of

dissolved CO_2 in the acetogenic phase while concentrations of dissolved CO_2 in methanogenic effluent were higher. There was no increase with respect to gas emissions.

Lower VFA loading rate conditions are illustrated in Figure 4.36. Conditions of 0.56 kg VFA/m^3-d had elevated levels of CO_2 in the acetogenic phase, emitted as gas, dissolved in effluent and as VFA, while the 0.63 kg COD/m^3-d condition had significantly higher concentrations only of VFA. In the methanogenic phase, the slightly higher OLR of 1.0 kg COD/m^3-d produced a positive net of biogas. However this was mainly in the CO_2, form, whereas the lower OLR of 0.64 kg COD/m^3-d produced increased levels of both CH_4 and CO_2. The final condition of 0.466 kg VFA/m^3-d produced almost no gases in the acetogenic phase though the dissolved CO_2 and VFA concentrations were elevated. In the methanogenic reactor the OLR of 1.3 kg COD/m^3-d (corrected for the difference in OLR) showed a significantly increased production of CH_4 along with increased CO_2 emissions. There were also increased concentrations of dissolved CO_2 in the methanogenic effluent.

These results are interesting overall. They appear to suggest limits to the bioconversion capacity of the bacteria. For instance, as discussed previously, in the VFA loading conditions that begin with initially high concentrations, acetogenic bacteria may have been limited in their capacity to convert CO_2 into additional acetate, VFAs or other by products. As seen in Figure 4.21 the OLR appears to be inversely related to the percentage increase of VFA. This may be due to a pH equilibrium that limits the acidity to functional levels. Baronofky et al. (1984) reported a pH equilibrium level of 5.0. As seen in Table 4.8, pH levels in the acetogenic reactor were consistently below 5, averaging 4.5 and ranging between 4.05 - 4.92. Ghosh et al. (1975) demonstrated successful acid digester performance maintaining a pH range between 5.5 and 6.0 while Eastman and Ferguson (1981) conducted experiments on rate limiting fermentation maintained at pH of 6.0. Since the pH in the current experiment was relatively much lower, it is possible that the excess acidic charge inhibited normal cellular functions such as optimal hydrolysis (discussed in section 4.3.3.2) and CO_2 conversion to acetate. It is also possible that there is a limiting component involved in the conversion of CO_2 to acetate, given the complexity it is difficult to determine what that is exactly.

Excess CO_2 was most often not converted, but emitted as gas or remained dissolved in the water. The equation $CH_3COOH \rightarrow CH_4 + CO_2$ predicts a 1:1 increase in methane production as well as carbon dioxide production (mass:mass) for each additional unit of acetate metabolized by acetotrophic methanogenesis. However, this was not consistently observed. The acetotrophic methanogens may have had a bioconversion limit. This may be limited by the number of the acetotrophs or their metabolism rate. Furthermore, methane yields were observed to increase in CO_2 conditions in the high VFA OLR groups, despite no corresponding increase in acetate. This suggests bioconversion may have been taking place through other pathways, possibly by metabolism of hydrogenotrophic methanogens in the methanogenic reactor. However, this conversion was not complete. It is possible that the feed rate did not correspond to the metabolism rate of the bacteria or perhaps the ratio with H_2 was insufficient (Chul-Lee et al., 2012). The significantly greater emissions of CO_2 in experimental conditions also suggest the need to reduce CO_2 levels to optimal concentrations, or optimal residence times that could be fully converted without excess emissions. However, these excess CO_2 emissions may be due to the metabolic processes and respiration of anaerobic bacteria due to their additional bioconversion activity. Although this is unavoidable, an aspect of gas recycling, discussed further in section 4.9.2 would allow for capture and additional bioconversion of CO_2 gases that are emitted from these processes.

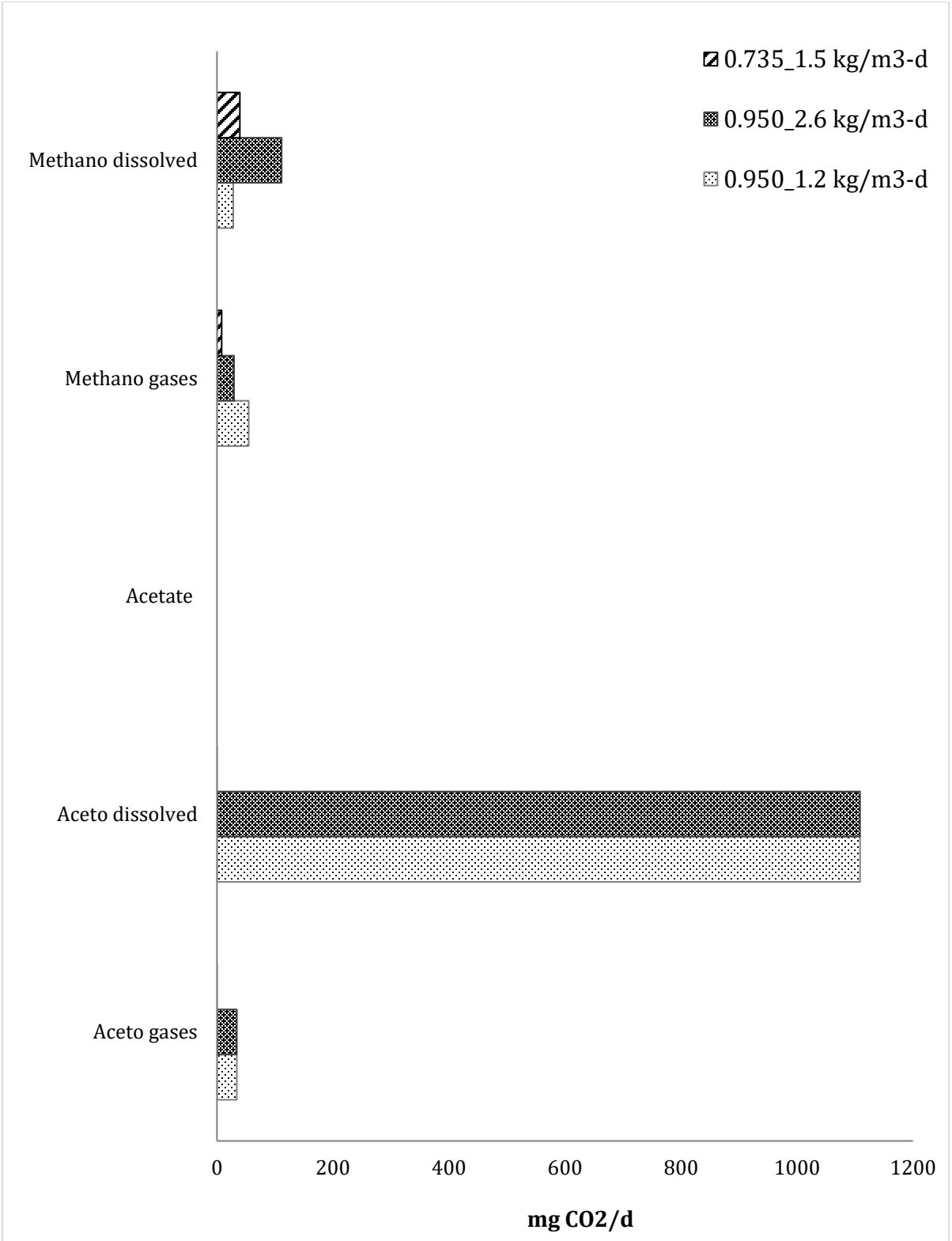


Figure 4.35 Net concentrations of CO_2 for each condition (CO_2 injected minus regular condition). High rate organic loading rates groups shown (0.735 and 0.95). Legend values correspond to OLR groups as VFA_COD kg/m^3-d (VFA for influent into acetogenic reactor and COD for influent into methanogenic reactor).

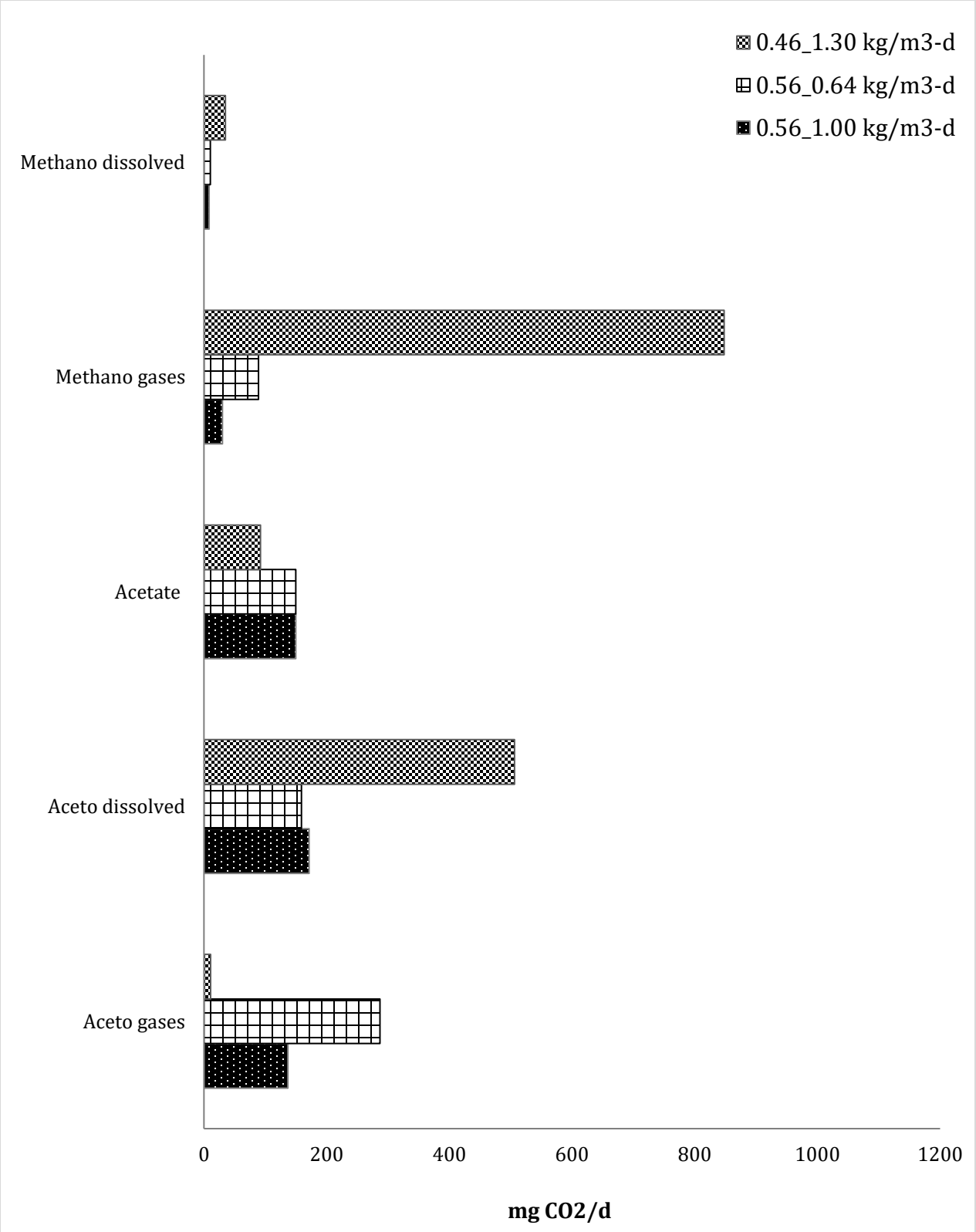


Figure 4.36 Net concentrations of CO_2 for each condition (CO_2 injected minus regular condition). Low rate organic loading rates groups shown. Legend values correspond to OLR groups as VFA_COD kg/m^3-d (VFA for influent into acetogenic reactor and COD for influent into methanogenic reactor).

4.6 Deinking Sludge Metal Analysis

Preliminary results from the continuous systems integrating deinking sludge were positive overall with no inhibition of methane production or reduction in COD removal efficiency. Given the potential benefits and risks associated with this deinking sludge to the AD system, a more detailed analysis of the composition and effects is discussed here.
It is generally believed that acetogenic bacteria are more resistant to heavy metal toxicity than methanogens (Chen, 1999; Zayed & Winter 2000; Chen et al., 2014). However, this depends on the specific interactions of the elements, their concentrations and the operating conditions. Nevertheless it is assumed that since, by design, the deinking sludge solids would remain in the acetogenic reactor, this first stage would assume the majority of the toxicity risk. At the same time, it is important to note that at a low pH such as exists in the acetogenic condition, metals become solubilized and could thus be transferred through the effluent to the methanogenic reactor. However, as will be discussed here, there is also potential for these metals to become neutralized in the second stage through a number of mechanisms.

An important aspect to consider is that total and soluble concentrations of elements and compounds may differ substantially in an anaerobic medium. Chemical reactions are incredibly complex; some may reduce available heavy metal concentrations by 1,000 times or more. Table 4.9 provides an example of how total and soluble concentrations can differ. In fact, heavy metals only lead to digester failure when the concentration of their free ions (in soluble form) exceeds a certain threshold concentration (Mosey & Hughes, 1975; Leighton & Forester, 1997b). Figure 4.37 illustrates a typical functional relation between the concentration of a salt or other element and its effect on the rate of a biological reaction. It can be essential within a certain range and then toxic past another point (called crossover concentration here).

Aside from the observed stability during experiments, there is much research that supports the possibility that the methanogenic reactor could provide protective effects from the soluble metals. Granular sludge, such as in the methanogenic reactor, has been shown to be an effective medium for biosorption of heavy metals from wastewaters.

Table 4.9 Concentrations of Inorganics Reported to be Inhibitory to Anaerobic Digestion (Parkin and Owen 1986).

Substance (1)	Concentration, in mg/L, at which Substance Is: Moderately inhibitory (2)	Strongly inhibitory (3)	Reference (4)
NA^+	3,500–5,500	8,000	64, 80
K^+	2,500–4,500	12,000	64, 80
Ca^{2+}	2,500–4,500	8,000	64, 80
Mg^{2+}	1,000–1,500	3,000	64, 80
Ammonia-nitrogen	1,500–3,000	3,000	63, 64, 80
Sulfide	200	200	64, 78, 80
Copper (Cu)	—	0.5 (soluble)	32, 94, 113
		50–70 (total)	27, 47
Chromium VI (Cr)	—	3.0 (soluble)	32, 94, 113
		200–260 (total)	27, 47
Chromium III	—	180–420 (total)	27, 47
Nickel (Ni)	—	2.0 (soluble)	32, 94, 113
		30 (total)	27, 47
Zinc (Zn)	—	1.0 (soluble)	32, 94, 113

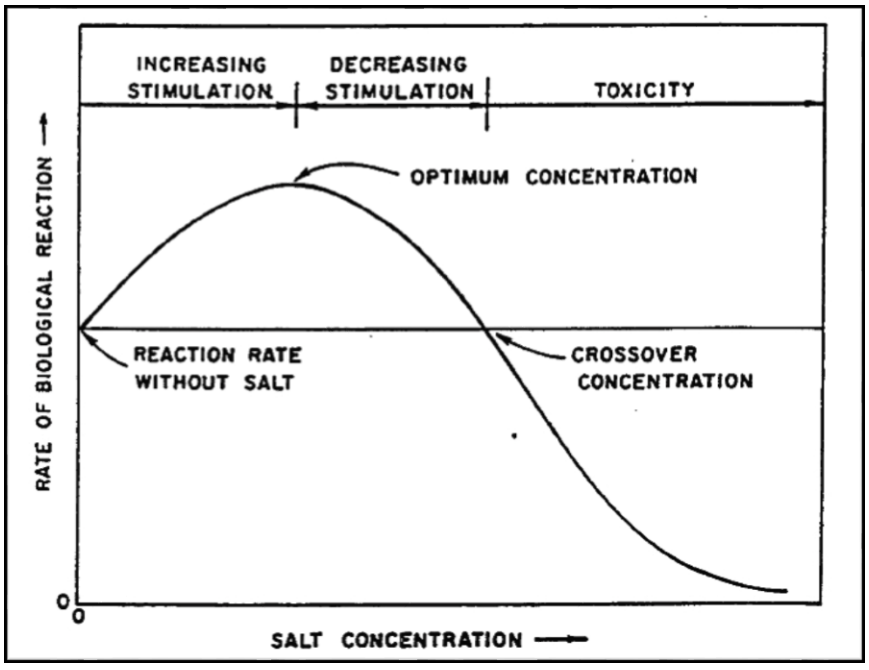


Figure 4.37 General effects of salts and other materials on biological reactions (McCarty, 1964)

Among other characteristics, this can be attributed to the particulate shape, compact porous structure, excellent settling ability, and high mechanical strength, of granular sludge. Viable anaerobic granules, treated with calcium, successfully biosorbed lead, copper, cadmium and nickel from aqueous solutions in continuous operation by ion exchange, complexation and precipitation mechanisms (Hawari & Mulligan, 2006a, Hawari & Mulligan, 2006b). An anaerobic sludge chemically modified as PO_4-biomass and Cl-biomass, operated in a continuous flow fixed-bed column operation showered successful remediation of (inorganic) arsenic contaminated water (Chowdhury & Mulligan, 2011). Another study demonstrated the potential for a two-phase anaerobic treatment system to treat Cr(VI)-contaminated effluent (Massara et al., 2008).

There may also be a protective effect of calcium alkalinity. High concentrations of calcium in the raw deinking sludge of 1.5×10^4 mg/kg, at a factory flow ratio 0.0.25 v/v (Table 3.4) could provide an advantage for precipitation of metals and salts.

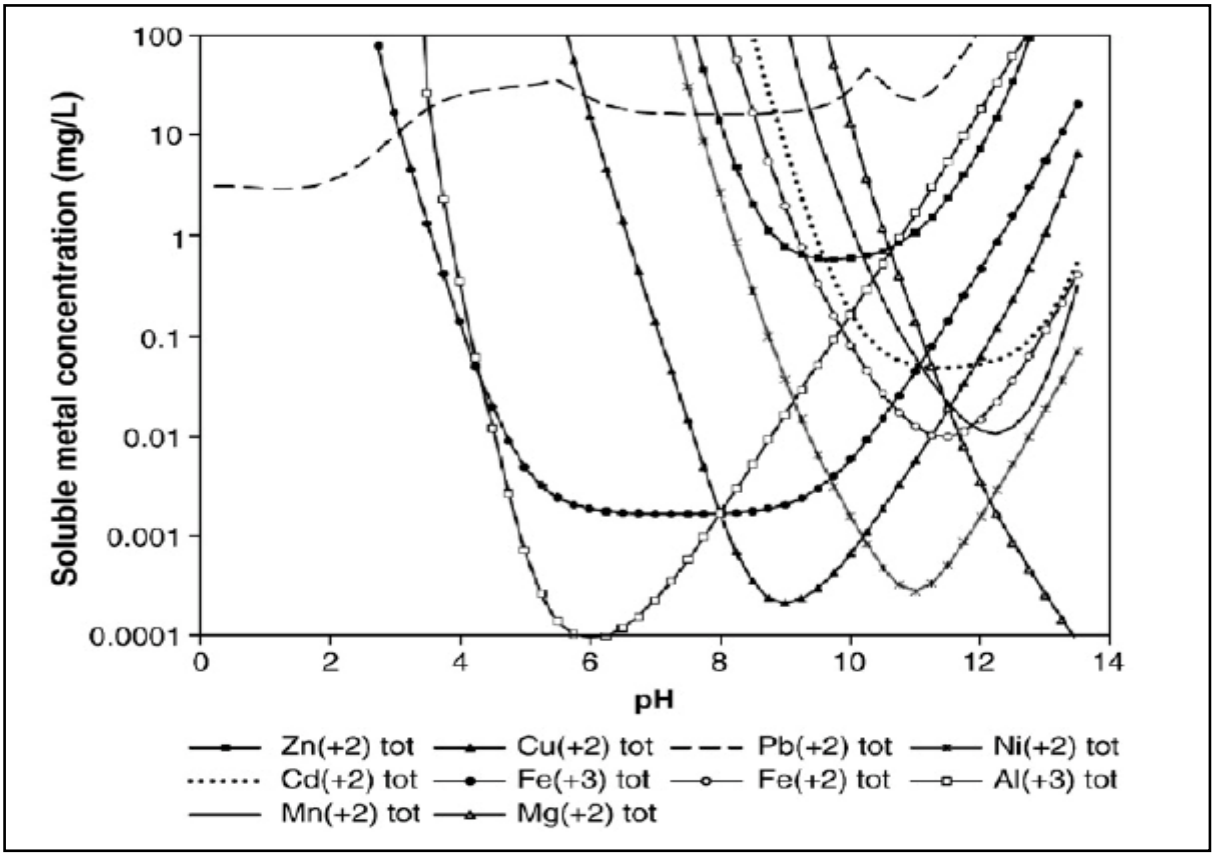


Figure 4.38 pH dependence of metal hydroxide solubilities (Lewis, 2010)

Assuming the calcium becomes solubilized and is present as calcium bicarbonate alkalinity, this could react to form aluminum hydroxide precipitate (EPA, 2000). Similarly this calcium alkalinity could react with chromium, which also requires hydroxide to precipitate, to provide further protective effects (Esmaeili et al., 2005). Figure 4.38 illustrates the complexity of these effects and how they vary with pH. As seen in the figure, effective precipitation of metals is often dependent on pH level and occurs in large part between pH of 7-10, overlapping with the methanogenic operating range.

Another factor, which may have contributed to the sustained health of the sludge, is the concentration of sulfate in the wastewater. As mentioned previously, it has been proposed that heavy metals only lead to digester failure when the concentration of their free ions (in soluble form) exceeds a certain threshold concentration (Mosey & Hughes, 1975; Leighton & Forester, 1997b) and this is directly related to the concentration of divalent sulfide ions present in the system. Sulfides have been consistently shown to remediate and provide protective effects from heavy metal inhibition by precipitation (Lawrence & McCarty, 1965; Masseli et al., 1967; Pichon et al., 1988; Jin et al., 1998, Zayed & Winter, 2000). In anaerobic reactors, sulfate (SO_4^{2-}) from the influent wastewater can be reduced to sulfide by the sulfate reducing bacteria consistent with Equation 4.1 (Koster et al., 1986).

$$SO_4^{2-} + 4\ H_2 \rightarrow H_2S + 2\ H_2O + 2\ OH^-$$
$$SO_4^{2-} + CH_3COOH \rightarrow H_2S + 2\ HCO_3^-$$

Equation 4.1

These sulfide ions are then free to bind to the metal cations and precipitate at the appropriate pH. The dependence of sulfide speciation on pH is illustrated in Figure 4.39, while the interaction of metal sulfide precipitation and pH is shown in Figure 4.40.

Rough initial calculations of the expected concentration of available sulfides in the methanogenic reactor account for sources from influent waste streams and biogas stripping and recovery which are expected to give an estimated 150-180 mg/L dissolved sulfide. Researchers have demonstrated that equimolar concentrations sulfide to heavy metal effectively protects against toxicity. Upon prolonged incubation, equimolar exposure

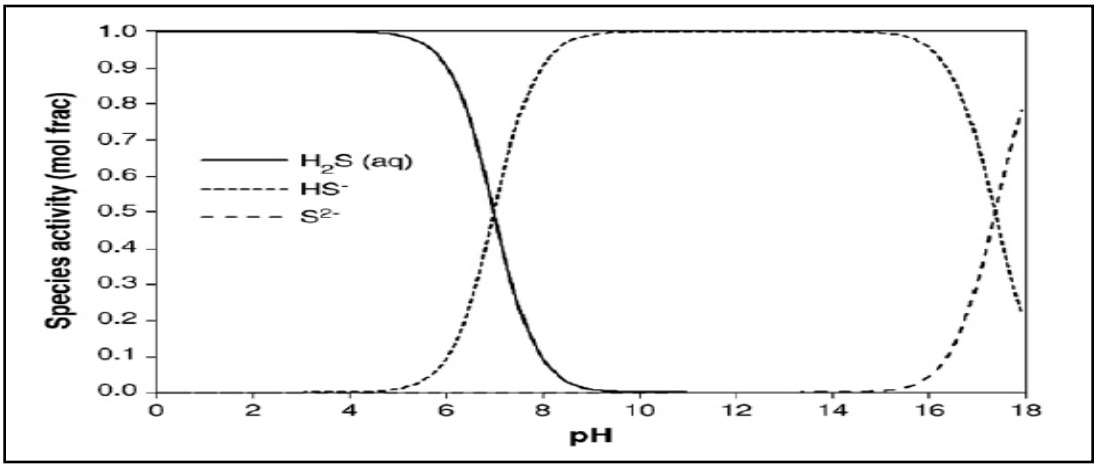


Figure 4.39 pH dependence of sulphide speciation

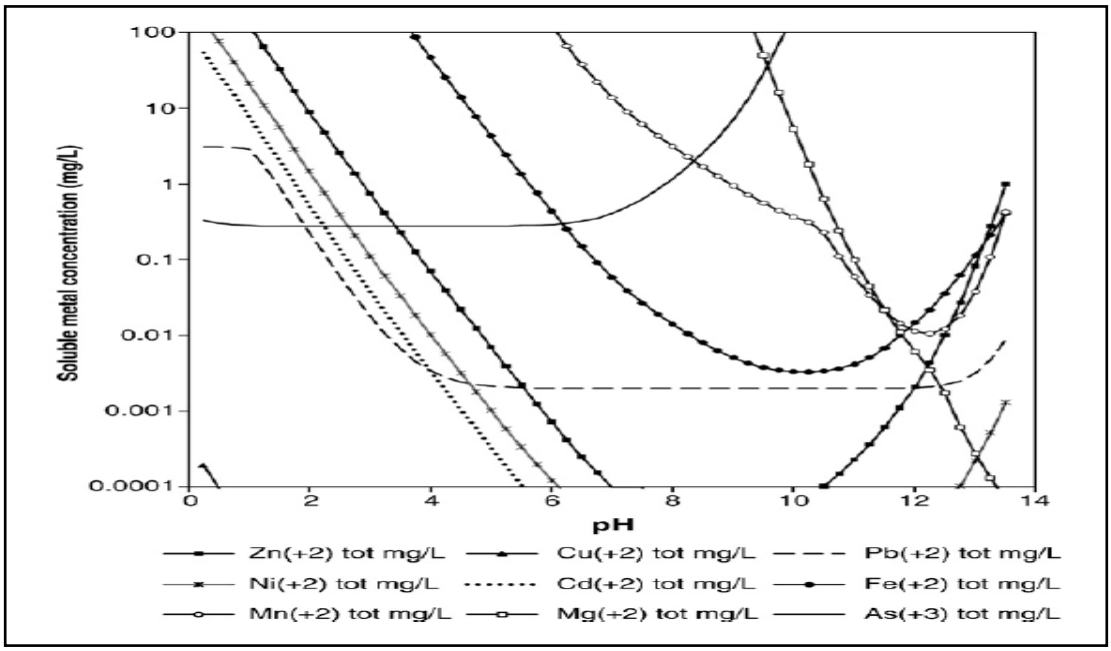


Figure 4.40 pH dependence of metal sulphide solubilities (Lewis, 2010)

to sulfate could prevent or remediate the 50% inhibition of methanogenesis that was observed in the presence of 10 mg $CuCl_2$, 40 mg $ZnCl_2$ and 60 mg $NiCl_2$ (Zayed & Winter, 2000). Researchers stipulated that sulfide precipitated the heavy metals as metal sulfides and by this way prevented heavy metal toxicity. Recovery failed, however, for copper chloride concentrations greater than 40 mg/L.

4.6.1 Solids Analysis

Nevertheless, the concentrations of metals in the solid sludge were measured for comparison and comprehensive analysis. Sludge samples of control, single-phase and acetogenic reactor of a 2-PAD conditions, were tested for metals and minerals at time 0 and on the last day of experimentation by ICP-MS. Results of elements that are relevant to anaerobic bacterial health are listed in Figures 4.41- 4.43.

Many interesting observations can be made from the results of the concentrations in solids across all conditions. Since the sample of liquid anaerobic sludge that served as the control, was obtained from a municipal treatment plant, it may have already contained elevated levels of metals (Jang et al., 2002). However, seeing as this liquid sludge was taken from an operational digester, it is arguably demonstrated that the concentrations of metals measured in the sludge allow for a population of anaerobic bacteria that are able to operate functionally. Concentrations in each experimental group have different implications. For instance, metals in the acetogenic reactor are subject to solubilization at the low pH and also can be wasted periodically, while metals may in the single phase reactor have a more permanent sludge residence, though sludge wasting is also possible from this reactor with the proper design. Moreover, the protective effects of precipitation given the pH related mechanisms and elemental interactions discussed in this section could be facilitated on the granular sludge in a single phase methanogenic reactor. Furthermore, among these metals, there are elements that have essential roles in the cellular function of anaerobic bacteria when present at certain levels and thus, would not cause a danger to the health of the reactor (Altas, 2009). For instance zinc and nickel are components of enzymes in anaerobic bacteria. Nickel can be essential for cellular function of anaerobic bacteria at concentrations between 300-600 mg/L, while higher concentrations can be inhibitory (Neis, 1999). In a second example, the presence of ferrous iron uptake systems seems to be important under anaerobic conditions because these bacteria can use Fe^{3+} as an electron acceptor (Ehrenreich & Widdel, 1994). Total volumes of deinking sludge loaded into reactors are listed in Table 4.10.

Table 4.10 Deinking Sludge Loading in Continuous Reactors at 0.025 ratio (v/v), total and daily volumes

	Days run	L total	L/d
Single Phase	29	0.39035	0.014
Acetogenic phase	15-20	0.75	0.05

Some results in the experimental conditions are notable and warrant additional monitoring. In most cases, concentrations of metals are lowest in the acetogenic condition, indicating some degree of solubilization in the acidic conditions. As illustrated in Figure 4.41, concentrations of copper were highest in the acetogenic sludge sample. This warrants some concern since it may be indicative of initial accumulation and VFA producing organisms in the acetogenic reactor have a very low tolerance for copper toxicity (Chen, 1999; Zayed & Winter, 2000). Cadmium concentrations could also potentially become a threat to reactor health if it is solubilized. The 9 mg/kg acetogenic reactor concentrations of cadmium are much higher than other conditions at 0.5 mg/kg (Figure 4.42). The limit of tolerance for acetogenic bacteria is 20 mg/L (Yu & Fang, 2001; Demirel & Yenigun, 2002). It is unclear how cadmium concentrations increased to such a degree in the acetogenic sludge since it is greater than initial raw concentrations. Chromium and nickel concentrations (Figure 4.42) are also slightly higher in the acetogenic reactor as compared to the single-phase reactor, though both were lower than in the control. Although the species of chromium cannot be specified by the ICP-MS solids analysis, it is worthwhile to monitor this level as Cr (IV) could potentially become toxic to acetogenic bacteria at 10 mg/L (Yu & Fang, 2001).

For the majority of metals, concentrations are lower for the single-phase as compared to the control condition. Although this may indicate a protective effect of precipitation, it is also a potential risk as the decreasing levels may ultimately reach concentrations that endanger bacterial health from deficiency. Concentrations of nickel for single phase operations are lower than the control (Figure 4.42) while those of zinc are higher than the control (Figure 4.41). It is worthwhile to monitor these concentrations as there are reports of toxicity as low as 1 and 2 mg/L for soluble zinc and nickel concentrations, respectively (Table 4.9). The concentrations of sodium and calcium in

Figure 4.43 are interesting because they are greatest for the single-phase condition. Though there are potential risks of toxicity at very high concentrations (Table 4.9), these elements may also aid in the protection of bacterial health by mechanisms discussed previously.

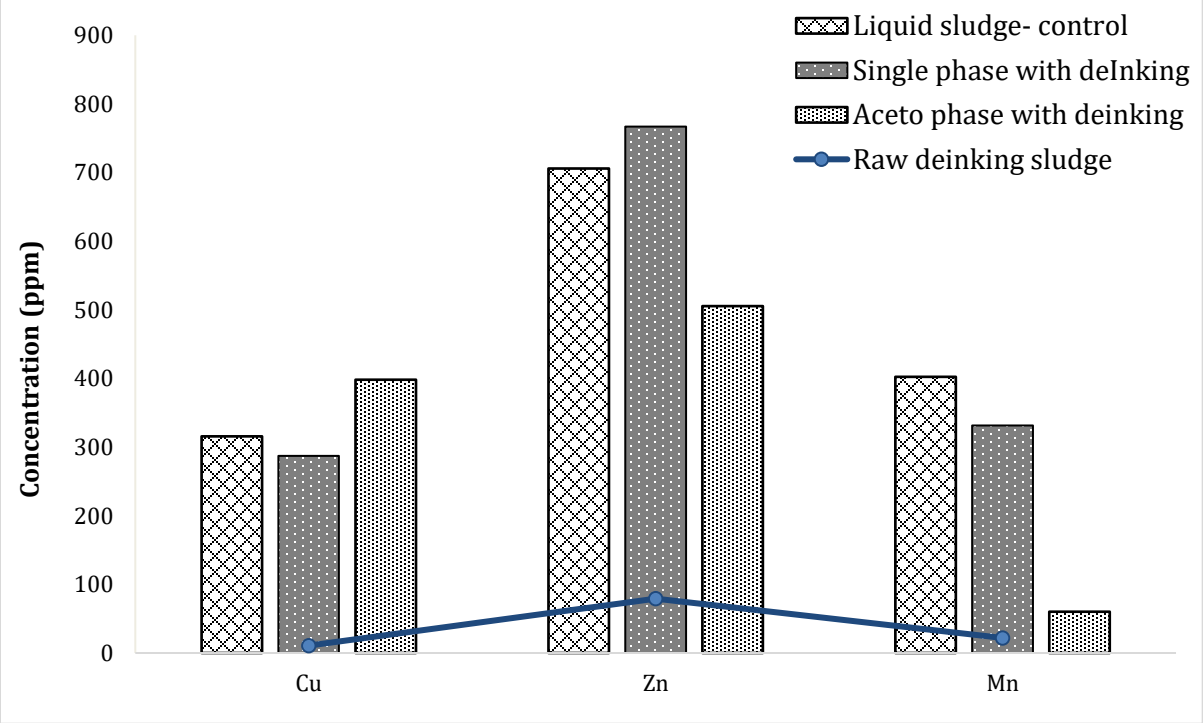


Figure 4.41 Concentrations of copper, zinc and manganese measured in sludge from 3 operating conditions, compared to concentrations of raw deinking sludge (0.025 v/v).

Potential complications do exist due to the high concentration of aluminum measured in all samples, since aluminum has been shown to react with SO_4^{2-} to synergistically inhibit all groups of bacteria (Cabirol et al., 2003). It was found that although an influent concentration of 2500 mg/L aluminum could be tolerated by gradually acclimatizing methanogenic bacteria, when combined with sulfate, the effects of aluminum were more inhibitory (Jackson-Moss, 1991; Cabirol, 2003). Although this factor warrants additional long-term monitoring, there are indications, such as non-competitive calcium precipitation, that it may not be a large risk. Combined, the factors discussed in this section demonstrate the potential of this system to allow for the sustained health and biogas production from the anaerobic bacteria.

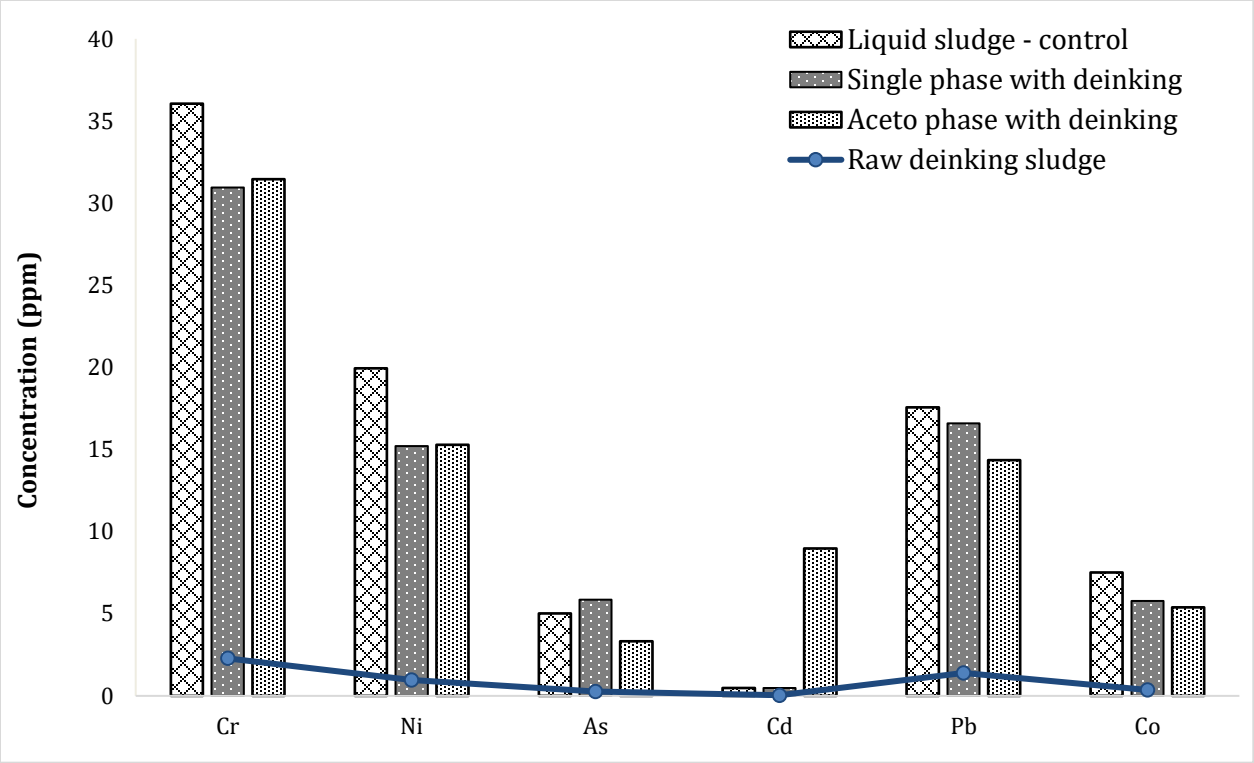


Figure 4.42 Concentrations of chromium, nickel, arsenic, cadmium, lead and cobalt measured in sludge from 3 operating conditions, compared to concentrations of raw deinking sludge (0.025 v/v).

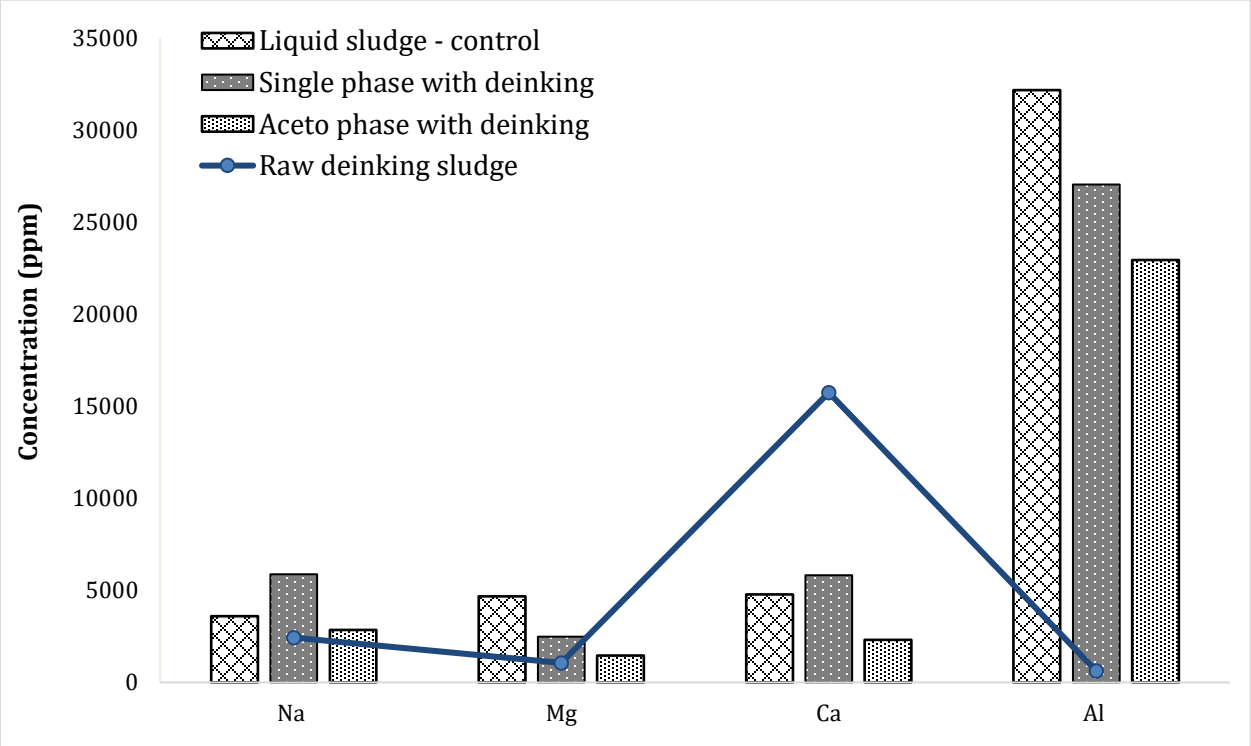


Figure 4.43 Concentrations of sodium, magnesium, calcium and aluminum measured in sludge from 3 operating conditions, compared to concentrations of raw deinking sludge (0.025 v/v).

4.6.2 Soluble Metal Concentration Analysis

Toxicity threshold limits refer to soluble concentrations and are usually reported in mg/L. However reported limits can only provide a rough guideline and are not absolute as there is a large range of reported levels of toxicity for specific elements and under different conditions. Table 4.11 shows an example of the range and variation within which toxicity can be observed. IC_{50} refers to the values of the half maximal inhibitory concentration. In other research, reported levels of copper toxicity have ranged from 5-4000 mg/L for methanogenic bacteria (Chen, 1999; Zayed & Winter, 2000). Toxicity levels for methanogenic bacteria from chromium have been reported as 250 IC_{50} mg/L for total (Lin & Chen, 1999) and 3 mg/L for soluble chromium IV (DeWalle et al., 1979). Toxicity for methanogenic bacteria from aluminum have been reported as high as 2500 mg/L (Jackson-Moss et al., 1989). Less research about toxicity for acetogenic bacteria has been reported compared to methanogenic bacteria perhaps due to their perceived robustness. Some notable findings from research have been reported as 18 mg/L IC_{50} for chromium in a batch test (Lin, 1993) and 1000 mg/L for aluminum (Cabirol et al., 2003). Reports of zinc toxicity to acetogenenic bacteria range from 5 mg/L to no toxicity at all (Zayed & Winter, 2000; Demirel & Yenigun, 2002). Overall, it can be said that the interactions are intricate and specific research needs to be done in each case to ascertain the effects.

Table 4.11 IC_{50} values of six heavy metals and comparison (Altas, 2009)

Bioactivity parameter	Product	Carbon source	IC_{50} ($mg\,L^{-1}$)					
			Cd	Cr	Cu	Ni	Zn	Pb
Methane production potential	Methane	Glucose	36	27	N.A.	35	7.5	N.A.
Methane production potential	Methane	VFA	330	250	130	1600	270	8000
Specific methanogenic activity	Methane	Starch	>550	630	158	118	97	N.A.
Specific methanogenic activity	Methane	Benzoate	150	210	175	100	110	N.A.
Hydrogen production potential	Hydrogen	Sucrose	3300	3000	30	1300	1500	>5000
Hydrogen production potential	Hydrogen	Glucose	N.A.	N.A.	350	N.A.	>500	N.A.
Hydrogen production potential	Hydrogen	Dairy wastewater	170	72	65	N.A.	120	N.A.
Specific methanogenic activity	Methane	VFA	7.7	N.A.	12.5	N.A.	16	67.2

Results of metal solubilization in the current study were only preliminary and limited to the 3 soluble metals: aluminum, chromium and partial results with iron. As seen in Figure 4.44 , soluble aluminum was measured at 1 mg/L from the acetogenic reactor with deinking and 0.127 mg/L without. The effluent of soluble aluminum from the methanogenic reactor was 0.025 mg/L with and 0.015 mg/L without deinking sludge while single-phase experiments showed the concentration to be 0.042 mg/L. Soluble chromium concentrations were slightly higher in the acetogenic effluent with deinking sludge at 0.826 mg/L compared to 0.775 mg/L without, while methanogenic effluent of soluble chromium was around 0.05 mg/L for both conditions. Single-phase experiments showed the soluble chromium concentration to be 0.083 mg/L. Soluble iron was measured at 0.409 mg/L in methanogenic effluent, reduced from an influent concentration of 4.42 mg/L, with deinking sludge (results not illustrated). Despite the limited data, results are positive, suggesting the action of protective mechanisms in the AD system. Both chromium and aluminum have been reported as resistant to precipitation with sulphide suggesting another mechanisms were responsible for the reduction in concentrations measured in the methanogenic effluent (Yu & Fang, 2001b; Cabirol et al., 2003). Measuring and tracking soluble metals would be an important part of a successful future system design.

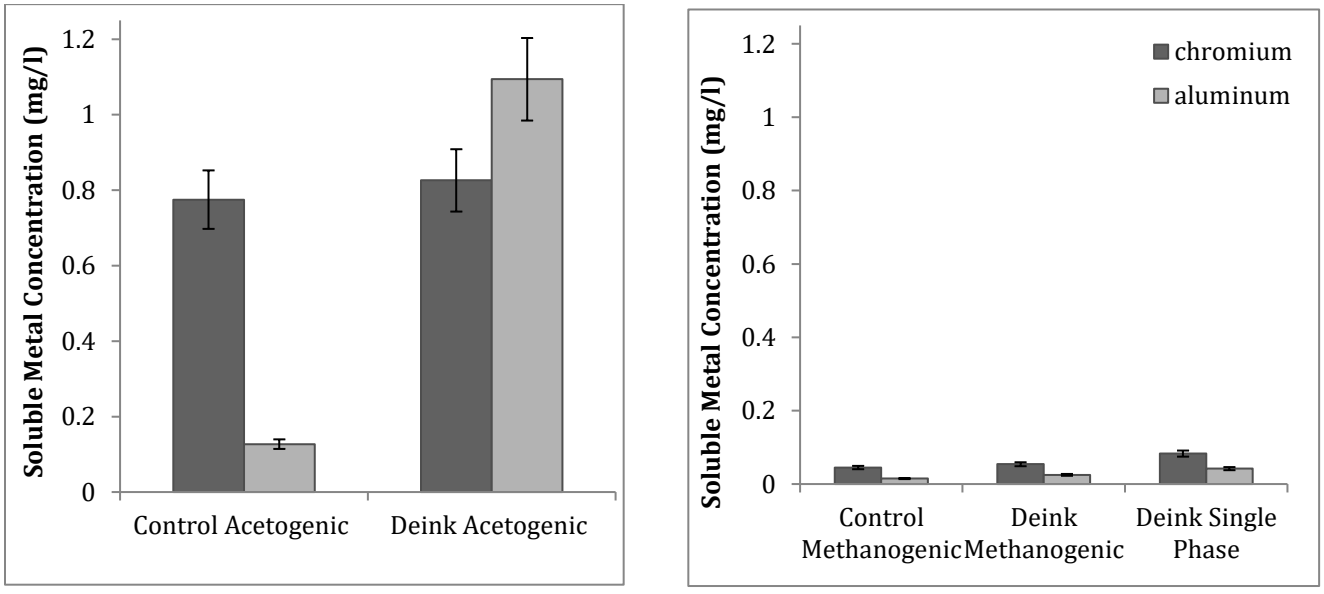


Figure 4.44 Soluble metal concentrations of chromium and aluminum in the effluent of the reactors, with and without integration of deinking sludge in the feed stream. Left illustrates acetogenic effluent, while right illustrates methanogenic effluent from single phase and 2-PAD reactors.

Overall, more accurate and comprehensive measurements of soluble metal concentrations, calcium alkalinity and sulphide would help to provide an indication of the protective potential of the proposed AD system and its feed streams against potential toxicity from the deinking sludge.

4.7 Biogas

Depending on the feed source being metabolized, biogas can contain significant quantities of undesirable compounds (contaminants), such as hydrogen sulphide (H_2S), ammonia (NH_3) and siloxanes and water vapour. These components can be problematic to thermocatalytic conversion devices and piping, causing corrosion, erosion, fouling and generating harmful environmental emissions. Biogas is typically employed in three ways: 1) burned directly in a boiler *in situ* to produce thermic energy for heating, 2) feed to engines for electric energy production, by turbine, piston or fuel cell engine at an efficiency of between 35-43%, or 3) in combined heat and power (CHP) cogeneration to capture heat produced upon generation of electricity (Centro Richerche Produzioni Animali, 2008; Weiland, 2010). The strengths and weaknesses of each option must be matched for suitability with the demands of the application. For instance, direct burning of biogas in a boiler does not require a high gas quality so has the advantage of simplifying operations although it is still generally recommended that hydrogen sulphide concentrations be reduced to prevent corrosion (Wellinger & Lindberg, 1999; Weiland, 2010).

In all cases, biogas produced at the plant would need at least some degree of scrubbing, either to protect pipes and engines and/or to adhere to Gaz Metro's specifications. Before it can be injected into the gas distribution line, biogas must meet the composition and characteristic standards as defined by BNQ 3672-100. Ultimately, the gas will be purified until it consists principally of dry biomethane and can be integrated seamlessly with the natural gas in the line. There are several biogas-scrubbing methods that have proven to be effective. Broadly, methods fall under two categories (Abatzoglou & Boivin, 2008):

- Physiochemical processes (reactive/non-reactive absorption, oxidation and molecular sieves)
- Biological processes.

One cost-effective means to scrub biogas emissions is a simple water column. This method has been shown to adsorb 80% of the H_2S from hog farm biogas in bench-top experiments (Lien et al., 2014). Figure 4.45 illustrates this concept and the relevant flows. Of course, this system would require a secondary, more robust scrubber to bring it to a higher standard. However, if used as an initial phase of biogas scrubbing, such a water column scrubber could have added benefits for the AD system. In addition to capturing H_2S, the water column could also dissolve CO_2 from the biogas emissions. As discussed in previously, hydrogenotrophic methanogens are able to biochemically convert excess CO_2 into CH_4 in different types of substrate water (Alimahmoodi & Mulligan, 2008; Abedi et al., 2015). This sulfide and carbon dioxide rich water could be recirculated into the methanogenic reactor to aid in the precipitation of soluble heavy metals from the de-inking sludge and at the same time could enhance methane production from the dissolved CO_2. Using the parameters outlined by Lien et al. (2014) as a framework, water level, gas flow rate, scrubbing time, pressure and water recirculation would be determined for maximum absorption of both gases. This initial scrubber would have the added benefit of lowering operational costs by removing the majority of the contaminant load in the biogas and lengthening the life of the consumable filter materials in the second, more robust scrubber.

4.8 Sludge wasting

Sludge wasting was not required for the entire duration of experiments, as it was negligible at the level of the small, 5-litre volume of the bench top reactor. Considering the suboptimal pH levels in the acetogenic reactor and the potentially inhibiting effects this may have had on hydrolysing bacteria, suggests that sludge degradation could be even more effective with pH adjustment to levels between 5 - 6. Nevertheless, the wasting of accumulated recalcitrant fixed solids in a full-scale reactor may periodically be required. As demonstrated in earlier sections, this would require proper assessment of acetogenic

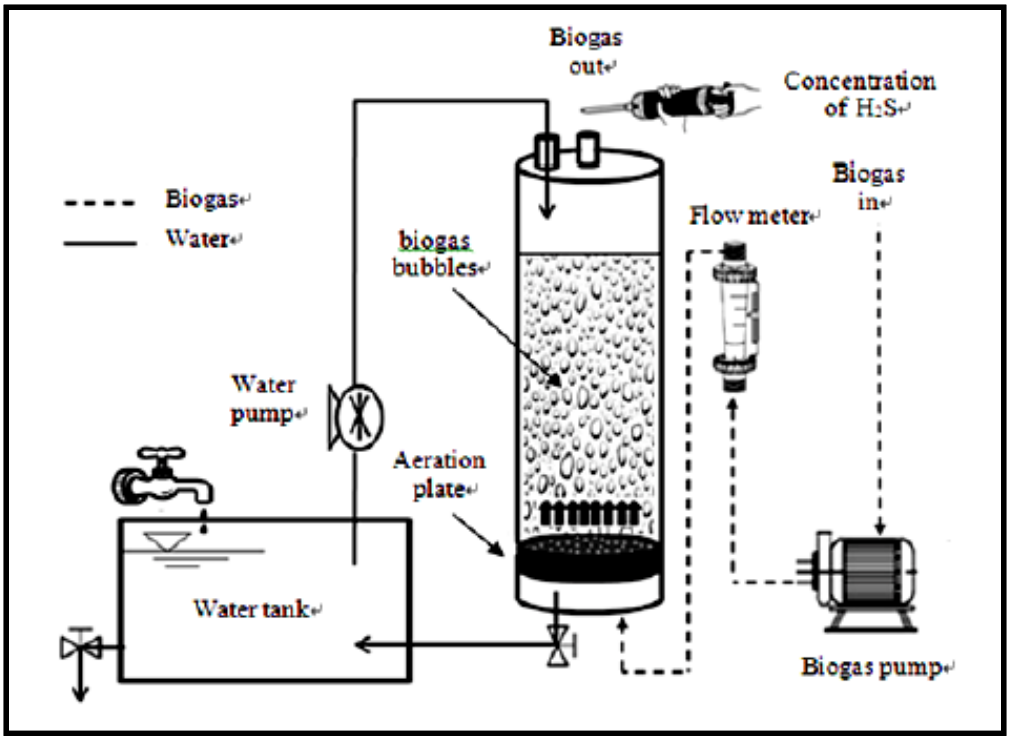


Figure 4.45 Schematic of desulfurization in biogas using water scrubbing (Lien et al, 2014)

sludge degradation in terms of rate and end products.. Equation 3.7 was used to calculate the mass of sludge that might be wasted each day. Assuming an acetogenic sludge concentration of 8 kg VSS/m^3, the SRT was found to be 60 days, which would require wasting of 975 kg dry solids/day (detailed in Appendix H). The granular sludge in the methanogenic reactor would not be expected to undergo any substantial sludge growth that would require wasting.

Although greatly reduced, there would also be some wasting expected from waste activated sludge reactor due to sludge growth. Given the same food to microbe ratio (F/M = 0.25), the reduction of influent water from 0.375 kg BOD/m^3, to 0.125 kg BOD/m^3, would result in wasting of approximately 400 kg dry solids/day, reduced from 1000 kg dry solids/day (detailed in Appendix H).

Besides the decrease in the biosolids quantity, the quality of the aerobic sludge often improves when partnered with AD; the number of filamentous bacteria causing bulking sludge in activated sludge plants is significantly reduced. This results in an improved settleability of the aerobic sludge and, consequently, a more stable and secure operation of

the activated sludge plant. Finally, due to the higher mineralization grade, dewaterability of aerobic sludge from activated sludge plants after anaerobic pre-treatment is often better than without anaerobic pre-treatment (Habets & Driessen, 2007; Meyer & Edwards, 2014). Table 4.12 lists a comparison of sludge production for aerobic versus anaerobic combined with anaerobic.

Table 4.12 Typical sludge production per ADT produced from recycle paper mill (Habets & Driessen, 2007)

Solids production	Complete aerobic treatment (kg TS/ADT)	Combined anaerobic/aerobic (kgTS/ADT)	Sludge savings (kg TS/ADT)
Biosolids (aerobic)	7.5	1.5	6.0
Inert solids (fibres)	1.5	1.5	0
Total sludge	9.0	3.0	6.0

4.9 Proposed System

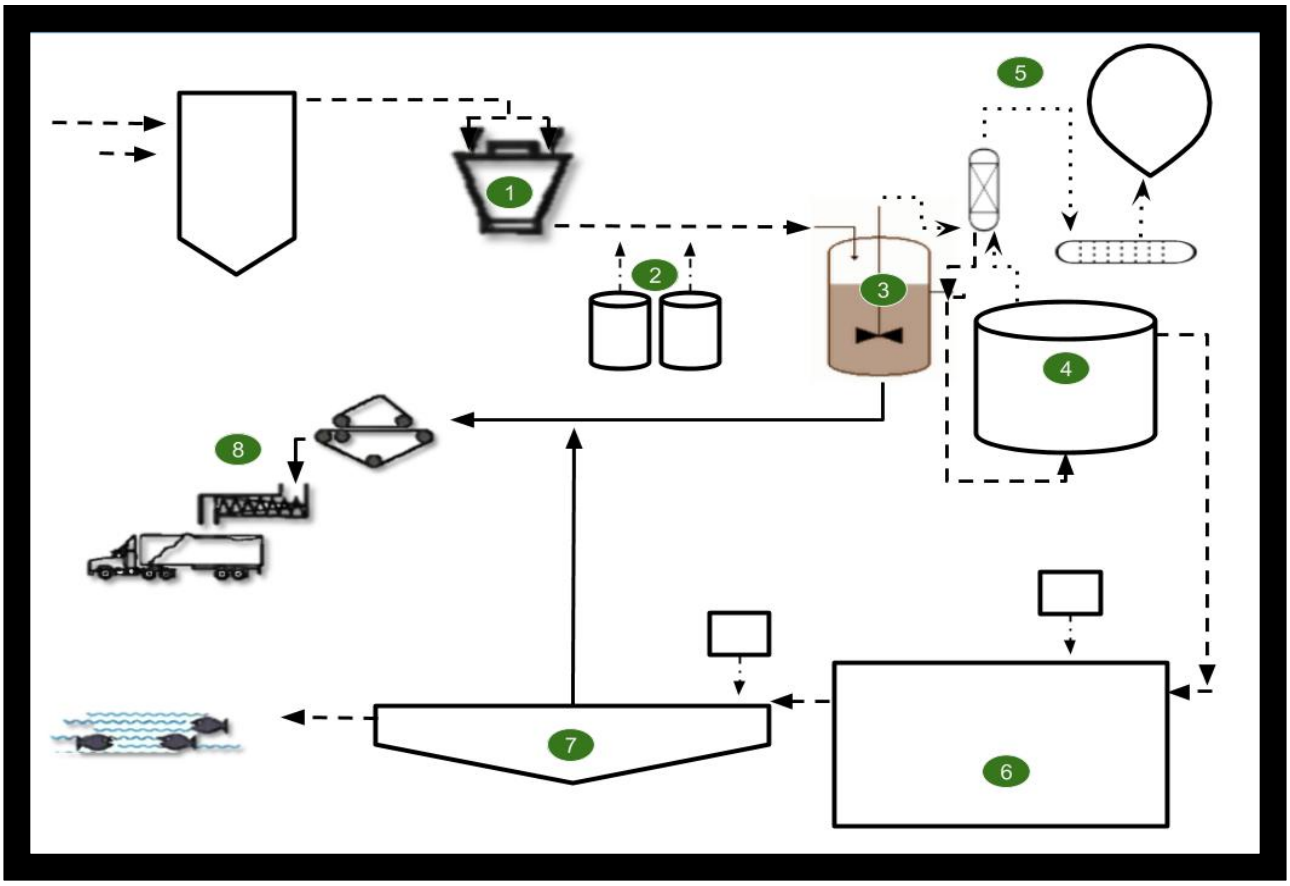
The Cascade treatment plant currently employs a waste activated aerobic system combined with stages of pretreatment (solids) to treat its water before discharge. Though this is very effective, the system can be costly due to requirements for aeration, high growth rate of aerobic bacteria and consequent dehydration and shipment for disposal of wasted sludge. The current research proposes the inclusion of an anaerobic digestion system in their waste stream treatment.

After consideration of a number of options, the best option is proposed to be: 2-phase system: influent wastewater was mixed at factory operating ratio with de-inking sludge at 6500 m^3/d + 165 m^3/d = 6665 m^3/d. A flow chart of the proposed system is illustrated in Figure 4.46. Influent wastewater would pass through the cooling tower during summer months. After nutrient and pH adjustment, the combined waste streams of

wastewater and deinking sludge would be directed to the first phase of the anaerobic treatment system, the mixed acetogenic reactor maintained at 38°C (temperature of influent wastewater). Experimental results indicate that a hydraulic retention time of 1 day appears to be effective to maintain acetogenesis. However, the mixed results from soluble and total COD suggest that additional retention time could be advantageous to facilitate solid degradation, estimated to be between 2 - 3 days. For the purpose of this analysis, it is assumed that adjusting pH to maintain levels between 5 - 6, would allow for successful biodegradation of soluble particles without requiring additional retention time or reactor volume. In that case, sufficient additional volume for mixing headspace and an estimate for solids retention time of 60 days would result in a total required volume of 10,000 m^3 for the acetogenic reactor. Efficient hydrolysis would allow for minimal sludge wasting from this phase, but could be a maximum of 975 kg fixed dry solids/d. These solids would be directed to the press and dehydration equipment.

Wastewater would then be pumped to the methanogenic reactor containing granular sludge maintained between 34- 37°C, assuming some heat loss from the previous stage. The hydraulic retention time of this reactor would aim to be 1.5 days, although depending on the efficiency of methane yield, and construction costs, it could be as long as 4.5 days. Assuming a retention time of 1.5 days, a maximum COD concentration of 6.7 kg/m^3, this would result in an organic loading rate of 4.5 kg/m^3-d from the acetogenic reactor. This OLR has the benefits of high methane yield, minimal reactor volume, and high COD reduction and avoids the risk of VFA accumulation and reactor failure. Accounting for headspace and sludge retention, a conservative estimate for required reactor volume is 20,000 m^3. The total volume for these reactors would be 30,000 m^3. The methanogenic effluent would then be directed to the waste activated sludge reactor which would enjoy lower operating costs due to the reduction of influent BOD from 375 to 125 mg/L and reduced aeration requirements and sludge wasting. Solids wasted would be directed to the press and dehydration equipment.

4.9.1 New Potential Flow Chart



#	Unit
1	Cooling tower
2	Nutrient and pH buffer adjustment
3	Acetogenic reactor
4	Methanogenic reactor
5	Biogas scrubbing and collection
6	Waste activated sludge reactor
7	Secondary clarifier
8	Sludge pressing, dehydration and disposal

Figure 4.46 Proposed flow chart of Cascades wastewater treatment plant at Kingsey Falls, QC that integrates the AD system. Figure includes reactors for 2-PAD system. Omitting unit 3 would yield a single-phase system. Unit descriptions are listed below figure.

4.9.2 Biogas infrastructure

A component of the proposed AD system that integrates de-inking sludge includes biogas-scrubbing system that is designed to treat the risk of soluble metal toxicity while also enhancing methane yield. A simple and cost-effective method to capture hydrogen sulfide in the biogas from both acetogenic and methanogenic reactors by using a water column to scrub the gas (Lien et al., 2014). In addition to the sulfate in the wastewater, it is proposed to capture the hydrogen sulfide and then to recirculate it back to the methanogenic reactor to stimulate precipitation. This water column has the added advantage of also potentially dissolving CO_2, which anaerobic bacteria can use to bio-convert into additional CH_4. A secondary, more robust, scrubbing system that removes water vapor and any residual impurities would be also required to bring the biogas up to the Gaz Metro distribution standards (BNQ 3672-100) in the case where biomethane would be sold into the natural gas line. These flows are outlined in the proposed flow schematic (Figure 4.46) and expanded in Figure 4.47.

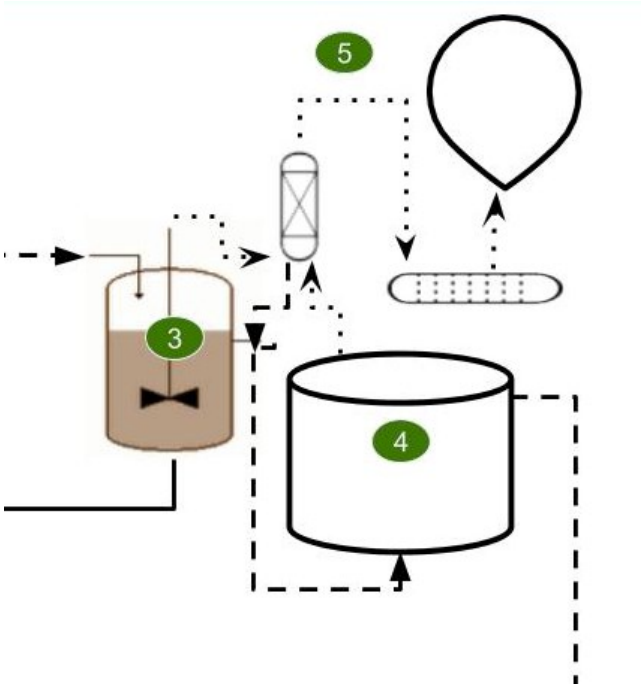


Figure 4.47 Gas flow from acetogenic (unit 3) and methanogenic (unit 4) reactors into water scrubbing column, to capture H_2S^- and CO_2 and then recirculate water to methanogenic reactor for precipitation and bioconversion respectively, while biogas continues to secondary scrubber and/or compressor to meet a more robust standard.

Chapter 5 COST ANALYSIS

Compared to aerobic forms of wastewater treatment, 2-stage anaerobic digestion typically has lower overall operational costs and creates biogas to use as energy. In order to assess the feasibility of such a project, least-cost, cost-benefit and project viability analyses were compiled (Energypaedia.info). The methane yield of a system is one of the principle factors to consider in determining the economic feasibility of adopting an AD system. However, this yield is most often not up to the theoretical standard of $0.35*(COD_i-COD_e) = CH_4$. As this study aims to demonstrate, it can be valuable to conduct bench-scale experiments to obtain more precise indications of the true methane yield potential of the waste stream substrate(s) as well as the characteristics of the effluents. The economic analysis presented in this section makes estimates using methane yields observed during experiments.

5.1 Legislative background

As mentioned in the introduction, recent federal and provincial legislative actions provide significant economic incentive for the adoption of such a project. The upcoming ban of organics in landfills (2020) combined with the required treatment of effluent and odour of such putrescible materials provides adequate incentive to proceed in developing the biogas avenue of the Climate Change Plan of Action (2013-2020) (Politique énergétique, 2015; MDDEP, 2012). At the same time, given the important role that natural gas plays in the Quebec economy, the government has been steadily working to ensure stable and secure access across the territory. Their strategy includes expanding the natural gas distribution network across the province, developing a liquidized natural gas network, and increasing the production of renewable natural gas (Audette, 2016).

In April 2016, the Ministry of Energy and Natural Resources submitted the 2030 New Energy Policy, which specified the need to diversify and improve the supply of energy, including natural gas. Upon submission of this policy report, the Minister of Environment, Sustainable Development and Parks (MDDEP) moved to request a review of measures that could improve the pricing practices of renewable natural gas. Using federal *modi operandi*

and other international models as case studies, the Régie de l'énergie commissioned proposal of a Feed-In-Tariff (FIT) rate structure that could properly valuate renewable natural gas in Quebec. The rate structure takes into account historical and projected demand, the volume of biogas produced, as well as costs avoided by using biogas, i.e. supply, gas compression, transportation rate, and carbon tax (Audette, 2016) . In parallel, Énergir (formerly known as Gaz Metro) submitted a request to the Régie de l'énergie to approve a contract to purchase 13 million m^3 of RNG/year for 20 years. This request was approved and since that time, Énergir has been actively seeking clients who may be able to provide them with sources of biogas and renewable natural gas to inject into their distribution network. These recent actions provide incentive to develop anaerobic digestion treatment at the Cascades facility so that the biogas produced from such a system could be connected directly to the existing Énergir natural gas distribution network.

5.2 Current Operating Costs

Cascades' current daily operating costs for their wastewater and deinking waste are illustrated in Figure 5.1. Sludge disposal is currently the highest cost at approximately 3110 CAD/day, followed by energy and nutrients each costing 690 and 660 CAD/day respectively. Flocculent costs approximately 140 CAD/day and the lowest cost is for heating the building at around 71 CAD/day. The total cost per day is approximately 4460 CAD/day.

5.3 Expected Biomethane Production and Additional Revenue

Cost estimates were calculated assuming methane yields for total COD between 0.14 - 0.16 m^3 CH_4/kg COD_{rmv} for each system, inline with experimental results. However, a higher methane yield of 0.21 m^3 CH_4/kg COD_{rmv} was also included for comparison purposes assuming the possibility of more efficient reactor, such as an expanded sludge bed, or increased retention time; this option is called 2-PAD+ and it is assumed to include deinking sludge in its waste stream. See Appendix E for an example of such a reactor.

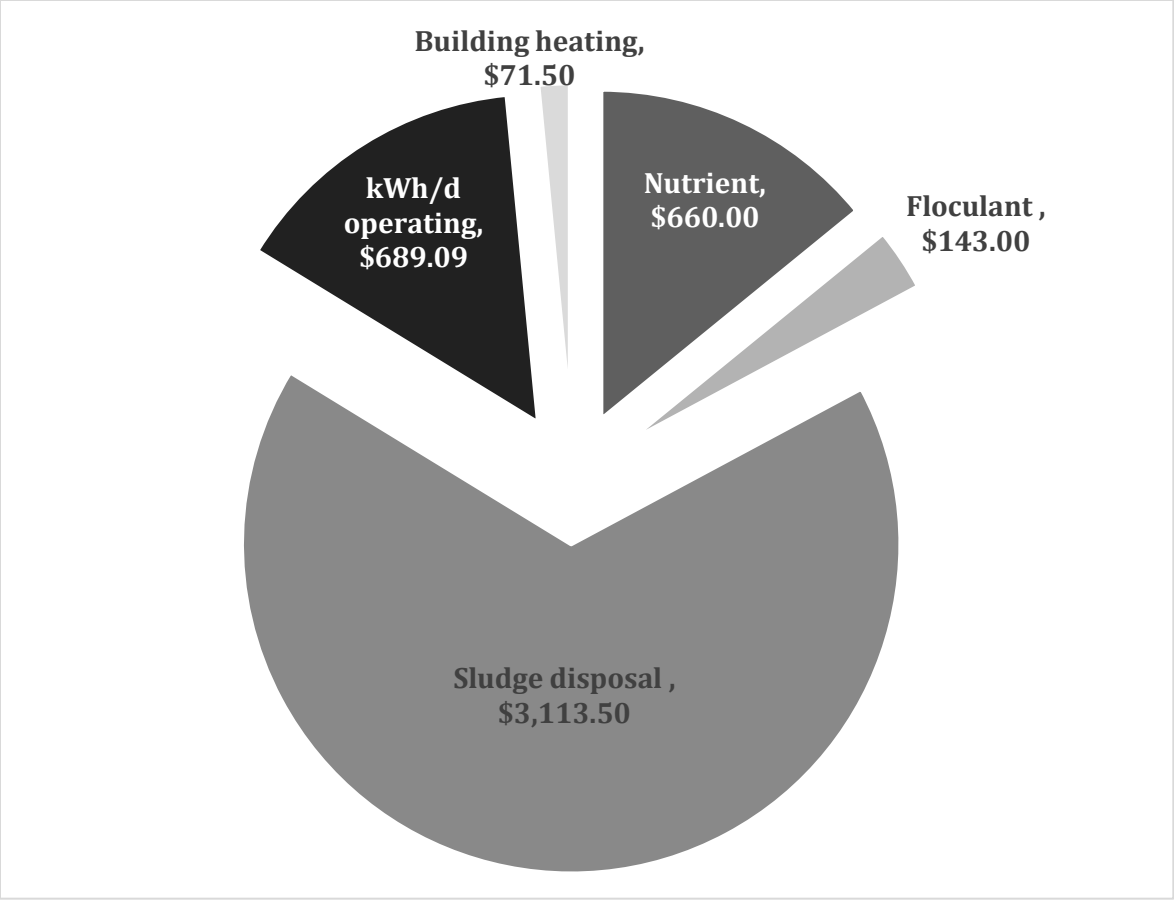


Figure 5.1 Daily operational costs including de-inking sludge treatment and disposal (CAD/day). Total cost = 4660 CAD/day (Appendix F).

Given current operating conditions of the plant and results, five options were assessed:

1. 2-PAD+ (with deinking sludge)
2. 2-PAD with deinking sludge
3. Single Phase with deinking sludge
4. 2-PAD
5. Single Phase

Based on experimental results, it is expected that the system could produce approximately 167 GJ/d from biomethane from an average yield of 0.15 m^3 CH_4/kg COD_{rmv}. This would produce a total of approximately 58 524 GJ/yr for the standard systems and 81 934 GJ/yr for the higher yielding (0.21 m^3 CH_4/kg COD_{rmv}) 2-PAD+ system (Appendix G). As shown in Table 5.1, these amounts of energy would put the subsidy at an estimated rate of return of 12$/GJ.

In the current study, 3 principle options for using biogas were considered, this energy could:

i) be sold in its entirety to the Énergir line,

ii) used in its entirety onsite for boiler heating or

iii) meet the energy needs of the treatment plant and sell the excess to Énergir.

These decisions would determine, more precisely, the amount that would be sold to Énergir at a subsidized rate. The cost-benefit analysis, comparing each option and each system design, is presented in this section.

Table 5.1 Example of a cross-tabulation of regressive subsidies based on various annual volume-modulated rates of return (TRG = taux de rendement global) for basic organic inputs. (Audette, 2016).

Grille d'analyse de TRG potentiel pour la période de démarrage de la filière 2017 à 2022					
	3154GJ/an (10m3/h) (100KW)	15768GJ/an (48m3/h) (500KW)	31 536 GJ/an (97 m3/h) (1000KW)	157680GJ/an (483 m3/h) (5000KW)	315360GJ/an (967 m3/h) (10000KW)
Subventions $->	70 %	50 %	40 %	20 %	0 %
TRG-4$/GJ[97]					
TRG-8 $/GJ					
TRG-10 $/GJ					
TRG-12 $/GJ					
TRG-14 $/GJ					
TRG-16 $/GJ					
TRG-18 $/GJ					
TRG-20 $/GJ					
TRG-22 $/GJ					
TRG-24 $/GJ					

Légende :

	Un TRG dans cette zone risque d'être insuffisant pour attirer les capitaux
	Un TRG dans cette zone attirerait les investissements requis
	Un TRG dans cette zone pourrait offrir une rentabilité élevée
	Un TRG dans cette zone offrirait une rentabilité très élevée

NB : Ce tableau est présenté à titre indicatif pour stimuler la réflexion, il devrait être ajusté et validé avec l'industrie et le véritable potentiel technico-économique pour le Québec.

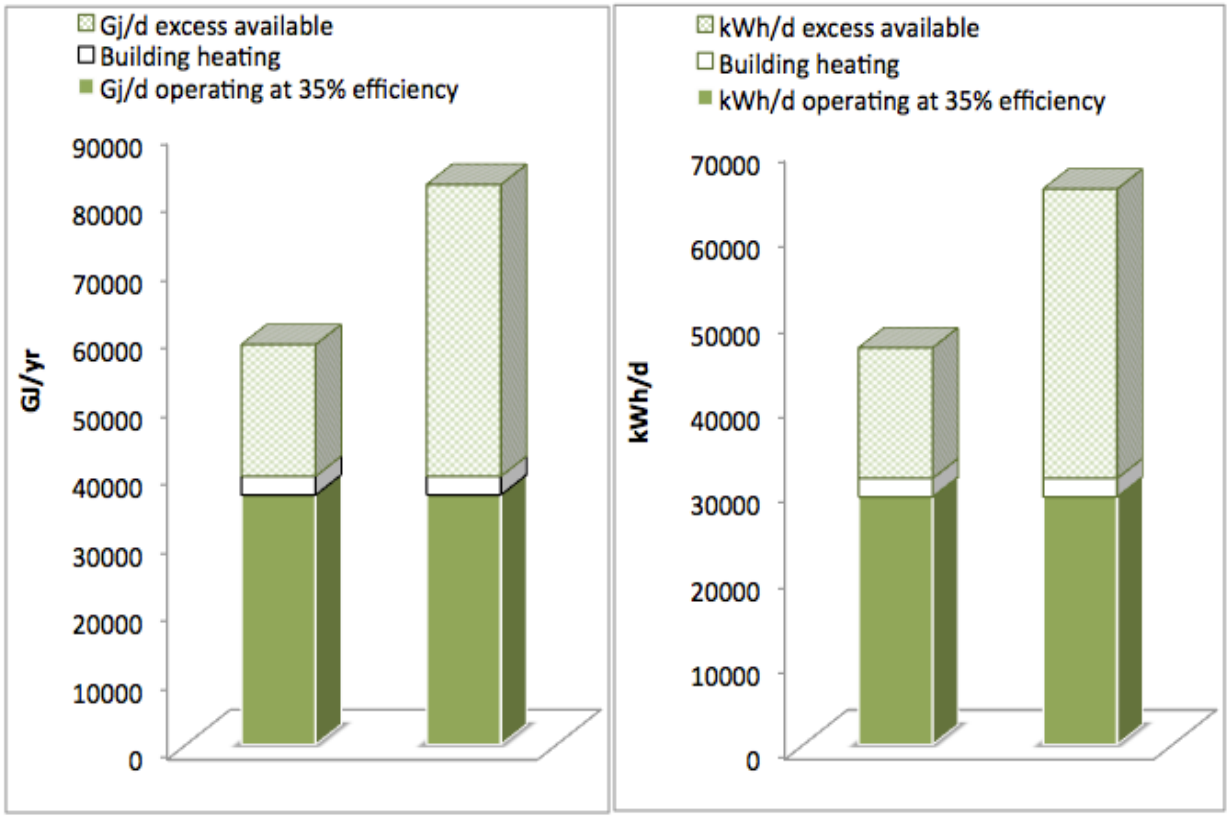


Figure 5.2 Total energy available from biogas for methane yields of 0.15 and 0.21 m^3 CH_4/kg COD. Columns indicate distribution of energy demands onsite. Left: gigajoules/year, right: kilowatt-hours/day (Appendix F).

Overall, it can be seen in Figures 5.3-5.6, systems integrating de-inking sludge offer much more economic incentive for adoption. The difference between 2-PAD and 2-PAD+ indicate that the methane yield of the reactor can also make a substantial difference in biogas production and revenue. It is worthwhile to note that there are a number of variables, such as those assumptions listed in Appendix F that could be subject to change. In all cases, construction costs for reactors' volume and required instrumentation, piping and pumps needs to be considered in comparing these options. Furthermore, these costs are expected to differ based on the system type and intended energy use. For instance, integrating de-inking sludge would require additional piping to relay the waste stream from one facility to another. Using gas onsite only may require lower quality standards but also might require additional piping to relay the gas. Operational costs are also estimated at very conservative levels, which may lead some options to appear less feasible than they are (Appendix G). These costs would need to be confirmed for a more complete analysis.

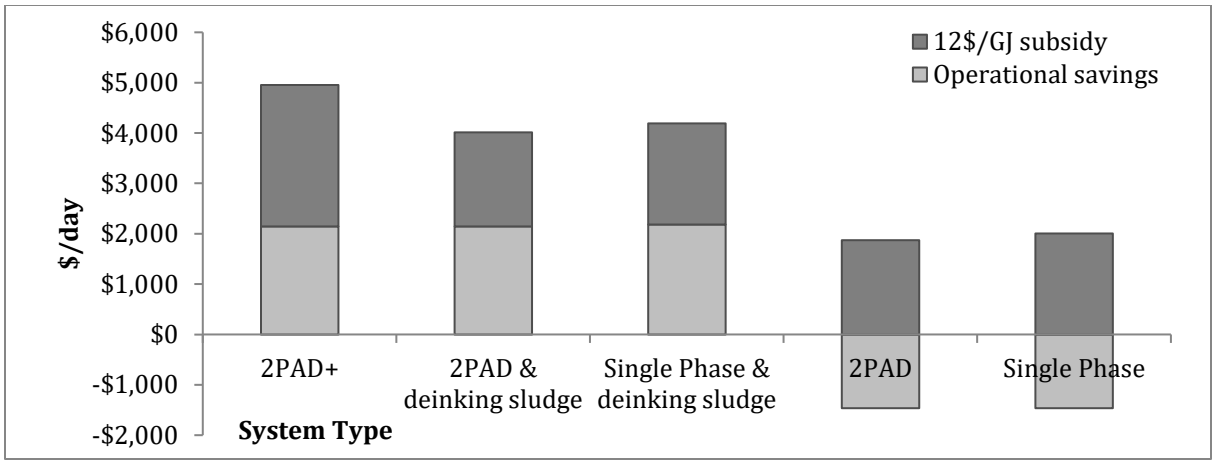


Figure 5.3 Changes in daily operational costs and potential revenue. Case where all bio-methane is sold to Énergir at subsidized rate.

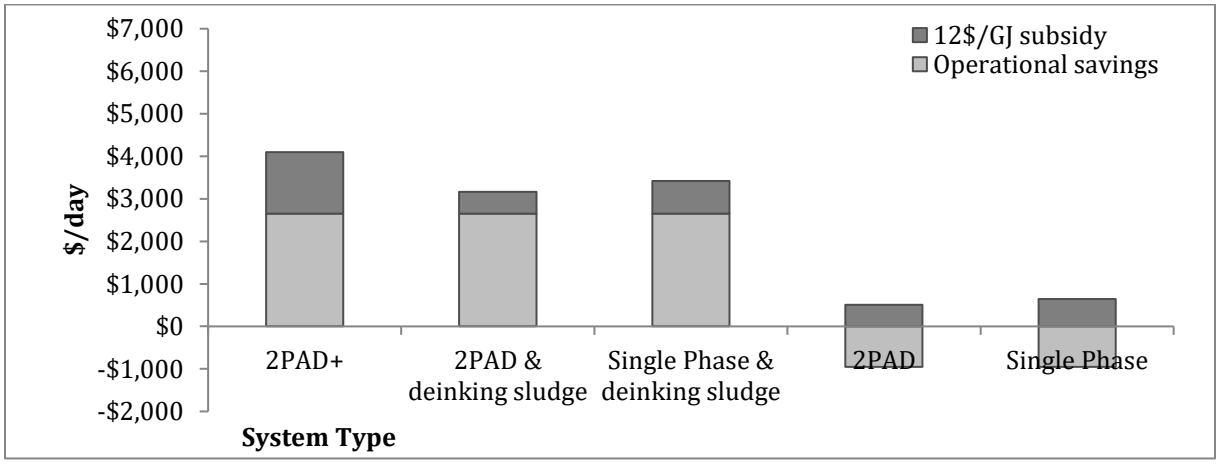


Figure 5.4 Changes in daily operational costs and potential revenue. Case where bio-methane is used to meet energy needs of the treatment plant and excess is sold to Énergir at a subsidized rate.

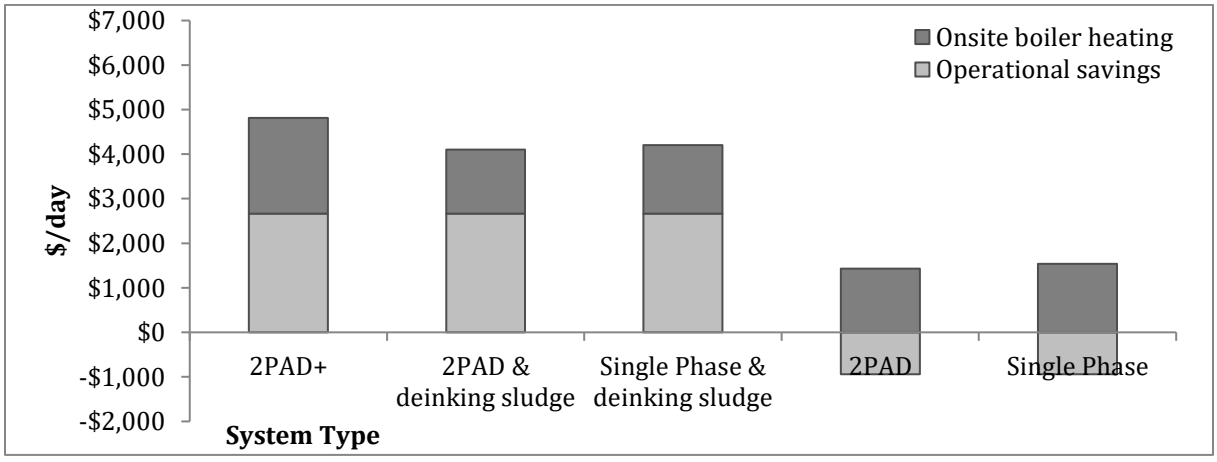


Figure 5.5 Changes in daily operational costs. Case where all bio-methane is used to meet the heating needs onsite of the treatment plant and other heating demands at the facility.

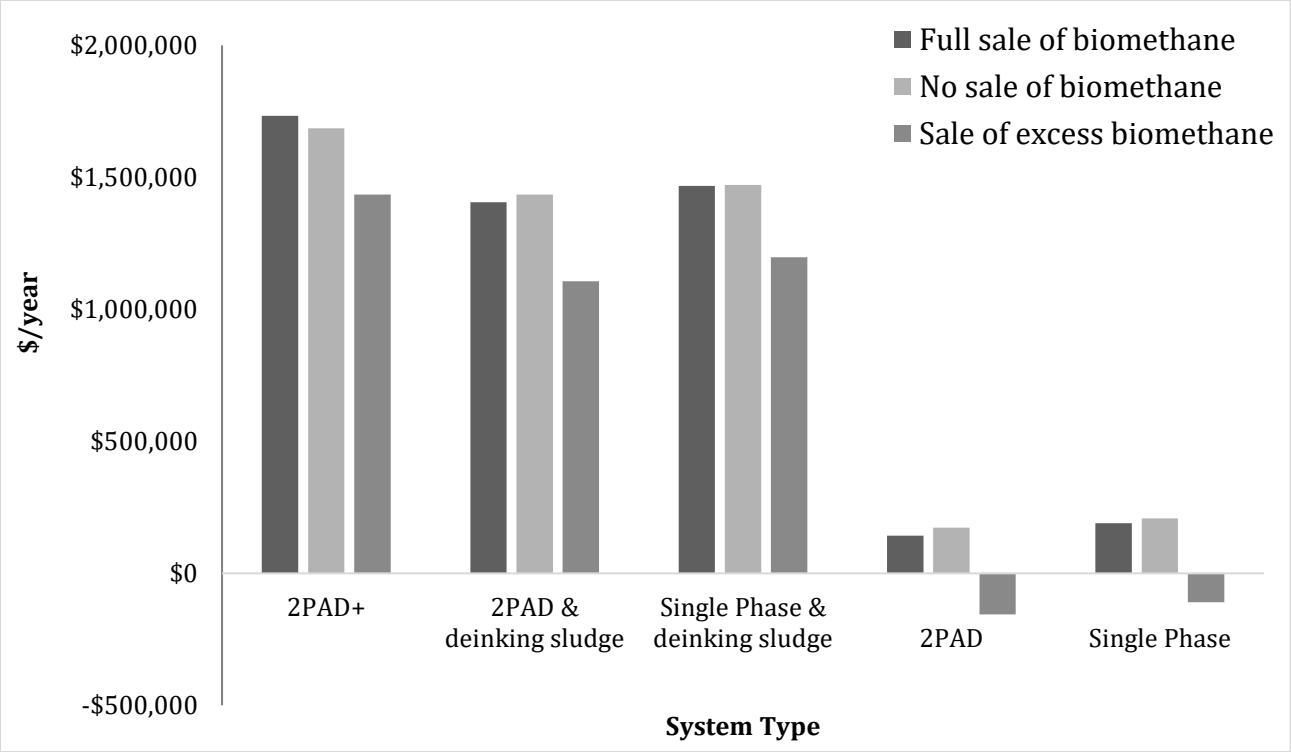


Figure 5.6 Additional revenue available per year as a result of system implementation. Depending on construction costs, these values can be used to determine number of years to recover costs in future viability analyses.

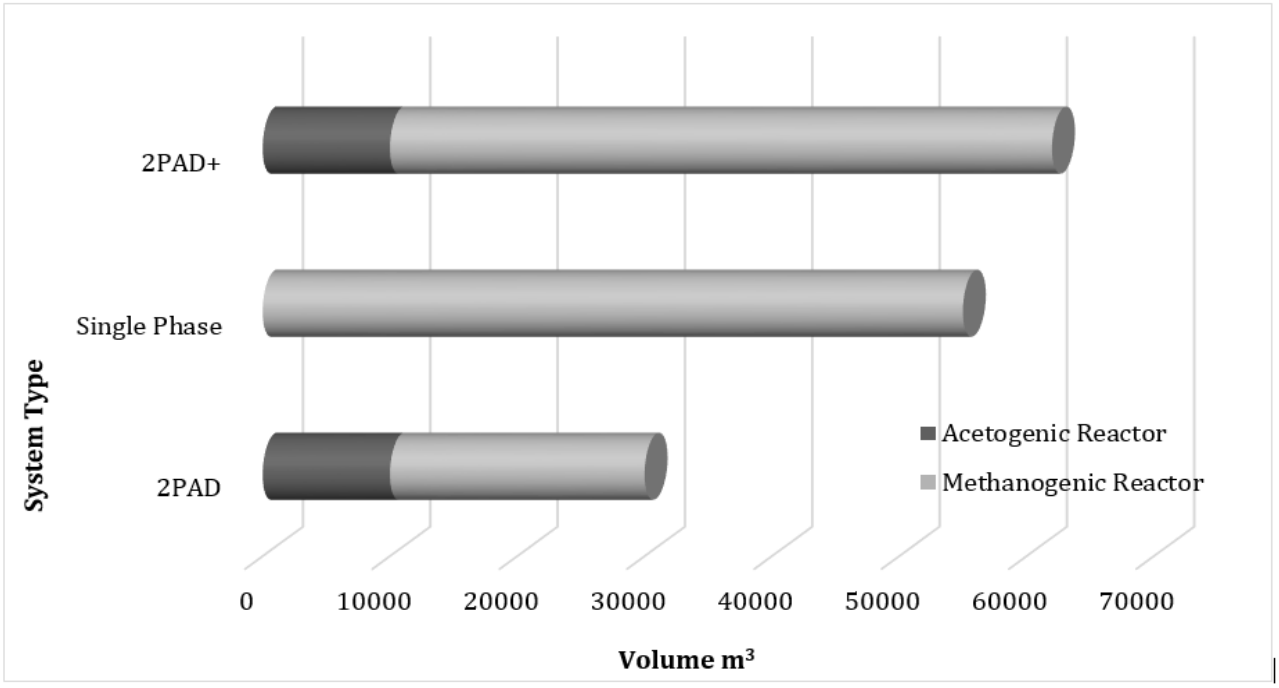


Figure 5.7 Required volumes for proposed system options in meters cubed. Acetogenic reactors represent RT of 1 day while methanogenic reactors represent RT of 1.5 or 4 days.

Comparing the options of single versus 2-PAD systems requires accounting for additional factors besides the expense of construction costs from reactor volume (Figure 5.7). A notable advantage of this system is the reduction of sludge creation and elimination of primary treatment. Single-phase systems are able to accept these solids, and can be designed to eliminate any recalcitrant matter, especially at wastewater solids concentrations of 0.35%. However, the efficacy of such degradation may be slower, if not less effective, than in a separate acetogenic phase. Moreover, if the de-inking sludge is integrated in the feed stream, it may be safer to separate methanogenic bacteria to a secondary phase in order to better regulate and monitor influent concentrations of metals and organics. It is generally believed that acetogenic bacteria are more resistant to metal toxicity than methanogenic bacteria.

Chapter 6 CONCLUSIONS

6.1 Conclusions

The main objective of this study was to determine the operational parameters and performance for the feasible integration of anaerobic digestion into the treatment operations of the Cascades paper recycling plant. The biomethane potential and biodegradability of the waste streams were measured by continuous operation in lab scale UASB reactors. Single and two phase conditions were compared in addition to a range of organic loading rates, temperatures, different types of sludge and feed streams. The potential to use recycling wastewater as a carbon sink was also investigated. In this way, the bioconversion potential of the AD system fed CO_2 saturated recycling wastewater was also investigated for effective increases in methane yield. The conclusions regarding experimental results and contributions to knowledge are summarized here.

6.1.1 Recycling plant waste streams in UASB continuous system

Single and two phase continuous operation were conducted. Performance was analyzed for COD removal as well as total and soluble methane yield.

6.1.1.1 Single phase conditions

Experiments using only granular sludge and influent wastewater were operated at temperatures of 38 and 45 °C and a retention time of 5 days. At an OLR of 1.24 kg tCOD/m^3-d and 0.60 kg sCOD/m^3-d, the 38 °C produced 0.16 and 0.35 m^3 CH_4 /kg COD_{rmv} for total and soluble COD respectively. At an OLR of 1.06 kg tCOD/m^3-d and 0.78 kg sCOD/m^3-d, the 45 °C produced 0.22 and 0.32 m^3 CH_4 /kg COD_{rmv} for total and soluble COD respectively.

Experiments using liquid sludge in the single phase condition were operated at 38 °C both with and without including deinking sludge in addition to the influent wastewater at a ratio of 0.025 v/v. Total COD produced a methane yield of 0.10 m^3 CH_4 /kg COD_{rmv} at an OLR of 1.20 kg tCOD/m^3-d for the condition with only wastewater, while soluble COD produced a methane yield of 0.18 m^3 CH_4 /kg COD_{rmv} at an OLR of 0.72 kg sCOD/m^3-d. Total COD produced a methane yield of 0.15 m^3 CH_4 /kg COD_{rmv} at an OLR of 1.33 kg tCOD/m^3-d

for the condition that included deinking sludge with wastewater, while soluble COD produced a methane yield of 0.23 m^3 CH_4 /kg COD_{rmv} at an OLR of 0.87 kg sCOD/m^3-d.

Reduction in COD was an average of 90% for all 4 conditions. Reductions in BOD were between 89-94% for all conditions except that which included deinking sludge and had a BOD removal of 81%.

6.1.1.2 Two phase condition Cascades operating parameters

This condition was designed to replicate the operating parameters of the Cascades plant to determine the effectiveness of continuous operations within those parameters. A 2-PAD system was fed influent wastewater and an operationally proportional flow of deinking sludge. The first phase was inoculated with acetogenic liquid sludge and maintained at a temperature of 38°C. The second phase used granular sludge and operated at a temperature of 37°C and maintained an organic loading rate of 1.50 kg tCOD/m^3-d and 1.05 kg sCOD/m^3-d. Total COD reduction was stable at 94% while methane yield ranged between 0.08 - 0.15 m^3 CH_4/kg COD_{rmv}. Soluble COD reduction was stable around 91% with a methane yield averaging 0.20 m^3 CH_4/kg $COD_{rmv.}$

6.1.1.3 Two phase conditions

Additional 2-PAD experiments were run to determine the effect of 5 organic loading rates (5, 2.46, 1.4, 1.07, 0.64 kg tCOD/ m^3-d and 3.0, 1.65, 0.90, 0.68, 0.52 kg sCOD/ m^3-d) and retention times (1, 2.25, 3.75, 4, 4.5 days) on COD removal and methane yield. Granular sludge was used in both the first and second phases. The first phase was operated at 25 °C and the second at 35 °C. Gas yields were highest for total COD, at 0.21 m^3 CH_4/ kg COD_{rmv} and for soluble COD at, 0.25 m^3CH_4/kg COD_{rmv}, at a retention time of 4.5 days. Shorter retention times between 1.0 - 4.0 days averaged gas production of 0.14 m^3 CH_4/ kg COD_{rmv} for total COD and 0.23 m^3 CH_4/kg COD_{rmv} for soluble COD. Removal efficiency of COD and BOD is stable and consistent around 90% for all conditions with the exception of an average removal efficiency of 78% for soluble COD at the shortest retention time, 1 day.

6.1.2 Bioconversion potential of UASB continuous system

The effectiveness of the AD system in bioconverting excess CO_2-injected wastewater was investigated in a series of experiments. Organic loading rates of both acetogenic and

methanogenic reactors were varied. Acetogenic loading rates were distinguished by loading rates of VFA concentration in the initial influent of: 0.46, 0.56, 0.735 and 0.95 kg VFA/m^3 –d, and methanogenic loading rates were distinguished by organic loading rates of COD which ranged between 0.64-2.6 kg COD/m^3-d.

6.1.2.1 Acetogenic conversion of CO_2 to VFAs

Conversion of excess CO_2 into acetate in the acetogenic reactor was monitored. Change (decrease) in pH was found to be inversely and proportionally related to an increase in VFA concentrations of control versus CO_2 conditions. All except 0.735 kg VFA/m^3-d loading rate groups showed an increased concentration of VFAs for the CO_2 condition though these effects were not pronounced and included increases in additional VFAs, namely butyric acid. A 28% increase in VFA levels (1% increase in acetate) in the CO_2 condition for the 0.95 kg VFA/m^3-d group corresponds to a 12% decrease in pH. A 7% decrease in VFA levels (16% decrease in acetate) in the CO_2 condition for the 0.735 kg VFA/m^3-d corresponds to a 21% increase in pH. A 2% increase in VFA concentrations (7% increase in acetate) of the CO_2 condition for the 0.56 kg VFA/m^3-d group corresponds to a 2% decrease in pH. Finally, a 12% increase in VFA concentrations (8% increase in acetate) of the CO_2 condition for the 0.46 kg VFA/m^3-d group correspond to an 11% decrease in pH.

6.1.2.2 Changes in biogas production and methane yields for total and soluble COD

Methane yields were calculated to access the effect of excess CO_2 on the bioconversion capacity of the AD system. Methane yields for soluble COD in the 0.95 kg VFA/m^3-d OLR group did show a 14% increase in yield for the CO_2 condition relative to the control condition, while the carbon dioxide yields were 45% greater than the control at the methanogenic OLR of 1.1 kg COD/m^3-d. The 0.735 kg VFA/m^3-d OLR group showed a 5% increase in the methane yield and a 9% increase in carbon dioxide yield for soluble COD in the CO_2 condition compared to the control at the OLR of 1.5 kg COD/m^3-d. The 0.56 kg VFA/m^3-d loading group showed a 12% increase soluble COD methane yield along with a 50% increase in carbon dioxide yield for the methanogenic OLR of 0.64 kg COD/m^3-d. At the methanogenic OLR of 1.0 kg COD/m^3-d the methane yield of soluble COD showed the CO_2 condition to have a greater methane (21%) and carbon dioxide (50%) yields. The

0.466 kg VFA/m^3-d OLR group show the methane yields from soluble COD to be 17% greater than the control while the carbon dioxide yields was 50% greater.

6.1.3 Observations of integrating deinking sludge

Concentrations of heavy metals and specific minerals were measured by ICP-MS in the sludge of continuously operating experiments that included deinking sludge. Three conditions were measured, control liquid sludge, acetogenic liquid sludge from 2-PAD and single phase liquid sludge.

6.1.3.1 Solid concentrations

Some results in the experimental conditions are notable and warrant additional monitoring. In most cases, concentrations of metals are lowest in the acetogenic condition indicating some degree of solubilization in the acidic conditions. Concentrations of copper and cadmium were highest in the acetogenic sludge sample at 400 and 9 mg/kg respectively. Concentrations of chromium and nickel are also slightly higher in the acetogenic reactor as compared to the single-phase reactor although both were lower than in the control.

6.1.3.1 Soluble concentrations

Results of metal solubilization in the current study were only preliminary and limited to the 3 soluble metals aluminum, chromium and partial results with iron. Soluble aluminum was measured at 1 mg/L from the acetogenic reactor with deinking and 0.127 mg/L without. The effluent of soluble aluminum from the methanogenic reactor was 0.025 mg/L with and 0.015 mg/L without deinking sludge while single-phase experiments showed the concentration to be 0.042 mg/L. Soluble chromium concentrations were slightly higher in the acetogenic effluent with deinking sludge at 0.826 mg/L compared to 0.775 mg/L without, while methanogenic effluent of soluble chromium was around 0.05 mg/L for both conditions. Single-phase experiments showed the soluble chromium concentration to be 0.083 mg/L. Soluble iron was measured at 0.409 mg/L in methanogenic effluent, which was reduced from an influent concentration of 4.42 mg/L, with deinking sludge.

6.2 Contributions to Knowledge

This research can provide valuable contributions for the paper recycling industry and renewable natural gas development. Solid waste created from deinking sludge is substantial and creates a major cost for the recycling industry. Successful co-digestion of this deinking sludge with wastewater from paper processing to reduce additional primary sludge and create renewable biomethane can make a large impact on industrial operations and costs. The results of this research present the final conditions which should be tested and monitored before scaling up to a pilot scale trial of the system. At the same time this research can also provide an industrial application and demonstration of the protective effects of granular sludge and additional mechanisms related to pH, sulfide and calcium hydroxide alkalinity that can eliminate risks of toxicity due to soluble heavy metals. Furthermore, this research provides evidence of the limits for solids degradation rate related to pH and VFA loading rate. The results of this study also provide information that can guide future attempts to bioconvert CO_2 to acetate and increase biomethane yield.

6.3 Recommendations for Future Work

Future progress would be carried out in the following steps.

1. Communication with industry to obtain better estimate of construction costs, operational costs, biogas scrubbing infrastructure and potentially new pipes for de-inking and gas heating.

2. Extended trials with optimal cost-performance conditions (minimum 10-15 RT each)
   2.1. Shortest retention times possible for 2 stages with highest methane yield.
      2.1.1. Aceto RT= 0.75 - 1 days Methano RT = 1.5 days or OLR ≤ 4kg/m^3-d (or best)

   2.2. Temperatures of 38/35°C
      Accurate rate of solids degradation in acetogenic phase and precision of any required wasting

2.3. Long-term effects of integrating de-inking sludge on acetogenic and methanogenic.

2.3.1. Monitor light and heavy metals (soluble and total)

2.3.2. Residual resins, hydrocarbons, if any

2.4. Ensure AD system effluent poses no risk to waste activated sludge bacteria

2.4.1. Monitor light and heavy metals (soluble and total)

2.4.2. Nutrient concentrations

2.4.3. Residual resins, hydrocarbons, if any

2.5. Biogas water column scrubbing experiments

2.5.1. Analysis of biogas emissions to determine H_2S concentration

2.5.2. Determine best column volume, water cycling and biogas flow through initial water scrubber to ensure optimal dissolution of H_2S and CO_2

2.5.3. Determine precipitation potential and if additional sulfide is required

3. Pilot scale construction and testing

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Appendices

Appendix A – HPLC Calibration Curves

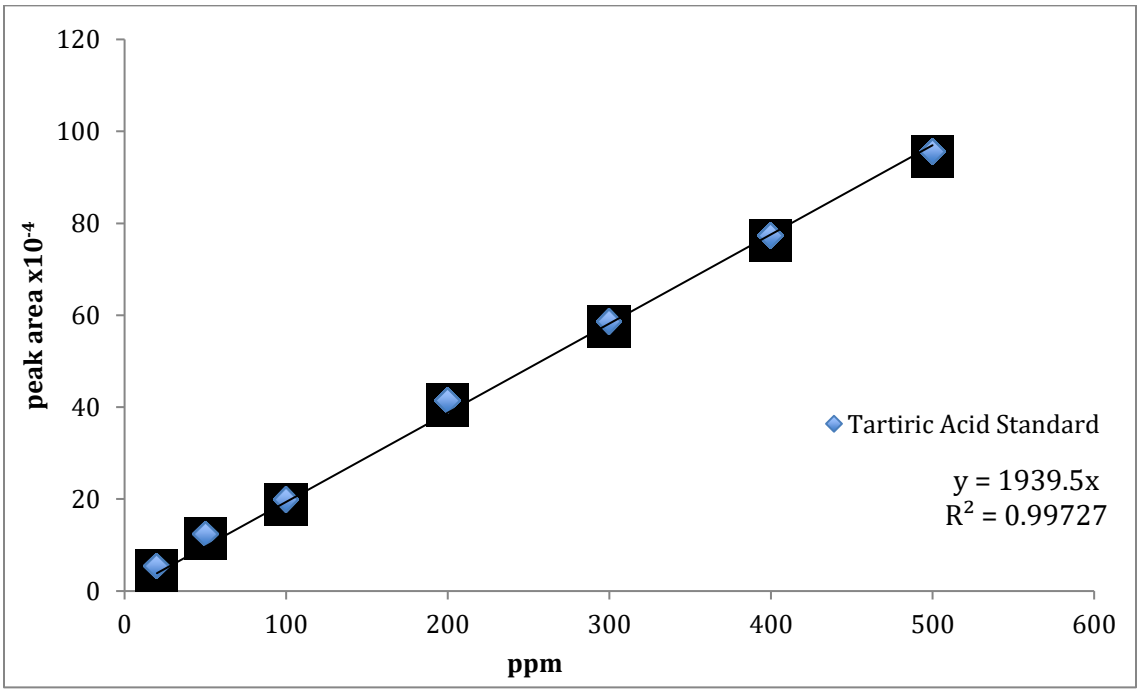


Figure A.1 Peak area vs. parts per million (ppm)for tartaric acid, calibration standard curve.

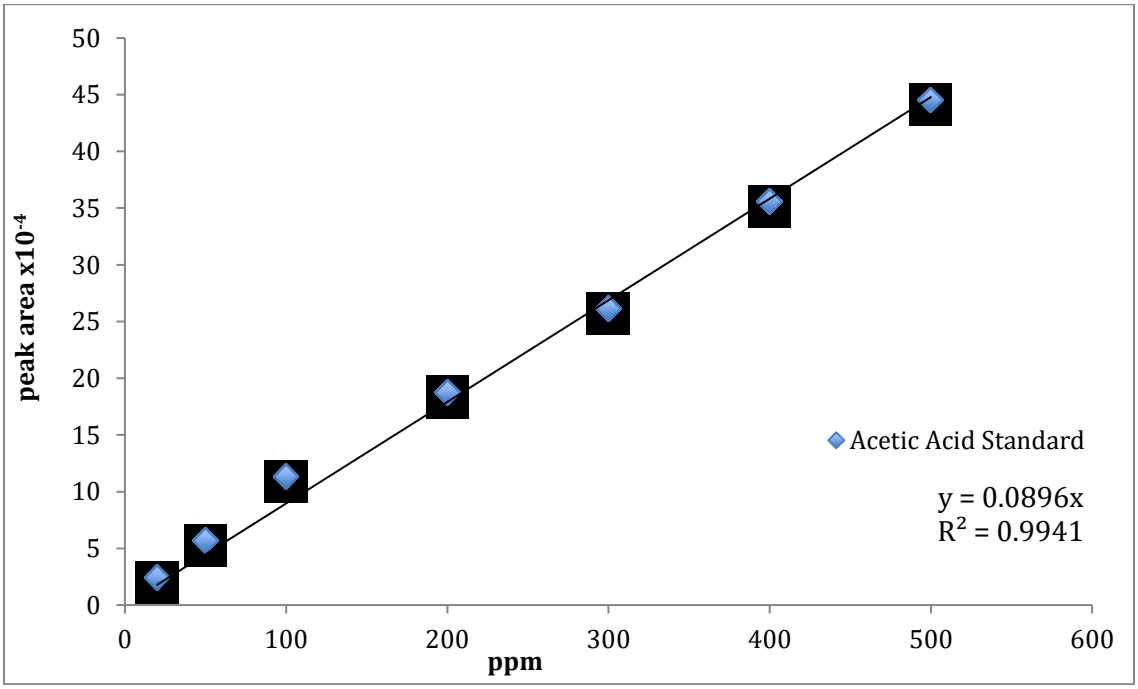


Figure A.2 Peak area va. parts per million (ppm) for acetic acid, calibration standard curve.

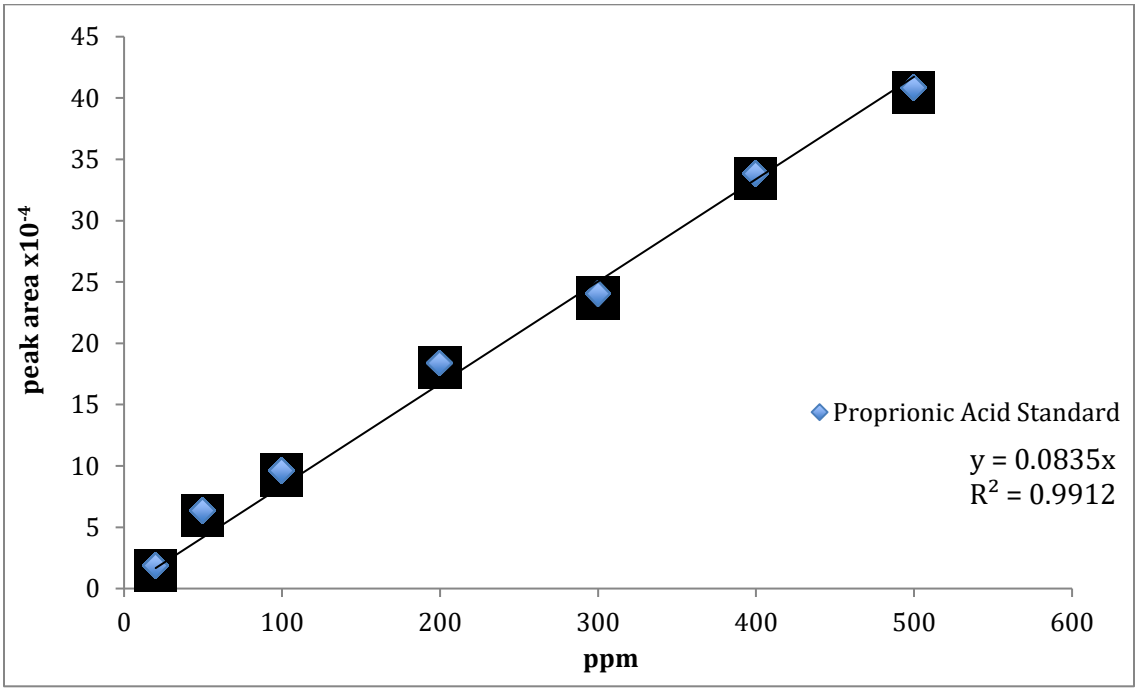


Figure A.3 Peak area vs. parts per million (ppm)for propionic acid, calibration standard curve.

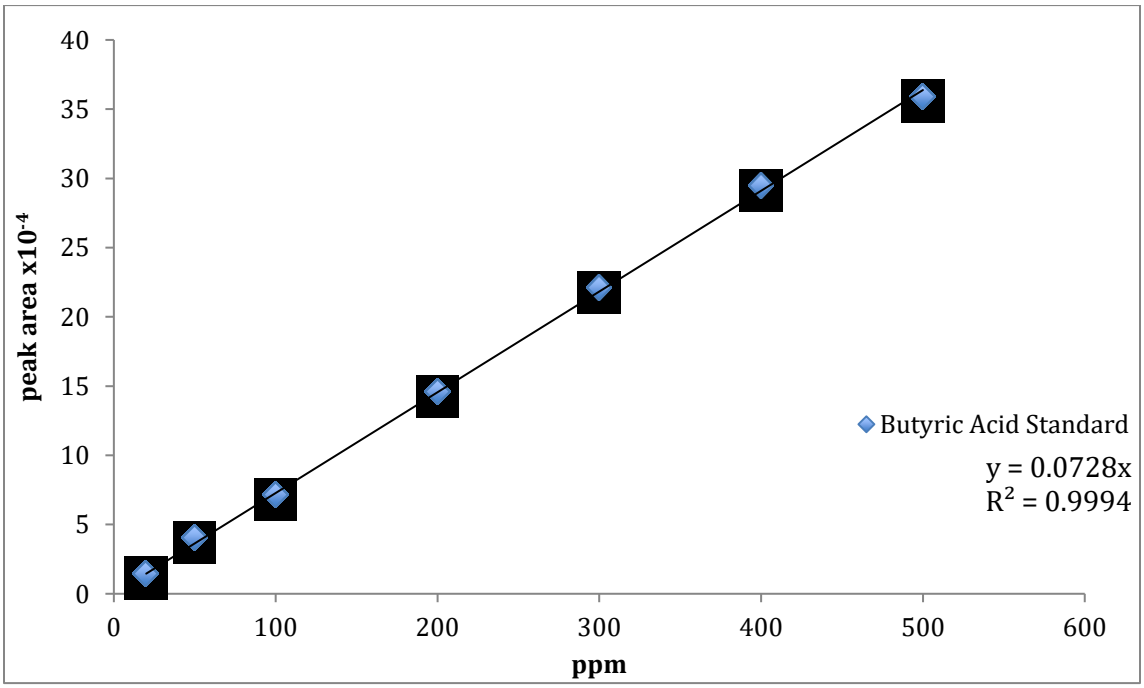


Figure A.4 Peak area vs. parts per million (ppm)for butyric acid, calibration standard curve.

Appendix B – Bench Top Continuous Reactor Details

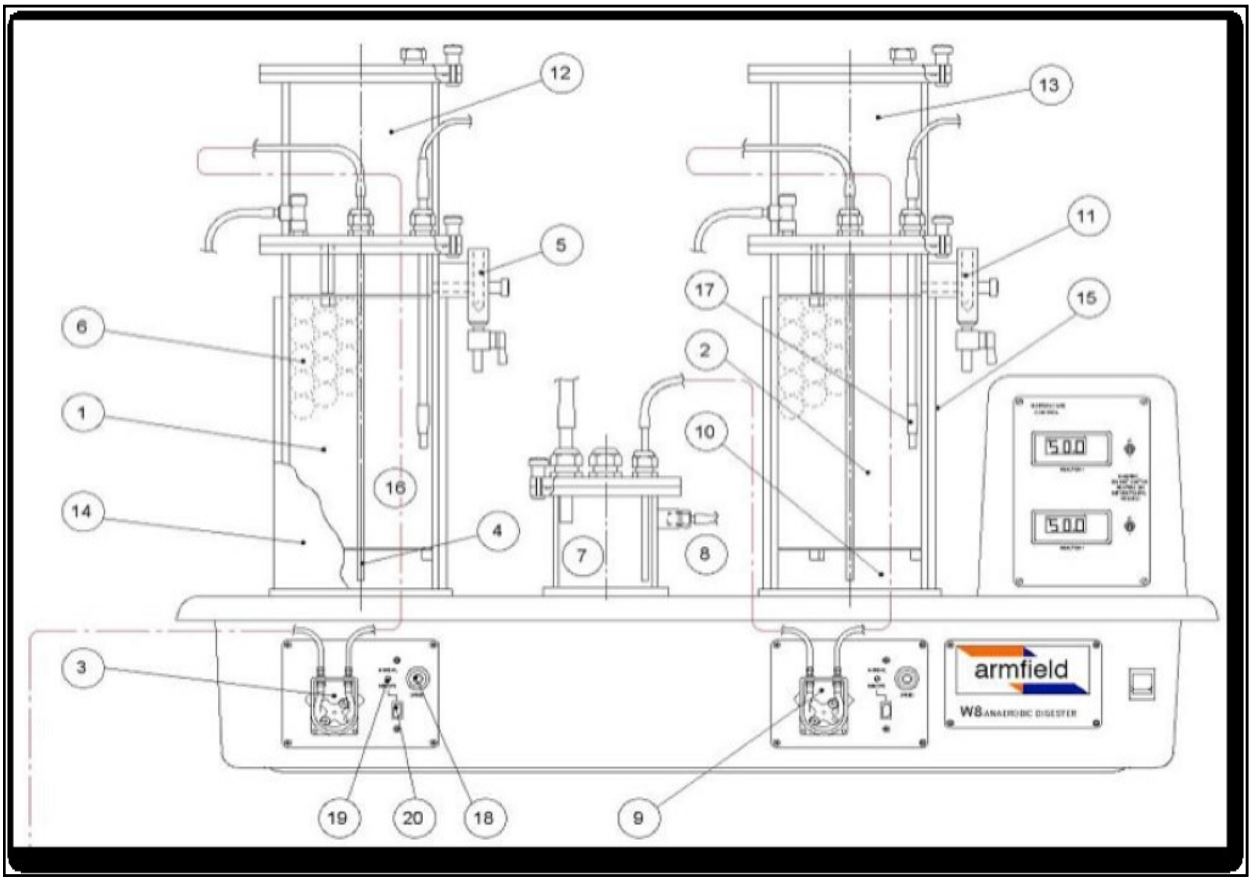


Figure B.1 Schematic diagram of Armfield W8 bench top continuous anaerobic digester

Each of the two reactors (1) and (2) has a total liquid volume of 5.0 litres. A packed section of 4.3 litres capacity in each reactor promotes good mixing of the feed with the biomass charge. The feed or influent is pumped by variable speed peristaltic pump (3) from a suitable container into reactor (1) through a central pipe (4) having an output near the base of the reactor. A 10-turn potentiometer (18) allows variation of pump motor speed to a maximum of 4.0 r.p.m. when the switch (19) is in the on position.

The liquid outlet from the reactor is through a gas seal (5) which ensures any gas produced cannot escape and also creates a liquid take-off which is lower than the operating level of the reactor to prevent any "scum" formed on the liquid surface being taken off.
The packing (6) used in the reactor is designed to promote as much movement as possible in the feed liquor as it slowly works its way to the outlet. The packing is necessary as the very low throughputs involved (typically 2.0 litres per day) would cause "channelling" and therefore poor contact with the bacteria in the reactor.

Liquor leaving the first reactor enters a buffer vessel (7). This vessel allows the first reactor

(1) to be operated at a higher throughput than the second reactor (2), the excess being taken off through overflow (8). Feed to the second reactor is by variable speed peristaltic pump (9) in an exactly similar way to reactor (1) through a central pipe (10) and leaving through a gas seal (11).

Any gas produced by the reaction taking place in reactor (1) or (2) is collected in 5 litre calibrated vessels (12) and (13) respectively. The gas collection is by water displacement through a constant head device which also creates a liquid seal between the gas tank and reactor.

Each reactor is heated to its operating temperature by electrical heating mats (14) and (15) that are secured using Velcro. A separate insulation mat covers the heating mat to reduce heat loss and prevent burns. The reactors must be operated with the insulation mats fitted. Temperature sensors (16) and (17) transmit the reactor contents temperature to 3-term controllers (A) and (F) which automatically adjust the electrical power to the mats to maintain the desired operating temperature at a constant level. Maximum operating temperature of both reactors is limited to 55°C but normal operation occurs typically at 37°C. The temperature sensors (16) and (17) located in reactors (1) and (2) respectively are connected to the temperature controllers in the console via BNC sockets (21) and (22) respectively. On the base of the plinth, a mains power input socket (24) is used for connecting the unit to the power supply using the mains cable provided. Output socket (23) is an extra mains power source used for driving external ancillary equipment up to 1 amp maximum such as additional instrumentation. (W8 Instruction Manual)

Appendix C - Standard Curves for Gas Chromatographic Method

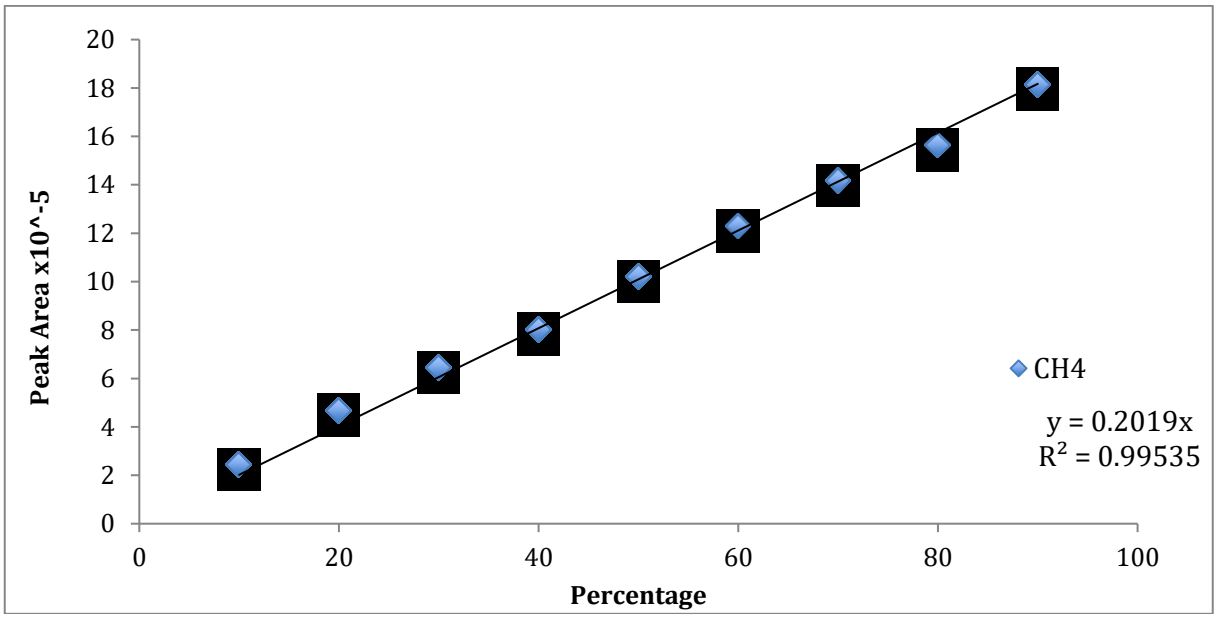


Figure C-1 Standard curves for gas chromatography peak calculation created by using pure methane (CH_4) gas.

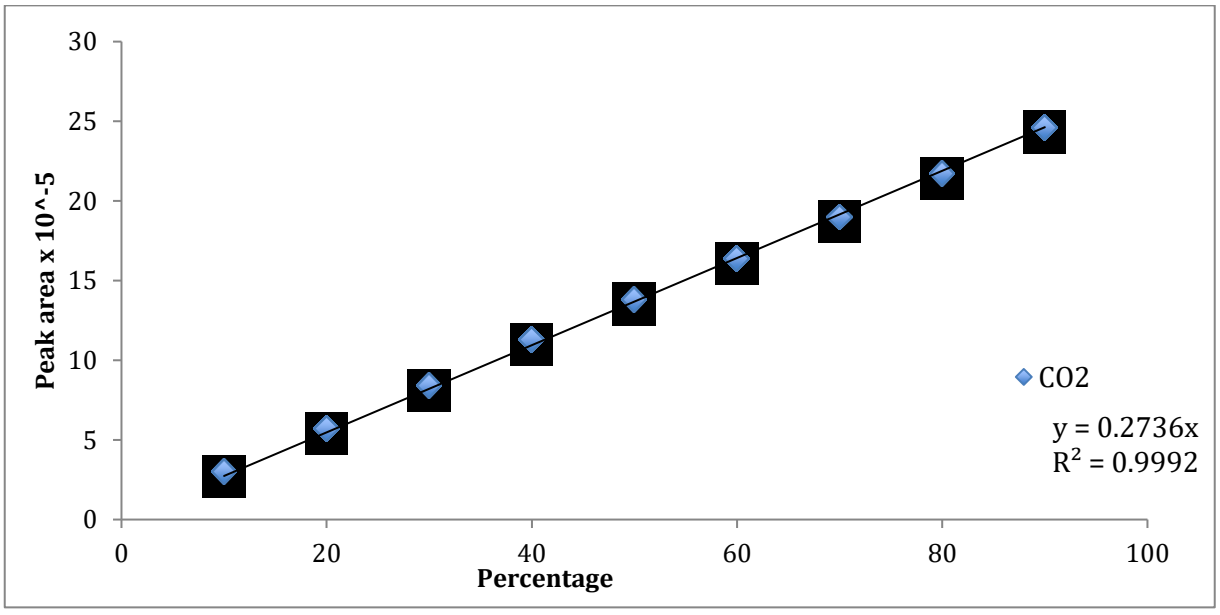


Figure C-2 Standard curves for gas chromatography peak calculation created by using pure carbon dioxide (CO_2) gas.

Appendix D – Carbon Dioxide Bioconversion Calculation

Example Calculation CO_2 Bioconversion [0.56 VFA_1.0 COD g/l condition]

CO_2 condition

Initial Influent		Acetogenic Reactor		Methanogenic Reactor	
		Gas Released		Gas Released	
		CO2	150	CO2	199
Dissolved		CH4	29	CH4	185
Titration	816	Dissolved		Dissolved	
Theoretical	2810	Titration	630	Titration	97
				Theoretical	
VFA	1317	VFA	1089	VFA	0

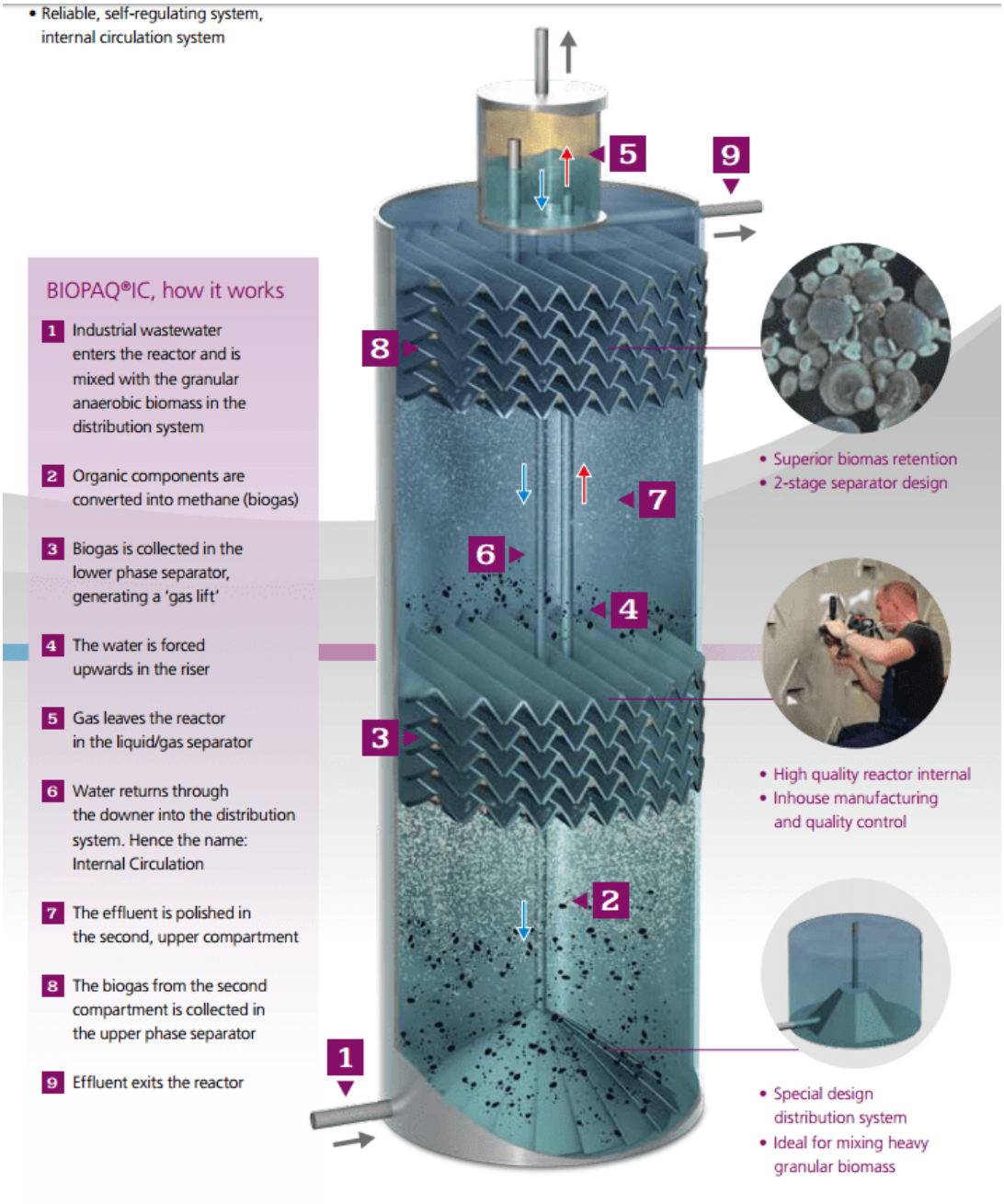
Control condition

Initial Influent		Acetogenic Reactor		Methanogenic Reactor	
		Gas Released		Gas Released	
		CO2	30	CO2	178
Dissolved		CH4	12	CH4	176
Titration	242	Dissolved		Dissolved	
Theoretical	1588	Titration	458	Titration	86
VFA	1224			Theoretical	79
		VFA	1048	VFA	0

Net value

Initial Influent		Acetogenic Reactor		Methanogenic Reactor	
Dissolved					
Titration	574	Gas Released		Gas Released	
Theoretical	1222	CO2	120	CO2	21
		CH4	17	CH4	9
	Total	Dissolved		Dissolved	
		Titration	171	Titration	11
				Theoretical	63
		VFA	42	VFA	0
		CO2 eqv	83		

Appendix E - Efficient UASB Design



Water Technologies Canada , 2018

Appendix F - Current Operational Costs

Hydro Energy Demand (rate of 0.0426 $/kWh)

Operation	m^3/d	kWh/m^3	kWh Day	$/day
Primary decanter				
Flottation	12500	0.29	3600	
Pumps and mixing		0.00	0	
Bioselecter			0	
(fluid pump, aerator, blower) Activated sludge	6500	1.59	10332.8	
Secondary decanter		0.00	0	
Press and dehydration wastewater and primary and secondary sludge	515	1.65	852	
Press and dehydration de-inking sludge	165	6.18	1020	
Biogas infrastructure	0			
Subtotal			15804.8	673.28

Natural Gas Energy Demand		kWh Day	$/day
Facility building heating		2160	71.43
Total Energy Costs/d			744.71

		Operation	ton/d	$/$m^3$ or ton	$ Day
100 mg/L BOD:4.5:0.75		**Nutrients**		0.11	660
99 mg/L	86%	urea		0.09	
16.5 mg/L	14%	PO4		0.02	

assume Q = 6000 m^3 after solids removal

	Flocculants	1812	0.08	143

Sludge wasting	Operation	ton/d	$/humid ton (30-45%)	$/Day
Primary and de-inking	**Sludge disposal costs**	123.4	25.00	3085
Production factor sludge/BOD: 0.45	**WAS disposal**	1.14	25.00	28.5
Total additional costs/d				3916.5

Appendix G - Proposed Operational Cost

Example Calculation (2-PAD system with de-inking sludge)

Operation	m^3/d	kWh/m^3	kWh Day	$/day
Hydro Energy Demand (rate of 0.0426 $/kWh)				
Primary decanter				
Flotation	0	0	0	
Pumps, mixing and monitoring	10500	0.29	3045	~~129.72~~
Bioselecter			0	
(fluid pump, aerator, blower) Activated sludge	6500	1.59	7235	
Secondary decanter		0.00	0	
Press and dehydration wastewater and primary and secondary sludge	4.59	1.65	7.57	
Press and dehydration de-inking sludge	0	6.18	0	
Biogas infrastructure	0			780[*1]
Subtotal			10 287	780 + 438

*1) 0.12$/$m^3$ inflation adjusted (18)

Natural Gas Energy Demand		kWh Day	$/day
Facility building heating		2160	71.43
Total Energy		12 447	1 290

		Operation	ton/d	$/$m^3$ or ton	$ Day
100 mg/L BOD:4.5:0.75		**Nutrients**		0.17	1105.00
99 mg/L	77%	urea		0.13	
16.5 mg/L	23%	PO4		0.04	
		Flocculants	795	0.08	63.60
		Biogas filter materials			14.30

Sludge wasting	Operation	ton/d	$/humid ton (30-45%)	$/Day
De-inking	**Sludge disposal costs**	0	25.00	0
Production factor sludge/BOD: 0.45	**WAS and Acetogenic disposal**	1.85	25.00	46.13
Total additional costs/d				1229$

Appendix H - Proposed Cost Calculation Assumptions

Ideal retention time interpolation from experiments
1 day acetogenic
1.5 days methanogenic (or 4.0 days for 2-PAD+ system)

Energy

Biogas		
Specific energy for CH_4	55	MJ/kg
Ideal gas law	35	MJ/m^3

Energy yield = (COD*Flow*Methane yield) *specific energy			
		GJ/d	GJ/yr (350 d/yr)
Average COD input (kg/m^3)	4.9		
Flow (m^3/day)	6500		
Methane yield (m^3 CH_4/kg COD)			
	0.14	156.065	54 623
	0.16	178.360	62 426
	0.21	234.098	81 934

Conversion Efficiency
Gas engine 35%
Boiler Heater 99%

Natural gas heating consumption 2160kWh/d

Operating costs? (0.12$/$m^3$) Richards 1996 inflation adjusted

Wastewater
COD = (0.267)BOD
Wastewater 1 : 0.025 de-inking sludge (v/v)
COD = (0.175)BOD

WAS sludge reduction for flocculent and wasting

Food/Microbe ratio (F/M) 0.25
Sludge production factor **0.45: BOD** (metric ton:metric ton)

	Current	Proposed	
BOD	0.375	0.125	kg/m^3
MLVSS of aerobic sludge	1.4	0.5	kg/m^3
Density	460	230	kg/m^3
WAS disposal	2.48	1.59	m^3/d
	1.10	0.37	metric ton/m^3
	1.14	0.48	metric ton wasted/d

*confirm if density would remain same with longer RT or if would reduce

Flocculent

WAS Q	1812	795	**m^3/d**
	0.08	0.08	**$/m^3**
	143	62.73	**$ Day**

*assume 0.2% solids from reduction in MLVSS

De-inking Q	165	0	**m^3/d**
	0.08	0.08	**$/m^3**
	13.20	0.00	**$ Day**

*confirm de-inking sludge quantity and cost

Aeration
Assume O_2 for aeration required is 1/2 of original, since bacteria are 1/3
(0.5 : 1.5) 5166.5 kWh

Nutrients adjustment*

	Influent WW	Current WAS treatment system	Proposed AD system		De-inking Sludge at Q ratio 0.025 v/v	
				Requires additional		Requires additional
COD (kg/m³)	6.70	2.20	6.70			
BOD (kg/m³)	2.54	0.60	2.54			
TN (kg/m³)	0.015	0.027	0.067	0.052	0.018	0.034
PO4 (kg/m³)	0.001	0.005	0.013	0.012	0.002	0.010
Urea		86%		83%		77%
PO_4^{3-}		14%		17%		23%
$/m³		0.11		0.28		0.17

For WAS (BOD:N:P) (100:4.5:0.75)

For Anaerobic Digestion (COD:N:P) (500:5:1)

*Bacterial Requirement

Increased cost estimate by same proportion as quantity increased
Potential problem for WAS bacteria: effluent from methanogenic reactor will have nutrients in the proportion 125:80:16.

Acetogenic Reactor – Solids

Wastewater + 0.025 (v/v) de-Inking sludge

	kg/m³	kg/d	m³/d*1	tons/d	3 days	7 days
					m³ required *3	
TSS	2.1	14700	42		126	294
Fixed solids*2	0.15	1050	3			
Wasting from Acetogenic at 30% humidity				1.4		

*1 Volume based on WW+deink density estimated at 350 kg/m³
*2 Residual solids that are not degradable by digestion assumed to be equal to fixed solids.
*3 Volume required for solids retention in reactor