ENHANCED ANAEROBIC DIGESTION OF MUNICIPAL WASTEWATER SLUDGE

ENHANCED ANAEROBIC DIGESTION OF MUNICIPAL WASTEWATER SLUDGE USING MICROBIAL ELECTROLYSIS CELLS

By:

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Abstract

In municipal wastewater treatment, anaerobic digestion is the slowest process requiring at least 15 day solids retention time (SRT). Treating only a small fraction of the total wastewater stream, anaerobic digesters require large reactor volumes and consistent heating (40°C). Thus, there is a growing need to investigate techniques to improve digestion efficiency. The long SRT requirement is a result of the time required for biological reactions such as hydrolysis and acetoclastic methanogenesis. There are numerous pretreatment methods which have so far been developed to particularly enhance hydrolysis. These pretreatment methods include thermalization, mechanical treatments, and chemical treatments. These methods aim to increase the degradability of the influent waste sludge which in turn will increase the efficiency of the digestion process. The goal of the research presented in this thesis is to enhance another limiting biological reaction: acetoclastic methanogenesis. Microbial electrolysis cell (MEC) technology was integrated into lab-scale anaerobic digesters in order to accelerate biosolids destruction under various SRT and temperature conditions. Various mathematical simulations were conducted using a developed steady-state ADM1 (Anaerobic Digestion Model No.1) model to further evaluate the performance of the digesters. The results of the research indicate that the proposed method is effective at shortened SRTs (e.g., 6 days) and can enhance the stability of anaerobic digestion when exposed to variations in temperature and influent composition.

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Nomenclature

CE	Coulombic efficiency (-)
COD	chemical oxygen demand (mg-COD/L)
ΔG°	Gibbs free energy (kJ/mol)
ΔH_{CH4}	heat of combustion of CH_4 (890.8 kJ/mol)
EAD	electrically-assisted digester
E_{ap}	applied electric voltage (V)
F	Faraday constant (96,485 C/mol)
GC	gas chromatography
I	electric current (A)
LCFA	long chain fatty acid
MEC	microbial electrolysis cell
MFC	microbial fuel cell
n_{CH4}	amount of CH_4 produced (mol)
R	Gas constant (8.314 J/mol/K)
r_E	energy recovery from the EAD (-)
SRT	solids retention time (days)
TSS	total suspended solids (mg-TSS/L)
V	volume of sludge (L)
VSS	volatile suspended solids (mg-VSS/L)
W_E	electric energy consumed to drive the MEC reactions (J)
W_{CH4}	energy recovered as CH_4 from the EAD (J)

Declaration of Academic Achievement

This dissertation consists of previously prepared material that has been submitted for publication in peer-reviewed scientific journals. The author of this dissertation is the primary author on each of these articles. As primary author, contributions included: experimental design, literature review, collections and analysis of data, development of mathematical models, and manuscript preparation. The thesis supervisor is the second author on each prepared material. He offered useful suggestions and his expertise during each phase of the research and manuscript preparation. Chapter 3 has been submitted to the *Journal of Environmental Informatics* and is currently being reviewed. Chapter 4 has been submitted to *Water Research*.

1. Introduction

1.1 Anaerobic digestion

Anaerobic digestion is an integral part of today's wastewater management and is used to treat waste streams from municipal, industrial and agricultural sectors. In municipal anaerobic digestion, the influent is the sludge produced from wastewater treatment facilities, including primary sludge and waste activated sludge (WAS). Anaerobic digestion thus serves two main purposes: first is to further break down these biosolids and the second is to destroy harmful pathogens (Grady et al., 2011). The destruction of biosolids is achieved through a series of biological reactions. Many of these reactions have relatively long time requirements, and the microbial kinetics favor mesophilic temperatures (35 – 40°C) over psychrophilic temperatures (<25°C). Therefore, in order to satisfy these requirements, anaerobic digesters must be operated with long solids retention times (SRTs) and heating systems. Despite treating a small fraction of the total wastewater stream (1-2%), large reactor volumes (~8000 m^3) are required to achieve those long SRTs (>15 days). Thus, there is a growing demand to develop efficient digestion systems which can alleviate high construction and operation costs of conventional methods.

1.2 Microbial electrolysis cells

A microbial electrolysis cell (MEC) is a bioelectrochemical system in which a small voltage (>0.3 V) is applied to stimulate a reaction (Liu et al., 2005; Logan et al., 2008). In an MEC, exoelectrogenic bacteria oxidize acetate at the bioanode and hydrogen

gas is subsequently produced at the cathode. Both hydrogen and acetate are key components in anaerobic digestion; thus, MECs have a potential to create synergistic effects when they are integrated into anaerobic digestion. For example, in conventional municipal digestion, acetate removal is slow and thus limits the overall biosolids destruction process. This rate-limiting role of acetate removal can be alleviated by the bioanode reaction in MECs.

1.3 Research objectives

The foremost research goal of the work presented in this thesis is to develop new digestion methods that mitigate the major weaknesses of conventional anaerobic digestion (i.e., slow acetate removal and heating requirement). The proposed digestion method integrates MEC technology with conventional anaerobic digestion in order to accelerate acetate removal. For the first part of this study (Chapter 3), an electrically-assisted digester (EAD) was equipped with MEC components alongside a conventional digester (control digester). Both digesters were operated at mesophilic temperatures under various SRTs and were fed with the combined sludge (mixture of primary and secondary sludge) collected from a local waste water treatment facility. The research objectives of the first part were to:

1) Demonstrate and prove the EAD concept using lab-scale digesters;
2) Evaluate the performance of the digesters by comparing their volatile suspended solids (VSS) and chemical oxygen demand (COD) removal;

3) Determine under what conditions the EAD effectively enhances the performance compared to a conventional digester; and
4) Determine the energy requirement since the EAD requires an external energy input for enhanced digestion.

The second part of this thesis (Chapter 4) was designed to examine the performance of a new lab-scale EAD at a psychrophilic temperature (22°C). A wide range of organic loading rates with secondary waste activated sludge (WAS) was also examined in the experiment. This study aimed to:

1) Examine whether the EAD was effective at a low temperature condition;
2) Examine the EAD's capacity to digest secondary waste activated sludge (WAS) as it is known to more difficult to digest compared to the primary sludge in wastewater treatment; and
3) Further investigate the effect MEC reactions have on organic acid concentration (i.e. acetate, propionate, butyrate and valerate).

In addition to the listed objectives, a steady-state mathematical model was developed to provide an in-depth understanding on individual microbial growth and biological reactions in the EAD.

1.4 Layout of thesis

Chapter 2 provides an extensive literature review of past studies on anaerobic digestion and microbial electrolysis cells. It also provides a description of the biological

processes in anaerobic digestion. Chapter 3 is dedicated to the first part of this research on demonstrating the proof-of-concept results on EAD applications at mesophilic conditions. Chapter 4 contains the findings from the second part of the research with further examination of the EAD at a psychrophilic temperature condition.

2. Literature Review

2.1 Anaerobic digestion

2.1.1 Introduction

Anaerobic digestion is a process which further treats wastewater and wastewater sludge. Its primary goal is to stabilize particulate organic matter by reducing volatile suspended solids (VSS) and chemical oxygen demand (COD) content (Grady et al., 2011). Anaerobic digestion has other additional benefits such as pathogenic deactivation and energy production in the form of methane gas (Metcalf & Eddy et al., 2004). Two vital design parameters for anaerobic digestion are temperature and solids retention time (SRT). There are three temperature ranges in which digesters are operated under: psychrophilic (<25°C), mesophilic (25 – 45°C) and thermophilic (45 – 65°C) (Connaughton et al., 2006). SRT refers to the average amount of time, usually measured in days, a solid particle would reside in the digester. Typically a minimum SRT of 15 days is required for conventional mesophilic digesters, while much longer SRTs are required for lower temperatures (Metcalf & Eddy et al, 2004). The rate of microbial growth in anaerobic digestion is temperature-dependent and as such the kinetics of their biological processes is affected by the operational temperature.

The COD removal and destruction of biosolids is achieved through a series of biological reactions. Hydrolysis is the first step of these reactions in which particulate matter (e.g., carbohydrates, proteins and lipids) are converted to soluble organics (e.g., sugars, amino acids, and long-chain fatty acids). Carbohydrates hydrolyze to

monosaccharides, proteins to amino acids and lipids to monosaccharides (~3 %) and long-chain fatty acids (~97 %) (Batstone et al., 2002). A precursor to hydrolysis is the disintegration of microbial cells into organic particles and a further breakdown of those particles into carbohydrates, proteins and lipids (Grady et al., 2011). This pre-hydrolysis step also generates insoluble inert organic matter. The hydrolysis reactions are usually catalyzed by extracellular enzymes such as cellulases, amylases, and proteases produced by acidogenic bacteria (Grady et al., 2011). For anaerobic digesters in municipal wastewater treatment facilities, the influent stream originates from primary sludge and the waste biomass from activated sludge processes and thus consists primarily of particulate matter. Proper hydrolysis of such particulate matter is thus essential because the following biological reactions require soluble organic substrates. Under mesophilic conditions, hydrolysis of carbohydrates and proteins is relatively quick requiring 1 – 3 days to create soluble sugars and amino acids; the decomposition of lipids is relatively slow, requiring 6 – 8 days (Grady et al., 2011). Therefore, if digester influent contains high concentrations of complex lipids, that particular hydrolysis step becomes a potential rate-limiting reaction, requiring a proper pretreatment of the influent sludge (Izumi et al., 2010; Ma et al., 2011; Valo et al., 2004).

Acidogensis is the next group of biological reactions containing both fermentation and anaerobic oxidation (also known as beta-oxidation). Fermentation is generally a very rapid reaction and requires only ~1 day to decompose sugars and amino acids to organic fatty acids and hydrogen gas. The primary products of fermentation are acetic acid,

propionic acid and butyric acid; the production of hydrogen gas via fermentation is generally small (Grady et al., 2011). The majority of the hydrogen gas production stems from anaerobic oxidation (beta-oxidation) which oxidizes long-chain and other organic fatty acids (e.g., propionic acid, butyric acid and valeric acid). The oxidation of long-chain fatty acids (LCFAs) follows the cyclical beta-oxidation process where acetate is released per cycle and water is reduced to hydrogen gas. This process requires about 4 days or more until the beta-oxidizing bacteria grow and attain sufficient population depending on the substrate (Grady et al., 2011). The beta-oxidizing bacteria responsible for the reaction are also known as syntrophic bacteria. The free energy ($\Delta G°$) of the anaerobic oxidation reaction is usually positive making it non-spontaneous; however, methanogens lower the acetate and hydrogen gas concentration. The thermodynamics of beta-oxidation is sensitively dependent on the amount of hydrogen gas. The partial pressure of hydrogen gas needs to be low enough for the beta-oxidation reaction to proceed, and thus hydrogen utilizing microorganisms are found in close proximity to beta-oxidizing bacteria. Acetate utilizing microorganisms are also reliant on acidogenesis reactions as they produce the carbon sources required for acetoclastic methanogenesis (Grady et al., 2011). This relationship between methanogens and beta-oxidizing bacteria is called obligate syntrophy.

The final step in anaerobic digestion is methanogenesis which is carried out by hydrogenotrophic and acetoclastic methanogens which belong to the Archaea domain. Hydrogenotrophic methanogens are classified into three orders: *Methanobacteriales,*

Methanococcales, and *Methanomicrobiales* (Grady et al., 2011). In hydrogenotrophic methanogenesis, hydrogen is used as the electron donor and carbon dioxide is used as the electron acceptor to produce methane (Metcalf & Eddy et al., 2004). Hydrogenotrophic methanogens are robust under various environmental conditions. They are also capable of quickly growing and utilizing hydrogen gas and producing methane in ~1 day (Grady et al., 2011). In contrast, acetoclastic methanogens grow and utilize acetate slowly. There are two known genera of acetoclastic methanogens: *Methanosarcina* species and *Methanosaeta* species. *Methanosaeta* species are the dominant methanogen found in anaerobic digesters requiring at least 12 days to begin utilizing acetate while the less-dominant *Methanosarcina* species requires 3 – 5 days (Grady et al., 2011). Although *Methanosarcina* species grow rapidly, their kinetic parameters lead them to be less dominant in anaerobic digesters. *Methanosarcina* species are considered to be copiotrophs (favouring high concentrations of substrate) whereas *Methanosaeta* species are oligiotrophs (favouring low concentrations of substrate). When anaerobic digesters are properly operating under long SRT conditions, the concentration of acetate is low which favours *Methanosaeta* species over *Methanosarcina* species. Approximately 70% of methane is produced via acetoclastic methanogenesis and the slow-growing *Methanosaeta* species which makes it a dominant rate-limiting reaction in municipal anaerobic digestion.

Conventional anaerobic digestion is effective at reducing biosolids content; however, there are certain drawbacks to achieve adequate effluent quality. Firstly, large

bioreactors (~8000 m^3) are required despite only treating a small fraction (~1–2% volume basis) of municipal wastewater. This large volume requirement is necessary to provide sufficiently long SRT conditions for the various rate-limiting reactions (e.g., acetoclastic methanogenesis). As a result, anaerobic digesters can have high construction cost. Secondly, the temperature requirement to maintain mesophilic conditions results in substantial energy consumption and expensive heating systems. Many domestic wastewaters are discharged at low ambient temperatures and are also low strength (Gatze et al., 2001). Thus, reducing the large volume and high temperature requirements are goals of many researchers who aim to improve the digestion of municipal wastewater streams.

2.1.2 Pretreatment methods to enhance anaerobic digestion of municipal solid waste

There is a certain demand to increase the efficiency of conventional anaerobic digestion of municipal wastewater sludge. One particular area of interest is to accelerate rate-limiting reactions (e.g. hydrolysis and acetoclastic methanogenesis). Various pretreatment methods have been developed to particularly enhance hydrolysis. Currently there are wide arrays of pretreatment options that have been implemented into full-scale anaerobic digesters including (Carrere et al., 2010):

- thermalization
- mechanical treatments (ultrasonic, lysis centrifuge, liquid shear, and grinding)
- chemical treatment (oxidation and alkali treatments)

The primary objective of these pretreatment methods is to increase degradability of the sludge which in turn would result in a higher energy recovery. Thermal techniques are typically energy intensive compared to other methods but are the most effective at enhancing VSS destruction (Carrere et al., 2010). Mechanical methods are generally less effective than thermal techniques but are less energy intensive. Oxidation (typically ozone and/or hydrogen peroxide) and alkali treatment (addition of NaOH, KOH or $Mg(OH)_2$) aim to enhance the amount of soluble organics as well as inactivate pathogens. Currently researchers are working on further improving these conventional methods or developing new techniques to enhance biosolids destruction and biogas production.

In a recent study done by Yu et al. (2014), electrochemical pretreatment was applied to further solubilize WAS. The primary objective of their work was to apply a novel pretreatment method to enhance the hydrolysis step of anaerobic digestion. Waste activated sludge was pretreated using a Ti/RuO_2 electrode pair (inducing water electrolysis) and a dosage of sodium hypochlorite (NaClO). The electrochemical pretreatment was successful at breaking up sludge flocs and destroying cell structures to create more soluble components. A pretreatment method similar to this is an electrical-alkali method where the same water electrolysis is paired with sodium hydroxide (NaOH). Zhen et al., (2014) investigated the electrical-alkali method and its ability to enhance hydrolysis and digestion of waste activated sludge. Their work also found positive results about the treatments ability to further enhance the lysis of cells and breakup of sludge flocs. Statistical analysis done by the researchers indicated that the electrical-alkali

pretreatment was able to increase the kinetics of the hydrolysis reaction. This study concluded that the VSS and TSS removal efficiencies were enhanced at applied voltages of 15 and 20 V but was not effective at lower applied voltages (5 and 10 V). Despite the increased solubilization, the electrical-alkali pretreatment used did not have a significant impact on the overall methane yield.

Another pretreatment method is to introduce zero valent iron (ZVI) to enhance the efficiency of hydrolysis. Feng et al., (2014) applied various doses of ZVI to waste activated sludge and found that ZVI effectively enhanced the decomposition of proteins and cellulose at doses between 1 – 4 g ZIV/L. The study concluded that the addition of ZVI increased the activities of enzymes such as protease, cellulase, acetate kinase (AK), phosphotransacetylase (PTA), butyrate kinase (BK), and phosphotransbutyrlase (PTB). A ZVI dosage of 4 g/L was most effective in their study while a dosage of 20 g/L did not further increase the activity of those enzymes; ranges between 4 and 20 g/L were not investigated. Their second key finding was that ZVI had potential to enhance the growth of hydrogenotrophic methanogens to further drive anaerobic processes such as methane production. This enhanced growth finding is unique because it shows that the ZVI is able to enhance two distinct groups of biological reactions in anaerobic digestion: hydrolysis and methanogenesis.

Pretreatment incorporating both sonication and thermalization has also been investigated (Carrere et al., 2010). Sahinkaya and Sevimli (2013) looked at combining

both pretreatments to enhance sludge disintegration efficiency. In this study, the sonication frequency was held constant at 20 kHz while the sludge was thermalized at temperatures ranging from 60 – 100°C. The authors found that for waste activated sludge, the sono-thermal pretreatment had synergistic effects with increased disintegration efficiencies compared to pretreatment with either sonication or thermalization. The optimal operating conditions were found to be a combination of 1-min sonication at 1.0 W/mL and a thermalization at 80°C for 1 hour. The methane production only increased at most by 13.6% however, which did not offset the high energy input required.

2.1.3 Comments regarding direction of current research

There are a number of published works that address the rate-limitedness of hydrolysis; however, that of methanogenesis has not been investigated mainly because there are practically no means to enhance or replace the reaction except for increasing temperature. Due to the slow-growing nature of *Methanosaeta* species, acetoclastic methanogenesis can be a dominant rate-limiting reaction in anaerobic digestion. Unlike the hydrolysis-enhancing pretreatment methods, implementation of new techniques to enhance a biological reaction such as methanogenesis can be difficult. Many of the pretreatment methods reviewed are performed on influent waste activated sludge before it enters the anaerobic digester. These methods do not directly interact with many of the microorganisms present and thus have low risks of compromising the stability of the digester. A technique which accelerates a particular reaction that occurs towards the end of the anaerobic digestion process (e.g., acetoclastic methanogenesis) should not compete

or interfere with other microorganisms present, creating another challenging problem. Such a potential interference is one of the main objectives of the research presented in this thesis. Thus in this thesis a new technique was introduced to mitigate the rate-limitedness of acetoclastic methanogensis without compromising the stability of the overall biological reactions in conventional anaerobic digesters.

2.2 Microbial electrolysis cell

2.2.1 Introduction

The microbial electrolysis cell (MEC) is an emerging technology that offers the ability to treat wastewater while simultaneously producing hydrogen gas (Liu et al., 2005; Rozendal et al., 2006). An MEC is an anaerobic bioelectrochemical cell that consists of a bioanode, cathode and an external applied voltage. At the bioanode, exoelectrogenic bacteria oxidize soluble organic matter (e.g., acetate) and transfer electrons to the anode as shown below (Logan et al., 2008):

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad (2\text{-}1)$$

At the cathode, hydrogen gas is produced via electrolytic water reduction (Logan et al., 2008):

$$2H^+ + 2e^- \rightarrow H_2 \quad (2\text{-}2)$$

In order to drive these coupled redox reactions, a small amount of electrical energy input is required (>0.3 V) (Liu et al., 2005; Rozendal et al., 2006; Logan et al., 2008). The

applied electric potential can vary based on the application of the MEC as long as it is below the threshold for oxygen evolution at the bioanode (1.23 V). MECs are generally inoculated with wastewater as it has a high concentration of bacteria (Logan et al., 2008). After an exoelectrogenic microbial community has been established on the bioanode, a wide variety of synthetic and real influents can be introduced for experimentation.

2.2.2 Studies using waste activated sludge in MECs and other bioelectrochemical systems

There are a number of previous studies that have focused on treating raw or digested wastewater in MECs and other similar bioelectrochemical systems. A microbial fuel cell (MFC) is similar to an MEC in that exoelectrogenic bacteria drive organic oxidation at the bioanode; a primary difference is that electrical energy is extracted in an MFC as opposed to being inputted. Rodrigo et al., (2007) produced electricity by feeding primary clarifier effluent to a set of MFCs. From this study, relevant conclusions were that the microbial community did not take long to develop and that the rate of energy generation was proportional to the influent COD. The first conclusion is significant because it shows that exoelectrogenic bacteria are naturally present in domestic wastewater so no additional start-up steps are required for bioelectrochemical system applications. The second conclusion demonstrates that high strength wastewaters (i.e. high COD), such as anaerobic digester influent, are suitable for bioelectrochemical applications.

In another study, waste activated sludge was fed to an MEC in order to evaluate hydrogen production as well as the MEC's ability to degrade various short-chain fatty acids (Liu et al., 2012). In this study, the waste activated sludge was pretreated using ultrasonic techniques. The substrate preference of exoelectrogenic bacteria was demonstrated with a wide array of substrates. The authors focused on analyzing the concentration change in acetate, propionate, n- and iso-butyrate as well as n- and iso-valerate which are commonly present in anaerobic digesters. The authors concluded that acetate and propionate were preferred substrates for exoelectrogens present in the MEC, followed by n-butyrate, iso-valerate and iso-butyrate. This conclusion demonstrates that exoelectrogens in an MEC can utilize the organics commonly found in wastewater sludge.

Lu et al., (2012) also fed waste activated sludge to a set of MECs. In this study, however, a focus was put on how alkaline pretreatment enhanced the hydrogen gas yield. The authors found that the rate of hydrogen production nearly doubled when the sludge influent was alkaline pretreated. This study once again demonstrated the ability of exoelectrogenic bacteria to utilize substrates found in waste activated sludge; however, their experimental results were dependent on chemical pretreatment.

Heidrich et al., (2014) operated a pilot-scale MEC (100 L) that was continuously fed with domestic wastewater. The MEC was operated year-round, providing insight on the effect of ambient influent temperature. In regards to temperature effects, the authors did not notice a significant impact of low temperature reducing MEC performance.

However the data was not conclusive as the authors were unable to determine if there was a statistical trend between performance of the MEC and changes in temperature with time. There is still a possibility that the high variance in data masked any temperature trend.

Another pilot-scale MEC (1000 L total volume) was continuously fed winery wastewater in order to determine whether current densities typically obtained in lab-scale experiments were obtainable from a scaled up version (Cuisick et al., 2011). A noticeable difference between lab-scale and pilot-scale MECs is the start-up time. This pilot-scale MEC required over 60 days for exoelectrogenic bacteria to sufficiently enrich the reactor. The authors increased the temperature (from 20 to 30°C) and volatile fatty acid (VFA) content after day 40 which appeared to accelerate the enrichment process. Once enriched, the pilot-scale MEC achieved 60% soluble COD removal and a maximum current density of 7.4 A/m^3. The maximum measured current density was 44% less than what was estimated to be possible and therefore finding new ways to increase current density is a challenge for pilot-scale MECs. Despite the slow enrichment and low current density, the authors were able to point out areas for improvement. One suggestion made was to operate the MEC at a temperature near 30°C during enrichment and then consider lowering it down to 20°C once steady current generation was established. Another design factor suggested to be improved upon was the cathode design as this was found to be a limiting factor to current generation in this pilot-scale MEC.

Carrera et al., (2013) continuously fed their lab-scale and pilot-scale MECs (50 mL to 10 L total volume) with synthetic and domestic wastewater. The smaller MECs (50 and 855 mL total volume) were a single chamber design whereas the 10 L MEC consisted of two 5 L chambers connected in series. The 10 L MEC was fed raw domestic wastewater and was operated under various hydraulic retention times (HRTs) ranged from 10 – 32 hours. Based on the measured results, the greatest COD removal was achieved at a 10 hour HRT. This study demonstrated that an MEC's performance is correlated to the organic loading rate.

The impact on organic loading rate and MEC design was further examined in another study by Carrera et al., (2013). In this study, tubular MECs (4 L total volume) were operated using low strength wastewater under various HRTs. This study established certain thresholds on organic loading rates which dictated when these particular tubular MECs could be more efficient (in terms of energy consumption) than certain aerobic treatment process. The results of this recent study need further investigation to determine whether those thresholds hold true for larger volumes or different designs.

So far, the pilot-scale studies discussed have simply increased the volume of their bioelectrochemical systems while maintaining the same configuration as typical lab-scale models. Jiang et al., (2011) conducted a unique study in which they operated a pilot-scale multi-anode/cathode microbial fuel cell (MAC MFC). Compared to typical MEC design which houses one anode per chamber, this design comprised of 12 anodes in a 20 L

reactor. This study once again demonstrated the relation between organic loading rate and performance. This study also showed that additional anodes can increase performance as long as there is a sufficient amount of substrate available.

2.2.3 Studies using MECs in anaerobic digestion projects

Since exoelectrogens are able to utilize organics found in wastewater sludge, researchers have begun to investigate combining anaerobic digestion and microbial electrolysis cell technology. In a recent study, Guo et al., (2013) digested sewage sludge in an MEC with applied voltages of 1.4 and 1.8 V; this voltage was found not to induce water electrolysis. The authors found that both hydrogen and methane productions were increased in a combined setup, by 1.7 – 5.2 fold and 11.4 – 14.6 fold respectively, compared to their control digester. The concentration of acetate, propionate and butyrate were also found to be lower in the combined digesters further confirming exoelectrogens ability to utilize these substrates effectively. Despite the increased biogas production, however, VSS removals were not significantly increased compared to the control digesters.

Bo et al., (2014) also coupled the MEC technology in an anaerobic digester using a stainless steel barrel-shaped reactor. In this setup, the stainless steel shell functioned as both the reactor body and the MEC cathode for hydrogen gas evolution. In this experiment, the authors were able to produce high-purity methane biogas (>98%) as well as increase the COD removal rate by three times. The high methane content was

theorized to be a result of an increase in hydrogenotrophic methanogen growth due to the increase in aqueous hydrogen production via the MEC cathode. Details regarding the influent characteristics were unknown which may have also attributed to the high methane content.

2.2.4 Comments regarding current research topics

Microbial electrolysis cells are an emerging technology that is versatile and capable of treating various wastewater sources and simultaneously producing hydrogen gas. MECs can be constructed with inexpensive materials such as carbon fiber brushes for anodes and stainless steel for cathodes. The electrical energy input can be offset by either the hydrogen (in an MEC system) or methane (in an anaerobic digester) produced, allowing the bioelectrochemical system to be energy neutral or an energy producer. However, many of the previous studies were performed with expensive pretreatment methods for efficient oxidation of substrates. Future research should continue pursuing potential applications of the MEC technology in practical wastewater treatment systems (i.e. anaerobic digestion) without expensive pretreatment.

2.3 Thesis objectives

Based on the previous studies discussed, the primary objective of the research presented in this thesis is to develop a unique and efficient anaerobic digestion system which integrates bioelectrochemical systems. Municipal anaerobic digestion can be costly despite only treating small fractions of total wastewater. Large reactor volumes can result

in high start-up costs and space limitations might result in a treatment facility being unable to perform digestion on-site; the transportation of sludge to other facilities adds additional costs. Thus, a main goal of this new digestion system is to reduce the reactor volumes required and therefore provide an option for smaller, yet effective anaerobic digesters. In order to reduce reactor volume without compromising efficiency of the digester the biosolids destruction process needs to be enhanced which means enhancing rate-limiting reactions. Many pretreatment methods enhancing hydrolysis have been extensively examined; however these methods can have high costs associated. Therefore, the research presented in this thesis aims to enhance other rate-limiting reactions such as acetoclastic methanogenesis and examine its effect on the digestion process. By utilizing unique MEC techniques it should be feasible to enhance other digestion reactions without the need for expensive pretreatments. The previously discussed studies have detailed the capacity of MEC technology to treat wastewater under various conditions and reactor designs. This thesis thus further examines the ability of MEC technology to integrate with conventional anaerobic digestion.

3. Lab-scale experiment and model study on enhanced digestion of wastewater using bioelectrochemical systems[1]

Abstract

Anaerobic digestion is the slowest process in municipal wastewater treatment, requiring at least 15 days of SRT (solids retention time). Here, we implemented microbial electrolysis cells (MECs) in anaerobic digesters to shorten the long SRT requirement. The MEC bioanode oxidizes acetic acid while the cathode produces H_2 gas. The electrode reactions can expedite acetic acid decomposition and thus enhance the rate of biosolids destruction because acetoclastic methanogenesis is known to be the rate-limiting step in conventional anaerobic digestion. A lab-scale electrically-assisted digester (EAD) with the MEC reactions was operated under a continuous fed-batch mode using raw wastewater sludge. Additionally, a steady-state model was developed by incorporating the MEC reaction in ADM1 (Anaerobic Digestion Model No.1 by International Water Association). In experiments, the EAD achieved 55 ± 1% VSS (volatile suspended solids) removal and 61 ± 2% COD (chemical oxygen demand) removal at a 6-day SRT while the control digester (built with the same electrode components but no MEC reactions induced) showed only 47 ± 5% VSS removal and 50 ± 4% COD removal at the same 6-day SRT. This result indicates that the SRT requirement can be substantially reduced by implementing the MEC reactions in mesophilic anaerobic digestion. Under a 14-day or 2-day SRT condition, however, the EAD did not show meaningful improvements on the COD and VSS removal compared to the control digester. Hydrogenotrophic methanogenesis was

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sufficiently rapid as H_2 gas was not detected in produced biogas. The mathematical simulation results demonstrated that the MEC reactions substantially reduce acetic acid concentration and thus supplement the slow acetoclastic methanogenesis reaction.

Keywords

Wastewater sludge digestion; mesophilic anaerobic digesters; bioelectrochemical systems; anaerobic digestion models; bioanode reaction models; exoelectrogenic bacteria; acetoclastic methanogenesis; energy recovery.

3.1 Introduction

Mesophilic anaerobic digestion is the slowest process in municipal wastewater treatment. Anaerobic digestion, treating only ~1% of total wastewater volume, requires significantly large reactors (~8000 m^3) to maintain a long retention time of 15 – 20 days (Metcalf & Eddy et al., 2004). As a result, construction and operation of anaerobic digesters are responsible for major expenses in wastewater treatment. The main objective of this study is to accelerate the rate of biosolids destruction so that the costs for anaerobic digestion can be reduced with smaller digester volumes and shorter solids retention times (SRT).

In mesophilic anaerobic digesters, destruction of biosolids is achieved through a series of biological reactions (Figure 3.1). Polymeric particulate organics (e.g., carbohydrates, proteins and lipids) are hydrolyzed into soluble organics (e.g., sugars,

amino acids and long-chain fatty acids). Hydrolysis of carbohydrates and proteins are generally quick (1 – 3 days) while hydrolytic decomposition of lipids is relatively slow, taking 6 – 8 days (Grady et al., 2011). Fermentation is usually fast, requiring only about 1 day for decomposition of sugars and amino acids into H_2 gas and volatile fatty acids (Grady et al., 2011). Beta-oxidation (anaerobic oxidation) needs about 4 days to start converting long-chain fatty acids into H_2 gas and acetic acid (Grady et al., 2011). The final step of biosolids destruction is driven by hydrogenotrophic and acetoclastic methanogens. Relatively rapid hydrogenotrophic methanogenesis starts oxidizing H_2 gas and producing CH_4 in about 1 day (Grady et al., 2011). However, acetoclastic methanogens grow slowly, requiring 3 – 5 days for *Methanosarcina* species and at least 12 days for *Methanosaeta* species to start utilizing acetic acid for CH_4 production (Grady et al., 2011). Approximately 70% of methanogenesis in anaerobic digestion is driven by acetoclastic methanogens (Grady et al., 2011), making acetoclastic methanogenesis the rate-limiting reaction for overall biosolids destruction. Also, the slowly growing *Methanosaeta* species are known to be more responsible than *Methanosarcina* species for the rate-limiting role of acetoclastic methanogenesis (Conklin et al., 2006). Hydrolysis can also be very slow when a large amount of lignocellulosic materials is present in sludge (Rittmann et al., 2001). However, acetoclastic methanogenesis is often considered to be the rate-determining reaction in domestic wastewater sludge digestion (Grady et al., 2011; Rittmann et al., 2001). Thus, we focused mainly on mitigating the rate-limiting effect of acetoclastic methanogenesis in mesophilic anaerobic digesters.

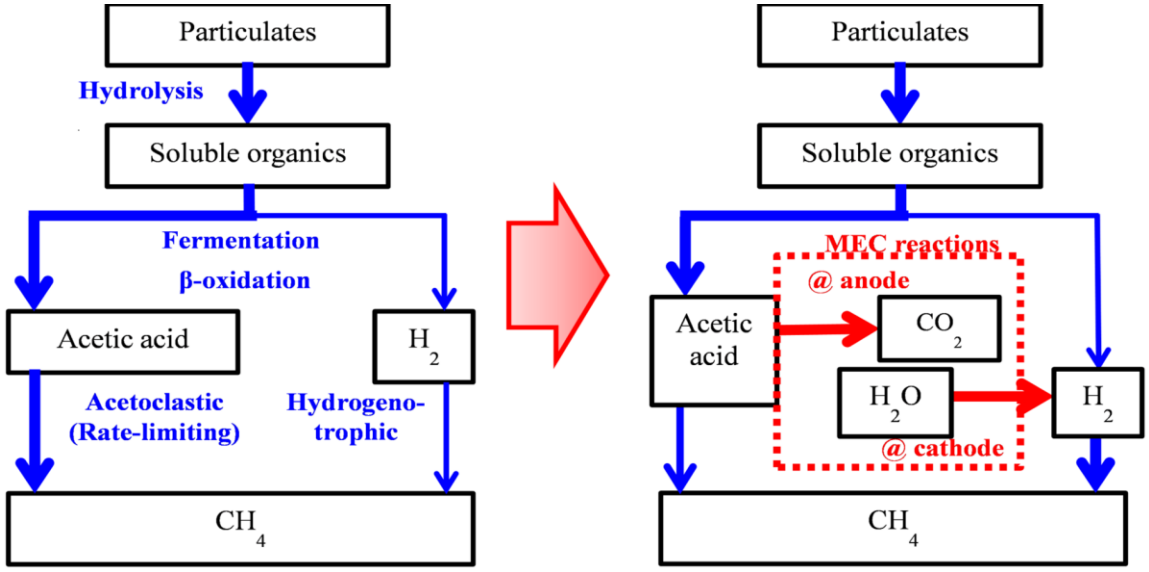

Figure 3.1: Changes in the reaction pathway for biosolids destruction by integrating MEC reactions into anaerobic digestion. (MEC: microbial electrolysis cell)

In this work, we aimed to expedite the decomposition of acetic acid by implementing microbial electrolysis cells (MECs) in mesophilic anaerobic digesters. At the MEC bioanode, exoelectrogenic bacteria oxidize organic fatty acids (including acetic acid) and the MEC cathode produces H_2 gas via electrolytic water reduction (Figure 3.1) (Liu et al., 2005; Rozendal et al., 2006; Logan et al., 2008). H_2 gas produced at the cathode will be rapidly utilized by hydrogenotrophic methanogens (Tice & Kim, 2014). As a result, the MEC reactions coupled with hydrogenotrophic methanogenesis can convert a certain fraction of acetic acid into CH_4 gas, creating an additional reaction pathway for the rate-limiting acetic acid decomposition step in anaerobic digesters (Figure 3.1). Therefore, the MEC electrode reactions implemented in anaerobic digestion can enhance the rate of biosolids destruction. In addition, the rate of the MEC reactions

can be monitored with electric current, allowing precise evaluation of their contribution to biosolids destruction.

The objective of this study is to demonstrate this new concept of integrating MEC technology with anaerobic digestion to expedite chemical oxygen demand (COD) and volatile suspended solids (VSS) removal. Eventually, we aimed to reduce the long SRT requirement of mesophilic anaerobic digesters and investigate how the shortened SRT conditions along with the MEC reactions affect other biological reactions, including acetoclastic and hydrogenotrophic methanogenesis. There are a number of previous studies where wastewater sludge or animal manure wastewater was treated in bioelectrochemical systems (Pham et al., 2006; Rodrigo et al., 2007; Liu et al., 2012; Lu et al., 2012; Guo et al., 2013; Ge et al., 2013; Tartakovsky et al., 2014; Tartakovsky et al., 2011). A recent study also showed enhanced CH_4 production and decomposition of individual organic acids by implementing an MEC in an anaerobic digester (Liu et al., 2013). Sasaki et al., (2010; 2011; 2013) provided H_2 gas by cathodic water electrolysis to enhance CH_4 production from various waste biosolids. Also, high purity CH_4 production (98.1%) was achieved by coupling MECs in anaerobic digesters (Bo et al., 2014). In addition to these synergistic effects demonstrated in the previous studies, we focused primarily on mitigating the rate-limiting role of acetoclastic methanogenesis in anaerobic digestion by introducing the additional acetic acid degradation pathway using MECs so that the long SRT requirement (15 days or longer) can be substantially shortened.

Another aspect of this study is to investigate the energy requirement since the MEC reactions are not spontaneously driven. The electric energy requirement of an MEC as an independent system is relatively small; thus, energy recovered as H_2 gas is usually greater than the applied electric energy (Cheng & Logan, 2007; Call & Logan, 2008; Hu et al., 2008). However, municipal wastewater sludge has relatively low ionic conductivity (~2 mS/cm) that can result in high resistive energy losses. On the other hand, biogas production (H_2, CH_4) is also driven by other biological reactions, such as fermentation, beta oxidation (anaerobic oxidation) and methanogenesis; therefore, the energy recovery with these reactions can be higher than that with only the MEC reactions. With these multiple factors influencing the energy consumption and recovery, we investigated whether the proposed electrically-assisted digesters can be operated as a net energy producer by comparing the energy requirement and energy production.

In our experimental system where anaerobic digestion is coupled with an MEC, biosolids destruction is achieved through a number of reactions, including hydrolysis, fermentation, beta-oxidation, acetoclastic methanogenesis, hydrogenotrophic methanogenesis, as well as electrolysis at the MEC cathode and oxidation at the MEC bioanode. Thus, it is practically impossible to monitor all of the individual reactions in experiments. Thus, we employed a numerical model to keep track of individual component concentrations and biological reactions in our experimental system. As International Water Association's Anaerobic Digestion Model No. 1 (ADM1) is widely used in anaerobic digestion model studies (Batstone et al., 2002), we built ADM1 and incorporated the electrode reactions in the model. Model simulation results were provided in this study to support our hypothesis that the MEC reactions partially supplement the rate-limiting

acetoclastic methanogenesis reaction in wastewater sludge destruction. The findings of this study will provide an improved method for wastewater sludge treatment using MEC technology and an approximation of the energy requirement for enhanced sludge treatment.

3.2 Material and Methods

3.2.1 Reactor construction

Two lab-scale anaerobic digester reactors, a control digester and an electrically-assisted digester (EAD), were constructed with MEC components in cylindrical polypropylene containers (total 250 mL with 240 mL of sludge volume and 10 mL of head volume) (Figure 3.2). Four carbon fiber brushes (2 cm diameter and 2.5 cm in length; Mill-Rose, OH) were pretreated in a muffle furnace at 450°C for 30 minutes (Wang et al., 2009) before they were located in each digester as bioanodes. Stainless steel mesh was used as the MEC cathode without any precious metal catalysts (total projected area of 150 cm^2, AISI 304, 100-mesh, McMaster-Carr, OH). The stainless steel mesh was rolled into a two-layer cylinder and placed around the interior wall of the reactor (Figure 3.2B). Plastic mesh (~1 mm thick) was placed between the anode brush and cathode to prevent electric short-circuiting. A nylon barbed tube fitting (McMaster-Carr, OH) was glued to the top of the reactor and connected to a plastic tube to collect biogas as previously demonstrated (Call & Logan, 2008). Another barbed fitting was placed near the bottom of the reactor to feed the reactor and draw digested sludge.

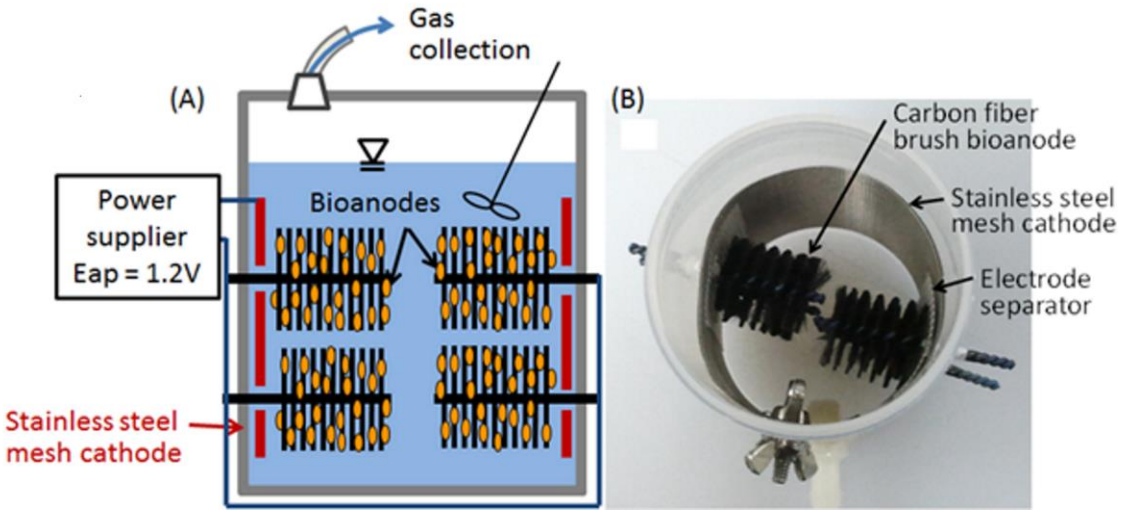

Figure 3.2: (A) Schematic design of an electrically-assisted digester (EAD); and (B) top view of the constructed EAD. (Continuous mixing provided using magnetic stirrers).

3.2.2 Reactor operation

The constructed digesters were inoculated with digested sludge effluent from a municipal wastewater treatment facility. After this one-time inoculation, the digesters were fed directly with a mixture of secondary (~60% by volume) and primary (~40% by volume) sludge collected from a nearby wastewater treatment facility. The collected sludge was stored at 4°C and was unaltered by any pretreatments. The influent total COD and VSS in the feed sludge were consistent throughout the digester operation: influent COD = 21.60 ± 1.70 g/L; and influent VSS = 11.98 ± 1.29 g/L. The COD/VSS ratio of 1.80 is higher than 1.42, indicating that the influent sludge contains a relatively large amount of soluble COD.

The control digester was operated as a typical mesophilic anaerobic digester without any electrode reactions by disconnecting the electrodes. The MEC reactions in the EAD were induced using an external power supplier (GPS-1850D; GW Instek, CA). The electric potential application (E_{ap}) was constant at 1.2 V in experiments while 0.6 V was applied during the start-up period. Both digesters were operated in a bench-top chamber at a constant temperature (39.4 ± 1.2°C). Note that this temperature condition is commonly applied in conventional mesophilic anaerobic digestion of municipal wastewater sludge (Metcalf & Eddy et al., 2004). Both digesters were continuously mixed using magnetic stirrers.

For a given SRT condition, the lab-scale digesters were operated under a continuous fed-batch mode where 120 mL (one half of the sludge volume) was regularly replaced with raw sludge. For instance, a 14-day SRT condition was achieved by feeding the digester every 7 days. Three different SRT conditions (14, 6 and 2 days) were examined. The digesters were operated for ~4 months (including the start-up period). The initial SRT was 14 days and was shortened down to 6 days and then 2 days. For each SRT condition, at least 4 fed-batch cycles were repeated and results from the last 3 fed-batch cycles were taken for discussion.

3.2.3 Experimental measurements

For each fed-batch cycle, raw and digested sludge samples were measured for total suspended solids (TSS), volatile suspended solids (VSS) and total chemical oxygen

demand (COD) in accordance with the standard method (Eaton et al., 2005). Conductivity and pH were measured using a pH and conductivity meter (SevenMulti, Mettler Toledo, Switzerland). The raw sludge pH was stable at pH 6.4 ± 0.2 throughout the experiment. The conductivity of raw sludge was relatively low at 2.2 ± 0.3 mS/cm.

Electric current in the EAD (*I*) was determined by measuring the electric potential drop across a 10-Ω resistor every 20 minutes using a multimeter and data acquisition system (Model 2700, Keithley Instruments, OH). Electric current (*I*) was normalized by the sludge volume in the reactor (240 mL) to calculate the volume-based current density (or specific current).

Biogas produced in each digester was collected using a gas bag (3 L capacity, Cali-5-Bond, Calibrated Instrument Inc., NY). Collected gas was analyzed for CH_4, CO_2, N_2, O_2 and H_2 using two gas chromatography (GC) instruments with a thermal conductivity detector (Varian Star 3400 CX, Agilent Technologies, CA). One GC was equipped with a Porapak-Q packed column (Chromatographic Specialties Inc., Canada) for the separation of CH_4, CO_2 and N_2 using helium as a carrier gas. The other was used to analyze for H_2 and O_2 using a Molecular Sieve 5a column with nitrogen as a carrier gas.

3.3.4 Efficiency and recovery calculations

Coulombic efficiency (*CE*) is the electron-based ratio of COD degraded by exoelectrogenic bacteria to the total COD removal (ΔCOD) throughout a fed-batch cycle as previously defined by Logan et al. (2006):

$$CE = \frac{8\int Idt}{FV\Delta COD} \tag{3-1}$$

I is the electric current in the EAD; *F* is the Faraday constant (96485 C/mol); and *V* is the sludge volume (240 mL). The electric energy consumed to drive the MEC reactions (W_E) was calculated by integrating the product of the electric potential application (E_{ap}) and resulting electric current (*I*) as (Logan et al., 2008):

$$W_E = \int IE_{ap}dt \tag{3-2}$$

The energy recovered as methane gas (W_{CH4}) was determined similarly by Logan et al. (2008) as:

$$W_{CH4} = n_{CH4}\Delta H_{CH4} \tag{3-3}$$

ΔH_{CH4} is the heat of combustion of methane (890.8 kJ/mol) (Haynes, 2013) and n_{CH4} is the amount of produced methane in moles. The methane production in moles (n_{CH4}) was approximated from ΔCOD as demonstrated in Metcalf and Eddy (2004):

$$n_{CH4} = V\Delta COD\left(\frac{1\ mol - CH_4}{64\ g - COD}\right) \tag{3-4}$$

Eq. (3-4) indicates that the amount of methane produced is proportional to the total COD removed in digesters. The conversion factor between mol-CH_4 and g-COD was found

from oxidation of methane ($CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$). The energy recovery ($r_E$) is the ratio between W_{CH4} and W_E as:

$$r_E = \frac{W_{CH4}}{W_E} \tag{3-5}$$

3.3.5 Numerical model development

A steady-state model was developed in order to simulate the rate of biosolids decomposition and microbial growth in the EAD and control digester in accordance with Anaerobic Digestion Model No.1 (ADM1) (Batstone et al., 2002). The developed model includes 21 model components (Table 3.1) and for each component, a steady-state mass balance equation was built with the kinetic rate expressions described in ADM1 (Table A1 in Appendix A). The system of steady-state mass balance equations (Appendix B) were solved simultaneously using fixed point iteration. The numerical model was verified with an example simulation result provided by Rosen & Jeppsson (2006) (Table C1 in Appendix C).

Table 3.1: Influent composition of sludge used for the mathematical model. Influent parameters were selected to match the total COD of the influent used in experimentation as well as the typical breakdown found in waste activated sludge.

Model component	Symbol	Influent (mg-COD/L)
Composites	X_c	12000
Particulate Inerts	X_{in}	100
Carbohydrates	X_{ch}	2000
Proteins	X_{pr}	4000
Lipids	X_{li}	2000
Monosaccharide Degraders	X_{su}	100
Amino Acid Degraders	X_{aa}	100
LCFA Degraders	X_{fa}	100
Valerate and Butyrate Degraders	X_{c4}	100
Propionate Degraders	X_{pro}	100
Acetoclastic Methanogens	X_{ac}	100
Hydrogenotrophic Methanogens	X_{h2}	100
Monosaccharides	S_{su}	1000
Amino Acids	S_{aa}	1000
Long Chain Fatty Acids	S_{fa}	50
Valerate	S_{va}	50
Butyrate	S_{bu}	50
Propionate	S_{pro}	50
Acetate	S_{ac}	1000
Hydrogen Gas	S_{h2}	0
Methane Gas	S_{ch4}	0

The model was further developed to include the MEC reactions in the EAD: acetate destruction at the bioanode (Eq. 3-6) and H_2 gas production at the cathode (Eq. 3-7) (Logan et al., 2008):

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad (3\text{-}6)$$

$$2H^+ + 2e^- \rightarrow H_2 \quad (3\text{-}7)$$

The rate of these electrode reactions was governed in the model by a fixed electric current density value. For example, 90 A/m^3 is equivalent to 644.74 mg COD/L/d for acetate destruction and H_2 gas production. For model simulation, the electric current

density was 90 A/m^3, which was the observed average current density during the experiment with SRT of 14 days, unless otherwise noted. The other kinetic parameters used in the mathematical model were taken from the International Water Association (Batstone et al., 2002) and adjusted for 39°C (Table 3.2). In the simulation, the total COD was assumed to be 24000 mg-COD/L which represents what was found during experimental operation of the EAD and control digester. This total COD value was approximately fractionated into individual components (Table 3.1) in accordance with previous model studies on wastewater sludge digestion (Cacho et al., 2002; Parker, 2005; Rosen & Jeppsson, 2006). Note that it is practically impossible to identify all of the individual components in ADM1 simulations.

Table 3.2: Kinetic constants used for mathematical model. Parameters selected were the suggested values for ADM1 (Batstone et al., 2002) and were adjusted for 39°C. The pH for both digesters was fixed at 7. Electric current density for was fixed at 90 A/m^3 for the EAD.

Model parameter	Symbol	Value	Unit
Max. specific disintegration rate	k_{dis}	0.595	d^{-1}
Microbial decay rate (all)	k_{dec}	0.0238	d^{-1}
Max. specific hydrolysis rate (all)	k_{hyd}	11.459	d^{-1}
Half-saturation value for sugar utilization	$K_{s,su}$	594.604	mg-COD/L
Max. specific sugar utilization rate	k_{su}	54.388	d^{-1}
Half-saturation value for amino acid utilization	$K_{s,aa}$	300	mg-COD/L
Max. specific amino acid utilization rate	k_{aa}	54.388	d^{-1}
Half-saturation coefficient for LCFA utilization	$K_{s,fa}$	400	mg-COD/L
Max. specific LCAFA utilization rate	k_{fa}	6.817	d^{-1}
Half-saturation value for butyrate/valerate utilization	$K_{s,c4}$	237.841	mg-COD/L
Max. specific butyrate/valerate utilization	k_{c4}	22.134	d^{-1}
Half-saturation value for propionate utilization	$K_{s,pro}$	131.607	mg-COD/L
Max. specific propionate utilization	k_{pro}	14.478	d^{-1}
Half-saturation value for acetoclastic methanogensis	$K_{s,ac}$	178.381	mg-COD/L
Max. specific acetoclastic methanogenesis rate	k_{ac}	9.514	d^{-1}
Half-saturation value for hydrogenotrophic methanogenesis	$K_{s,h2}$	0.0114	mg-COD/L
Max. specific hydrogenotrophic methanogenesis rate	k_{h2}	35	d^{-1}
Yield of sugar degraders	Y_{su}	0.1	-
Yield of amino acid degraders	Y_{aa}	0.08	-
Yield of LCFA degraders	Y_{fa}	0.06	-
Yield of butyrate/valerate degraders	Y_{c4}	0.06	-
Yield of propionate degraders	Y_{pro}	0.04	-
Yield of acetoclastic methanogens	Y_{ac}	0.05	-
Yield of hydrogenotrophic methanogens	Y_{h2}	0.06	-
Fraction of inert particulate from composite decomposition	$f_{i,xc}$	0.3	-
Fraction of carbohydrate from composite decomposition	$f_{ch,xc}$	0.2	-
Fraction of protein from composite decomposition	$f_{pr,xc}$	0.2	-
Fraction of lipid from composite decomposition	$f_{li,xc}$	0.3	-
Fraction of LCFA from lipid decomposition	$f_{fa,li}$	0.95	-
Fraction of valerate from amino acid decomposition	$f_{va,aa}$	0.23	-
Fraction of butyrate from sugar decomposition	$f_{bu,su}$	0.13	-
Fraction of butyrate from amino acid decomposition	$f_{bu,aa}$	0.26	-
Fraction of propionate from sugar decomposition	$f_{pro,su}$	0.27	-
Fraction of propionate from amino acid decomposition	$f_{pro,aa}$	0.05	-
Fraction of acetate from sugar decomposition	$f_{ac,su}$	0.41	-
Faction of acetate from amino acid decomposition	$f_{ac,aa}$	0.4	-
Fraction of H_2 gas from sugar decomposition	$f_{h2,su}$	0.19	-
Fraction of H_2 gas from sugar decomposition	$f_{h2,aa}$	0.06	-

3.2 Results

3.2.1 VSS and COD removal in the EAD and control digester

The MEC reactions expedited biosolids destruction under relatively short SRT conditions. The electrically-assisted digester (EAD) achieved 55 ± 1% VSS removal at an SRT of 6 days (Figure 3.3A). At the same SRT condition, the control digester showed only 47 ± 5% VSS removal. When the SRT was sufficiently long at 14 days, the VSS removal was 61 ± 1% for the EAD and 64 ± 2% for the control digester. At a very short SRT condition of 2 days, the VSS removal was similar (37 – 39%) between the EAD and control digester. These results indicate that the MEC reactions enhance the VSS removal only under a certain SRT condition (i.e., 6-day SRT).

The MEC reactions also improved the COD removal at the 6-day SRT. When the SRT was sufficiently long at 14 days, the COD removal was consistent at ~65% between the EAD and control digesters (Figure 3.3B). As the SRT was shortened to 6 days, the control digester showed a substantial drop in COD removal from 66 ± 0.5% to 50 ± 4%. However, the decrease in the COD removal for the EAD was relatively small from 65 ± 0.3% to 61 ± 0.9%, implying that the MEC reactions can expedite organic destruction in anaerobic digestion of wastewater treatment sludge. Similar to the VSS removal result, the very short SRT condition (2 days) made the MEC reactions ineffective for additional COD removal in the EAD compared to the control digester.

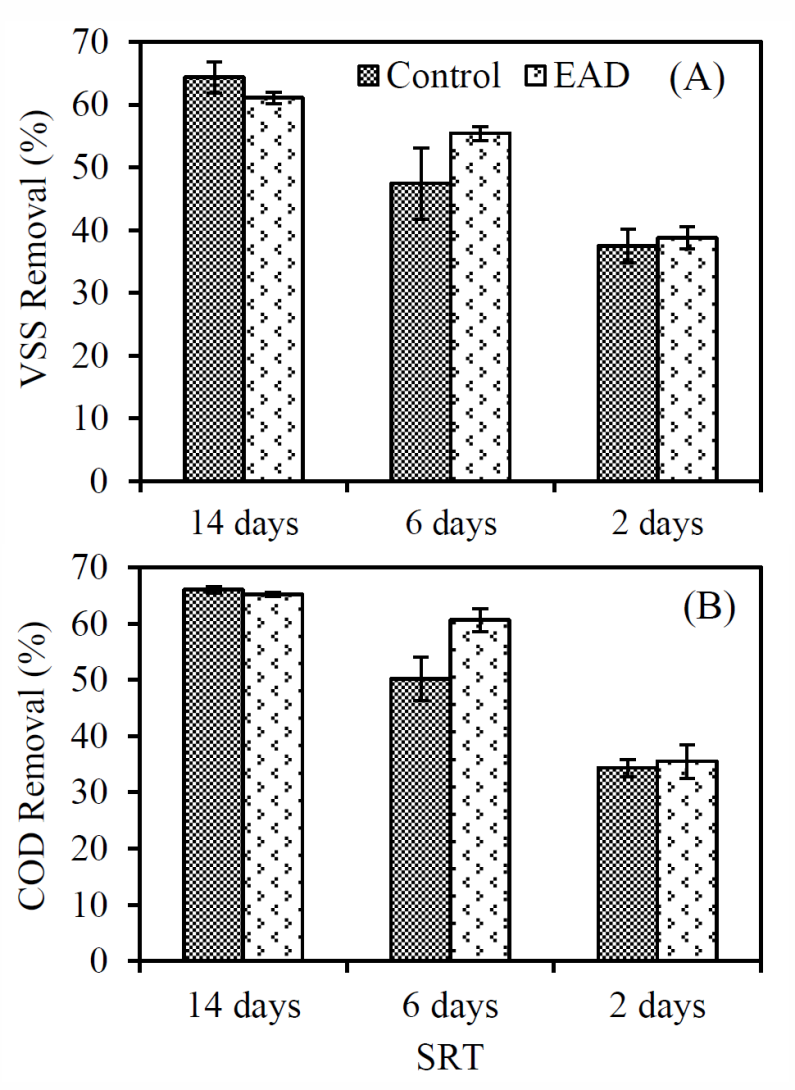

Figure 3.3: Effects of MEC reactions on (A) VSS removal and (B) COD removal. The error bar indicates the magnitude of the standard deviation (n = 3). (Control digester effluent: pH = 7.5 ± 0.1 and conductivity = 3.9 ± 0.3 mS/cm; EAD effluent: pH = 7.4 ± 0.2 and conductivity = 3.5 ± 0.3 mS/cm).

For the 6-day SRT condition, the VSS destruction rate in the EAD was 0.94 kg-VSS/m^3/d, which is 16% greater than 0.81 kg-VSS/m^3/d in the control digester. The COD destruction rate was also greater in the EAD (1.9 kg-COD/m^3/d) by 19% compared to 1.6

kg-COD/m^3/d found in the control digester. Note that the average VSS and COD loading rates under the 6-day SRT condition were 1.86 kg-VSS/m^3/d and 3.36 kg-COD/m^3/d, respectively.

3.2.2 Electric current in the EAD

The electric current in the EAD was affected by SRT conditions because the SRT governs the organic loading rate and thus determines the concentration of soluble substrates for exoelectrogenic microorganisms (Figure 3.4). At an SRT of 2 days, the volume-based current density (specific current) remained high and stable over a continuous fed-batch cycle (between 140 and 190 A/m^3) because the relatively high organic loading rate (11.61 kg COD/m^3/d) maintained high concentration of soluble substrates for the exoelectrogens. The electric current density was similar in magnitude and trend between SRTs of 6 and 14 days (Figure 3.4). It was high at the beginning of each continuous fed-batch cycle (~150 A/m^3) and it rapidly decreased down to ~50 A/m^3. The current density (specific current) result indicates that acetic acid (or volatile fatty acids) was rapidly consumed in the EAD under a 6- or 14-day SRT while the acetic acid concentration was maintained high throughout the fed-batch cycle for 2-day SRT. In a separate experiment, an addition of sodium acetate in the EAD was immediately responded with high electric current (data not shown) confirming that current in the EAD is dependent on the concentration of acetic acid. Exoelectrogenic bacteria are known to prefer acetate as a substrate compared to other complex organics even though

bioelectrochemical systems have been examined with various types of wastewater (Cheng et al., 2007; Chae et al., 2009; Pham et al., 2006; Liu et al., 2005).

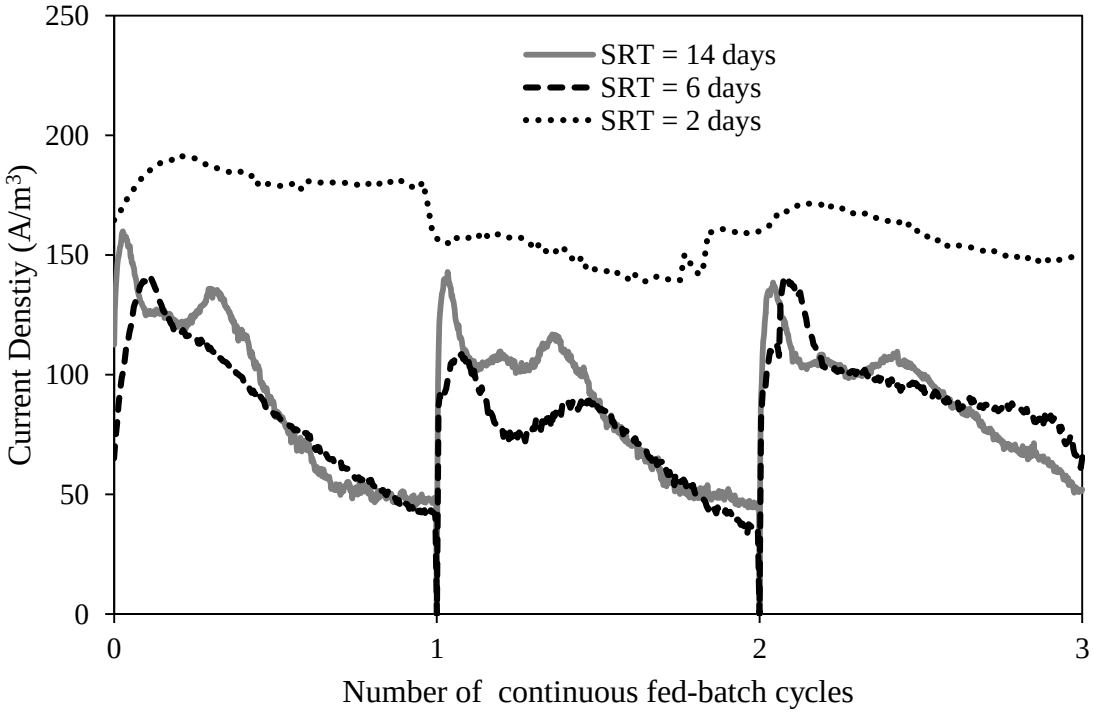

Figure 3.4: Effect of SRT on electric current generation in the EAD. The *x*-axis (number of continuous fed-batch cycles) was prepared by normalizing time by the length of fed batch cycle; thus, one cycle unit is 7 days (14-day SRT), 3 days (6-day SRT) and 1 day (2-day SRT). (Electric current density (specific current) obtained by normalizing electric current by the sludge volume in the EAD, 240 mL).

Note that the conductivity of the influent sludge was consistent at 2.2 ± 0.3 mS/cm throughout the experiment. The conductivity increased slightly in the EAD digester to 4.1 ± 0.1, 3.5 ± 0.2, and 2.8 ± 0.2 mS/cm for the 14-, 6-, and 2-day SRT conditions, respectively. This gradual increase in the conductivity with the increasing SRT can be explained by the increasing amount of soluble compounds (e.g., organic fatty

acids) with time mainly driven by hydrolysis of particulate organics. Even with this increasing effluent conductivity with the increasing SRT, the current density was higher (140 – 190 A/m^3) for the 2-day SRT than that under the 6- or 14-day SRT (40 – 160 A/m^3) (Figure 3.4), indicating that the low sludge conductivity was not a controlling factor for the electric current generation in the EAD. As discussed in the previous paragraph, the acetate concentration determined the magnitude of the electric current.

3.2.3 Coulombic efficiency and energy recovery

The Coulombic efficiency (*CE*) in the EAD was relatively high at 30% for the 14-day SRT (Figure 3.5), indicating that 30% of the removed COD was contributed by the MEC reactions. At the 6-day SRT condition, the *CE* was substantially reduced down to 16% and this reduced *CE* can be explained by the shortened time for the MEC reactions (from 7- to 3-day continuous fed-batch cycle) which increased the organic loading rate from 1.53 kg COD/m^3/d (14-day SRT) to 3.36 kg COD/m^3/d (6-day SRT). Since the magnitude of electric current density was similar for both the 6- and 14-day SRT conditions, the bioanode of the EAD oxidized acetate at a similar rate. Since the organic loading rate was roughly doubled, the *CE* dropped by roughly one half. However, when the SRT was further decreased from 6 to 2 days, the *CE* was maintained at 14% (Figure 3.5) because the electric current was substantially boosted (Figure 3.4) and the COD removal dropped from 61 to 35% (Figure 3.3). The energy recovery (r_E) was high above 300% under the 6- and 2-day SRT conditions while it was relatively low at ~160% at the 14-day SRT condition (Figure 3.5).

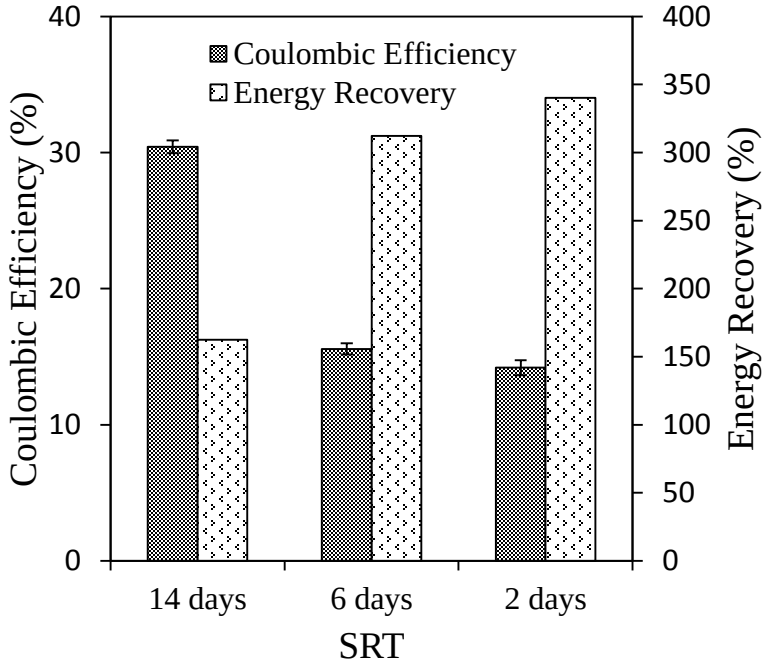

Figure 3.5: Coulombic efficiency (CE) and energy recovery (r_E) in EAD operation.

3.2.4 Gas composition

H_2 gas was not detected in the GC analysis and the biogas consisted mainly of CH_4 (50 – 60%) and CO_2 (40 – 50%) (Figures D.1 & D.2 in Appendix D) both in the EAD and control digesters. In addition, the biogas fractions were not affected by the changing SRT conditions. Unlike the very high CH_4 fraction (98.1%) reported in a recent study (Bo et al., 2014), the CH_4 content in the biogas was not affected by the MEC reactions as the gas composition was the same between the EAD and control digester. This result implies that the MEC reactions do not change the resulting ratio between CH_4 and CO_2 even though they not only altered the reaction pathways (Figure 3.1) but also

accelerated the overall rate of biosolids destruction (Figure 3.3). This inconsistent CH_4 fraction result with the previous study needs further investigation in future study (Bo et al., 2014). Note that the MEC reactions were considered to increase the CH_4 content and decrease the CO_2 fraction because the cathode reaction produces H_2 gas and hydrogenotrophic methanogenesis ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$) consumes CO_2 (Bo et al., 2014).

3.2.5 Model simulation results

In the numerical model simulation, the EAD removed more total COD than the control digester for SRTs below 8 days (Figure 3.6A). At an SRT of 5 or 6 days, the EAD showed the greatest improvement with 12% more COD removal compared to the control digester. Due to the additional acetate removal by the MEC bioanode, the acetoclastic methanogen population (X_{ac}) in the EAD was consistently lower than that in the control digester for the SRT of 7 days or longer (Figure 3.6B). Even with the lower acetoclastic methanogen population, the effluent acetate concentration (S_{ac}) was distinctively lower in the EAD than that in the control digester (Figure 3.6C), indicating that the bioanode successfully replaces the role of acetoclastic methanogens in biosolids destruction. Note that the rapid increase in the acetate concentration for 3 days (S_{ac}) can be explained by active fermentation of sugars and amino acids. The concentration of hydrogenotrophic methanogens (X_{h2}) on the other hand was consistently higher in the EAD due to the enhanced H_2 gas production at the MEC cathode. In both digesters, H_2 gas (S_{h2}) was rapidly consumed by hydrogenotrophic methanogens, resulting in very low H_2 gas

concentration below 0.005 mg-COD/L for all of the examined SRT conditions (Figure 3.6C). When the SRT was very short (<3 days), the fermenting and beta-oxidizing microorganisms (X_{su}, X_{aa} and X_{fa}) were not sufficiently enriched.

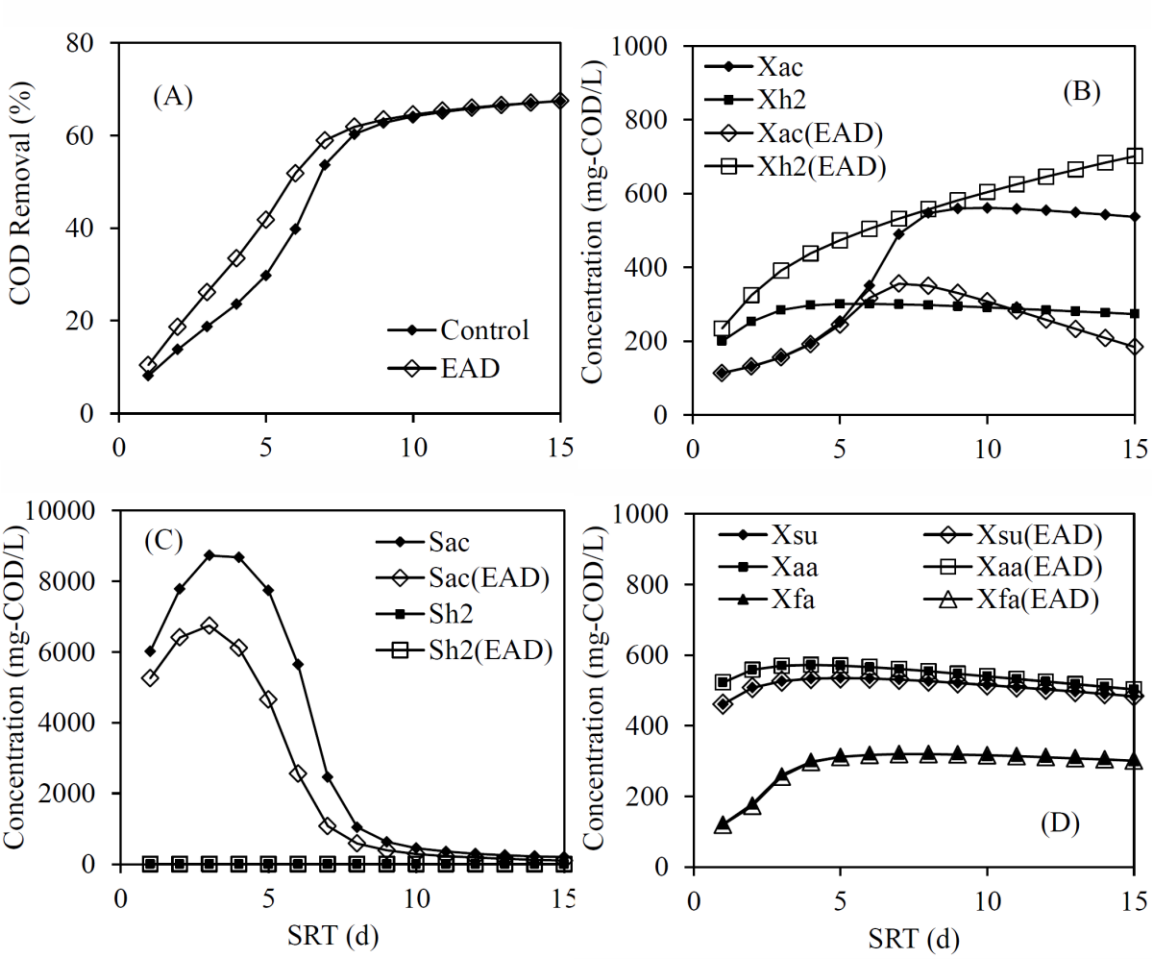

Figure 3.6: Mathematical simulation results for (A) total COD removal; (B) methanogen population; (C) acetate and H_2 gas concentration; (D) acidogenic bacteria population. Electric current density was fixed at 90 A/m^3 for the EAD. The influent composition is given in Table 3.1 and the kinetic constants are listed in Table 3.2.

Note that the model simulation results are provided to support our main hypothesis: the MEC reactions supplement the rate-limiting acetoclastic methanogenesis reaction and thus enhance the rate of biosolids destruction in anaerobic digestion. Due to difficulties in analyzing the individual solids components (X_c, X_{in}, X_{li}, X_{ch}, X_{pr}, X_{c4}, X_{fa}, X_{aa}, X_{su}, X_{pro}, X_{ac}, X_{h2}) in the sludge used in the experiment, precise comparison between experimental results and model simulations was not conducted in this study. However, it should be emphasized that the total COD removal are consistent between the experimental results (Figure 3.3B) and model simulations (Figure 3.6A) within a reasonable range.

3.3 Discussion

3.3.1 Insignificant contribution by MEC at 14- or 2-day SRT

In this study, the MEC reactions (acetate oxidation and H_2 gas production) were implemented to partially replace acetoclastic methanogenesis. Since hydrogenotrophic methanogenesis is sufficiently rapid in converting H_2 gas into CH_4, the MEC reactions coupled with hydrogenotrophic methanogenesis can be considered to play the same role of acetoclastic methanogenesis (i.e., conversion of acetic acid into CH_4). At the 14-day SRT condition, the *CE* of 30% indicates that the MEC reactions contributed 30% of the total COD removal in the EAD, replacing a significant fraction of acetoclastic methanogenesis. Even with this substantial contribution, the EAD did not show noticeable improvements in the VSS and COD removal compared to the control digester (Figure 3.3). This experimental observation is consistent with the negligible improvement

in the COD removal in the model simulation results under long SRT conditions (SRT > 8 d) (Figure 3.6A). This negligible improvement can be explained by the fully enriched acetoclastic methanogen population (X_{ac}) in the control digester (Figure 3.6B). Thus, the bioanode competes with acetoclastic methanogens for a limited amount of acetate (Figure 3.6C) rather than supplementing acetoclastic methanogenesis; as a result, the additional acetate removal by the bioanode in the EAD did not improve the overall COD and VSS removal compared to the control digester.

Under a 2-day SRT condition, hydrolysis is a dominant rate-limiting reaction. Hydrolysis is driven by extracellular enzymes produced by both fermentative and beta-oxidizing microorganisms (Grady et al., 2011; Halalsheh et al., 2011; Meng et al., 2015; Kim et al., 2012). These microorganisms are not fully enriched at the very short 2-day SRT condition (Figure 3.6D), making hydrolysis a dominant rate-limiting reaction. Since the overall reaction is bottlenecked by the hydrolysis step, the addition of the MEC reactions in the EAD did not bring meaningful improvement in COD and VSS removal compared to the control digester (Figures 3.3 and 3.6A).

3.3.2 MEC contribution to expedited biosolids destruction at 6-day SRT

MEC reactions expedited biosolids destruction with the 55% VSS removal and 61% COD removal in 6 days in the EAD (Figure 3.3). This result indicates that the MEC reactions (i.e., acetate oxidation at the bioanode and H_2 production at the cathode) successfully supplemented the role of acetoclastic methanogens, which is substantially

limited at the 6-day SRT condition. Produced H_2 gas at the MEC cathode was rapidly converted into CH_4 by hydrogenotrophic methanogens as H_2 was not detected in the gas chromatography analysis. The observed *CE* during the 6-day SRT operation (Figure 3.5) indicates that 16% of the removed COD went through the MEC reactions. Thus, the MEC reactions are responsible for ~10% of the total COD removal in the EAD (product of *CE* = 16% and COD removal = 61%). This 10% contribution is consistent with the difference in the COD removal between 61% in the EAD and 50% in the control digester.

The mathematical model results are also consistent with the experimental observation as the EAD outperforms the control digester only for the SRTs around 6 days (Figure 3.6A). In the EAD, the acetate concentration was significantly lower than that in the control digester (Figure 3.6C), indicating that the bioanode reaction successfully supplemented the slow acetoclastic methanogenesis reaction (Figure 3.1). While we did not analyze the experimental samples for acetate concentration, in another set of experiments we observed consistently lower acetate concentration in the EAD by 30 – 40% compared to that in the control digester (Asztalos and Kim, 2015).

The improved VSS removal can be indirectly attributed to the reduced acetate concentration (S_{ac}) in the EAD (Figure 3.6C). The reduced acetate concentration makes fermentation reactions more thermodynamically spontaneous, providing a more favorable environment for fermentative microorganisms to grow (McCarty, 1975). Since hydrolysis is driven by enzymes excreted by these microorganisms, the rate of VSS removal is

consequently enhanced. This indirect enhancement to hydrolysis requires further attention in future studies and should be implemented in future mathematical models as the current ADM1 employs a simplified kinetic equation for the hydrolysis step (Grady et al., 2011).

3.3.3 Acetoclastic methanogenesis at 6-day SRT

During the digester operation at the 6-day SRT, acetoclastic methanogenesis made a relatively small contribution to biosolids destruction compared to a 14-day SRT operation. There are only two known microbial genera for acetoclastic methanogens: *Methanosarcina* and *Methanosaeta*. *Methanosarcina* requires an SRT of at least 3 – 5 days for enrichment while *Methanosaeta* species are even slower, requiring a minimum of 12 days to initiate active methanogenesis from acetate oxidation (Grady et al., 2011). While *Methanosaeta* species are slow growers they are known to utilize acetate more effectively than *Methanosarcina*. For instance, the half saturation constant was found to be 90 mg COD/L for *Methanosaeta* and 320 mg COD/L for *Methanosarcina* (Conklin et al., 2006). This trend in the half saturation coefficient was confirmed in a review article with of 0.1 – 1.2 mM (acetate) for *Methanosaeta* and 3.0 – 4.5 mM for *Methanosarcina* (Aiyuk et al., 2006). The greater half saturation coefficient values indicate that *Methanosarcina* cannot actively utilize acetate at a low concentration, making *Methanosarcina* species less responsible for acetoclastic methanogenesis than *Methanosaeta* in anaerobic digesters (Lui & Whiteman, 2008). In our experiments at the 6-day SRT, *Methanosarcina* was present while most of the *Methanosaeta* was washed

out from the digesters because of their slow enrichment (>12 days). Therefore, acetoclastic methanogenesis driven only by *Methanosarcina* made a relatively minor contribution to the overall biosolids destruction. Because of this limited contribution by acetoclastic methanogenesis, the biosolids destruction in the control digester dropped substantially from 64 to 47% in the VSS removal when the SRT was decreased from 14 to 6 days (Figure 3.3A). While *Methanosaeta* and *Methanosarcina* species are not separately reflected in ADM1, the simulation result showed a consistent trend with the experimental observation as the population of acetoclastic methanogens (X_{ac} in Figure 3.6B) was not sufficiently high under the 6-day SRT condition, leaving the large amount of residual acetate in the control digester (S_{ac} in Figure 3.6C).

Even though acetate concentration was not measured in this study, it is evident that the MEC bioanode dominantly consumes acetate (Liu et al., 2005; Logan et al., 2006; Logan et al., 2008). As a result, low acetate concentration was consistently reported in anaerobic digestion systems coupled with the MEC reactions (Liu et al., 2012; Guo et al., 2013; Wang et al., 2014; Choi and Ahn., 2014; Asztalos and Kim, 2015). These literature articles strongly indicate that the presence of the MEC reactions reduces acetate concentration and thus leads to supplementing the rate-limiting acetoclastic methanogenesis reaction.

3.3.4 Sufficiently rapid hydrogenotrophic methanogenesis

It should be emphasized that H_2 gas was not detected in the GC analysis throughout the experiments. This GC analysis result is consistent with the simulation result as the H_2 concentration (S_{h2}) was always very low, below 5×10^{-3} mg-COD/L (Figure 3.6C). In addition, simulation results show that the H_2 concentration in the EAD was higher than that in the control digester by a subtle difference ($<7.5 \times 10^{-3}$ mg-COD/L). This absence of H_2 in the collected biogas indicates that hydrogenotrophic methanogenesis was sufficiently rapid compared to the rate of H_2 gas production by the MEC cathode reaction, beta-oxidation (anaerobic oxidation) and fermentation. Hydrogenotrophic methanogens was sufficiently enriched in 3 days (Figure 3.6B). In addition to their rapid growth, the rate of their metabolic consumption of H_2 gas was found to be very fast. The depth of liquid sludge in the built digester was 7 cm and the MEC cathode was placed vertically around the inner wall of the digester. Thus, the average travel distance of H_2 bubbles produced at the cathode is 3.5 cm before they reach the gas-liquid interface. Therefore, the absence of H_2 gas means that hydrogenotrophic methanogenesis was sufficiently fast to achieve complete consumption of H_2 gas while the gas bubbles travel the short distance (3.5 cm).

3.3.5 Estimation of acetic acid concentration using electric current in EAD

The magnitude of electric current in the EAD directly indicates the activity of exoelectrogenic bacteria which utilize volatile fatty acids (mainly acetic acid) as a substrate (Liu et al., 2005; Cheng et al., 2007; Chae et al., 2009). As such; the trends in

volume-based current density (specific current) throughout a cycle describe the change in volatile fatty acids concentration in the EAD. At the 2-day SRT condition, the consistently high current density (140 – 190 A/m^3, Figure 3.4) indicates that a relatively high concentration of acetic acid was maintained throughout the continuous fed-batch cycle. The trends in the electric current were very similar between the 14- and 6-day SRT conditions. This similarity indicates that the acetic acid concentration under the 6-day SRT was as low as that under the 14-day SRT, proving that the MEC reactions successfully replaced acetoclastic methanogenesis and kept the acetate concentration low. As a result, the COD removal was relatively unaffected (from 65% to 61%) in the EAD with the decreasing SRT from 14 to 6 days while the COD removal substantially dropped from 66% to 50% in the control digester.

3.3.6 Potential retention of slowly growing microorganisms near bioanodes

When the EAD and control digester was autopsied after 4 months of experimental operation, very thick biofilms (about the diameter of the graphite fiber brush of 2 cm) were formed on the brush anode in the EAD. A recent study performed by De Vrieze et al., (2014) concluded that such anode biofilms increase the retention of slowly growing microorganisms (e.g., acetoclastic methanogens) in the EAD and thus enhance the rate of anaerobic digestion. However, in our study, such thick biofilms were not observed on the graphite brushes in the control digester, indicating that solid retention near the graphite brush did not play an important role in enhancing the rate of anaerobic digestion in this study. This inconsistent result from De Vrieze et al., (2014) can be explained by the use

of a different type of anode materials that cannot hold biomass long enough without electric current.

3.4 Conclusions

The electrically-assisted digester (EAD) demonstrated promising results by removing 55% VSS and 61% of total COD under a 6-day SRT. These results were achieved by the implementation of MEC reactions in which exoelectrogenic bacteria supplemented acetic acid uptake as acetoclastic methanogens were limited under this relatively short SRT. The EAD provided enhanced performance compared to the control digester under a 6-day SRT. When the SRT was 14 days, acetoclastic methanogens in the digesters were enriched enough such that the contribution by MEC reactions in the EAD (30% of total COD removal) did not improve the overall VSS and COD removal compared to the control digester. At the 2-day SRT condition, anaerobic reactions other than acetoclastic methanogenesis (e.g., hydrolysis) limit the overall rate of biosolids digestion, preventing the additional acetic acid removal by the MEC reactions from accelerating the overall biosolids destruction. Based on our lab-scale experiment results, the EAD was shown to effectively accelerate wastewater sludge digestion for SRT conditions near 6 days. The rate of hydrogenotrophic methanogenesis was found to be sufficiently rapid in the EAD. As a result, H_2 gas was not detected in the collected biogas even with the additional H_2 gas production from the cathode. The energy recovery (r_E) as methane gas was more than three times the electric energy consumed to drive the MEC electrode reaction. This finding indicates that the energy requirement in the EAD is not

high, showing promising potentials for practical applications in wastewater treatment facilities.

A mathematical model was also built by modifying ADM1. The simulation results showed that the MEC reactions successfully decrease acetic acid concentration. This finding supports our hypothesis: the MEC reactions supplement the rate-limiting acetoclastic methanogenesis reaction. Also, the simulation results showed improved COD removal in the EAD only for relatively short SRT conditions (< 8 days).

The results demonstrated that the MEC reactions can be integrated into conventional mesophilic anaerobic digesters at a lab-scale to enhance the destruction of VSS and COD. Further work is required to determine whether this system can be properly up-scaled from a 250-mL reactor and if the EAD can be used with various grades of influents (e.g., thickened wastewater sludge or high-strength agricultural wastewater). This study showed relatively limited experimental information as individual fatty acid concentration, including acetic acid, was not provided. Thus, the suggested mechanism on how the MEC reactions improve biosolids destruction needs to be further investigated in future study.

3.5 Acknowledgements

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4. Psychrophilic digestion of waste activated sludge using microbial electrolysis cells[2]

Abstract

This study examined the effects microbial electrolysis cell (MEC) reactions on psychrophilic anaerobic digestion. Two lab-scale digesters, a control digester and an electrically-assisted digester (EAD – equipped with an MEC bioanode and cathode) were operated at psychrophilic temperatures under three solids retention times (SRT = 7, 10 and 14 days). The MEC bioanode directly oxidizes acetate while hydrogen gas is produced at the cathode. The MEC reactions in the EAD reduced the concentration of propionic, n-butyric and iso-butyric acids. The EAD is thought to lower the concentration of these short-chain fatty acids by direct oxidation at the bioanode as well as indirectly by improved beta-oxidation. The VSS and COD removal was higher in the EAD by 5 – 10% compared to the control digester for the 7- and 14-day SRT conditions. When compared to mathematical model results, this improved COD removal in the EAD at psychrophilic temperatures was equivalent to that with conventional digesters at mesophilic temperatures. The magnitude of electric current in the EAD was governed by the organic loading rate while conductivity and acetic acid concentration showed negligible effects on current generation. The waste activated sludge is thought to contain large amounts of lipids and other complex polymeric substances which resulted in high CH_4 content (95%) in the biogas from both the EAD and control digester.

[2] Manuscript submitted to *Water Research*

Keywords

Psychrophilic anaerobic digestion; bioelectrochemical systems; exoelectrogenic bacteria; anaerobic digestion model no. 1; short-chain fatty acids

4.1 Introduction

In municipal wastewater sludge treatment, anaerobic digestion is typically operated under mesophilic conditions at temperatures ranging from 35 – 40°C (Metcalf & Eddy, et al, 2004). To maintain this temperature requirement for large sludge volumes, a substantial amount of energy is therefore required. However, low temperature conditions below 35°C slow down the biosolids destruction with reduced rates of microbial growth and biogas production (Connaughton et al., 2006). As a result, lower temperature digesters require a substantially long solids retention time (SRT) for adequate performance. For example, a digester operated at 20°C would require an SRT of approximately 40 days (Metcalf & Eddy et al., 2004). However, there are a number of benefits of operating digestion systems at a lower temperature, such as reduced energy input and significantly reduced digester construction cost without heating systems and insulation walls, allowing small wastewater treatment facilities to operate sludge digesters. Thus, in this study the primary objective was to examine the performance of an electrically-assisted digester (EAD) operating at psychrophilic (<25°C) conditions.

In anaerobic digestion, the destruction of biosolids is achieved through a series of biological reactions (Figure 4.1). Under mesophilic conditions, the hydrolysis of carbohydrates and proteins is relatively quick, requiring 1 – 3 days; while lipids require 6 – 8 days for hydrolytic decomposition (Grady et al., 2011). Many studies have reported that if digester influent contains a large amount of complex lipids, the hydrolysis step starts to govern the overall rate of biosolids destruction (Ariunbaater et al., 2014; Izumi et al., 2010; Ma et al., 2011; Valo et al., 2004). Hydrolyzed soluble organics (monosaccharides, amino acids and long chain fatty acids) are decomposed to short chain organic acids and hydrogen gas in acidogenesis reactions, such as fermentation and beta-oxidation. Acetoclastic methanogenesis and hydrogenotrophic methanogenesis are the final steps converting acetate and hydrogen gas to methane gas, respectively. Hydrogenotrophic methanogens are known to rapidly convert hydrogen gas to methane in less than 1 day (Grady et al., 2011). Acetoclastic methanogenesis, however, requires a substantially long time as acetoclastic methanogens need 3 – 5 days (*Methanosarcina* spp.) and at least 12 days (*Methanosaeta* spp.) to start utilizing acetate (Grady et al., 2011). The majority of acetoclastic methanogenesis is driven by *Methanosaeta* species which result in the process being another rate-limiting step in anaerobic digestion. When the digester influent contains easily degradable substrates with a small amount of lipids, acetoclastic methanogenesis has been reported to be the dominant rate-limiting step (Ariunbaater et al., 2014; Grady et al., 2011; Rittmann & McCarty, 2001). In domestic wastewater sludge digestion, the influent does not typically contain high levels of

complex substrates which results in acetoclastic methanogenesis being the key rate-limiting step.

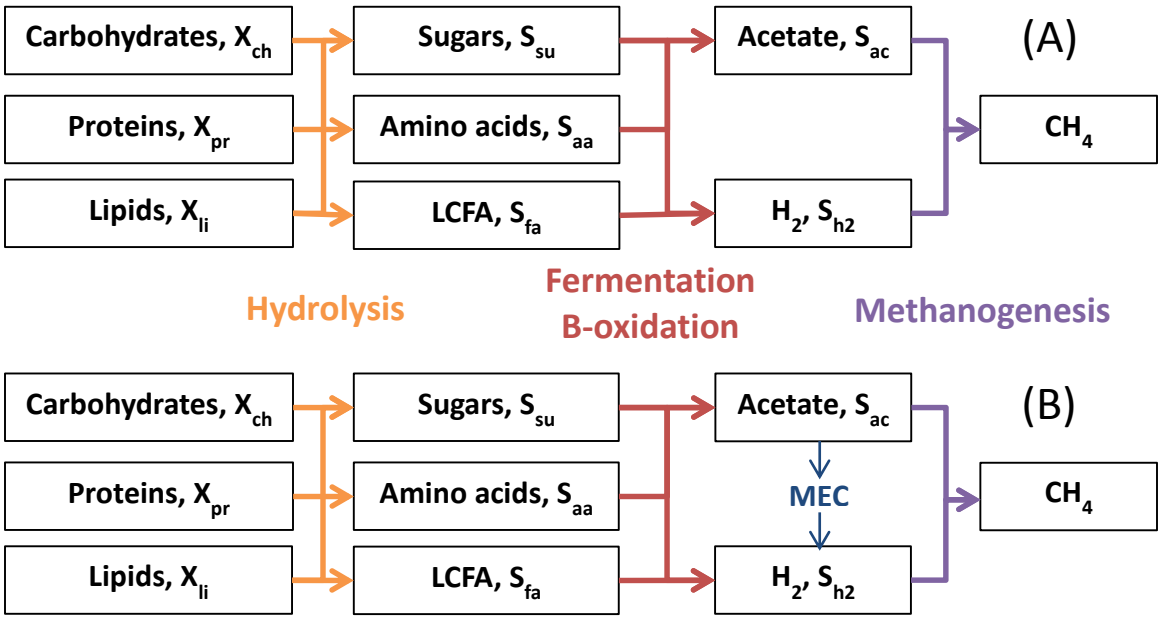


Figure 4.1: Reaction pathways for biosolids in (A) conventional anaerobic digestion and (B) electrically-assisted digestion.

In this study, microbial electrolysis cell (MEC) technology was integrated into a lab-scale anaerobic digester in order to expedite the rate of biosolids destruction as previously described (Asztalos and Kim, under review) but under even lower temperature conditions (20°C). An MEC consists of a bioanode and cathode that are electrically connected with an applied external power supplier (Liu et al., 2005; Rozendal et al., 2006; Logan et al., 2008). Acetate is oxidized by exoelectrogenic bacteria at the bioanode and hydrogen gas is produced at the cathode via electrolytic water reduction. Hydrogenotrophic methanogens quickly convert the produced hydrogen gas into methane gas. The MEC bioanode oxidize a certain portion of the available acetate in the digester

and creates an additional pathway for acetate removal as previous demonstrated in the EAD (electrically-assisted digester) under mesophilic condition (40°C) (Asztalos and Kim, under review). While expedited volatile suspended solids (VSS) and chemical oxygen demand (COD) removals were demonstrated, decomposition of organic acids (acetic acid, propionic acid, butyric acid and valeric acid) in the EAD was not clearly investigated. Experimental examination of such organic acids is necessary to ensure proper destruction of long-chain fatty acids (LCFAs) via beta-oxidation.

We also built a numerical model similar to Anaerobic Digestion Model No.1 (ADM1) developed by the IWA Task Group (Batstone et al., 2002) with the addition of the MEC component (Asztalos and Kim, under review). The numerical model allowed us to examine a variety of components and microbes under various SRT, temperature and electrical current conditions. For example, experimental results on improved digestion performance with the MEC reactions under psychrophilic conditions were compared with conventional digester performance under various temperature conditions using the model. Based on this comparison, we were able to discuss the energy requirement for the MEC reaction and that for heating wastewater sludge to attain mesophilic conditions.

4.2 Materials and Methods

4.2.1 Reactor construction

Two lab-scale anaerobic digestion reactors, a control digester and an electrically-assisted digester (EAD), were constructed with MEC components. The reactor bodies

were made out of a thick polypropylene block in which a cylindrical hole (6.5 cm diameter and 6.5 cm depth with the effective liquid volume of 180 mL) was drilled. Two end-plates were fastened to the top and bottom of the bodies using metal tie rods and nuts placed along the perimeter of the reactor bodies (Figure 4.2). Three carbon fiber brushes (2 cm diameter and 2.5 cm in length; Mill-Rose, OH) were pretreated in a muffle furnace at 450°C for 30 minutes (Wang et al., 2009) before they were placed in each digester as bioanodes. A single layer of stainless steel mesh was used as the MEC cathode without the use of any precious metal catalysts (total projected area of 135 cm^2, AISI 304, 100-mesh, McMaster-Carr, OH). The stainless steel mesh was wrapped around the interior wall of the reactor. The three bioanodes were fit through the top end-plate with an average distance of ~2 cm from the stainless steel mesh cathode. A small hole was drilled through the top plate to allow for feeding and withdrawing solution from the digesters. A plastic tube was glued to the top plate for biogas collection

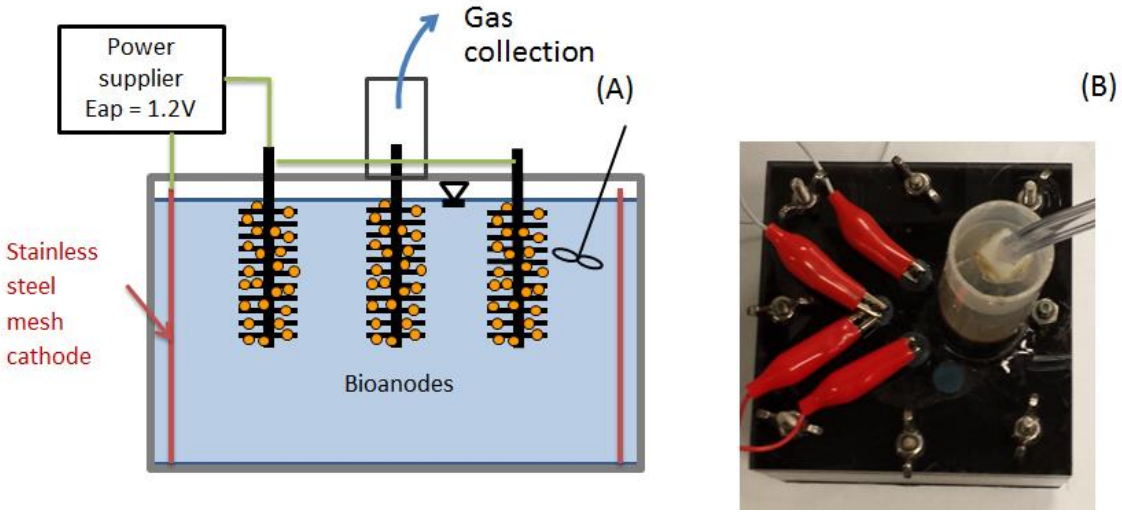


Figure 4.2: (A) Schematic of electrically-assisted digester. (B) Top view of the lab-scale electrically-assisted digester.

4.2.2 Reactor operation

The constructed digesters were started with an influent containing 50% digested sludge from other lab-scale reactors and 50% secondary sludge (WAS) from a local municipal wastewater treatment facility. After this one-time start-up cycle, the digesters were directly fed with WAS. The collected sludge was stored for up to two weeks at 4°C and was unaltered by any pretreatments. The composition of collected WAS from a local wastewater treatment facility was not consistent with substantially varying chemical oxygen demand (7.89 ± 1.88 g COD/L) and volatile suspended solids (5.20 ± 1.20 g VSS/L) throughout the 5-month experiment.

The digesters were operated under psychrophilic conditions (22.5 ± 0.5°C) and were continuously mixed using magnetic stirrers. The MEC reactions in the EAD were induced using an external power supplier (GPS-1850D; GW Instek, CA) while the control digester was operated as a typical anaerobic digester by disconnecting the electrodes. The electric potential application (E_{ap}) was 0.8 V during the EAD start-up for ~2 weeks and then held constant at 1.2 V throughout the experiment.

Three different SRT conditions (7, 10 and 14 days) were examined in the experiment. For a given SRT condition, the digesters were operated under a continuous fed-batch mode where 90 mL (one half of the effective reactor volume) was replaced with untreated secondary sludge (i.e., WAS from the local facility). For instance, a steady-state 14-day SRT condition was achieved by feeding the digester every 7 days.

The gas head-space in the reactors was purged using nitrogen gas at the beginning of each fed-batch cycle. The initial SRT was 7 days and was lengthened to 10 days and then finally 14 days. For each SRT condition, at least 5 fed-batch cycles were repeated and results from all cycles were taken for analysis and discussion.

4.2.3 Experimental measurements

For each cycle, influent and effluent sludge samples were measured for total suspended solids (TSS), volatile suspended solids (VSS) and total chemical oxygen demand (COD) in accordance with the standard method (Eaton et al., 2005). The sludge was also analyzed for conductivity and pH (SevenMulti, Mettler Toledo, Switzerland). The raw sludge pH was stable and neutral throughout the experiment at 6.8 ± 0.3. The conductivity of the sludge was also stable and relatively low at 1.26 ± 0.2 mS/cm.

Short-chain fatty acids were analyzed using a flame ionization detector-gas chromatography (FID-GC) instrument (Varian CP-3800) equipped with a Stabilwax-DA column (Restek Corporation, PA). Prior to the FID-GC analysis, the sludge samples were centrifuged at 7000 RPM for 10 minutes and the supernatant was then acidified using a 3% vol. phosphoric acid solution as previously described (Eaton et al., 2005)

Biogas produced in each digester was collected using a gas bag (250 mL capacity, Cali-5-Bond, Calibrated Instruments Inc., NY). Collected biogas was analyzed for CH_4, CO_2, N_2, O_2 and H_2 using two thermal conductivity detector gas chromatography (TCD-

GC) instruments (Varian Star 3400 CX, Agilent Technologies, CA). One TCD-GC was equipped with a Porapak-Q packed column (Chromatographic Specialties Inc., Canada) for the separation of CH_4 CO_2, and N_2 using helium as a carrier gas. The other TCD-GC was used to analyze H_2 and O_2 using a Molecular Sieve 5a column with nitrogen as a carrier gas.

Electric current in the EAD (*I*) was determined by measuring the electric potential drop across a 10-Ω resistor every 20 minutes using a multimeter and data acquisition system (Model 2700, Keithley Instruments, OH). The electric current was normalized by the effective sludge volume in the digester (180 mL) to calculate the volume-based current density (or specific current).

4.2.4 Efficiency and recovery calculations

As defined by Logan et al. (2006), Coulombic efficiency (*CE*) is the ratio of the COD degraded by exoelectrogenic bacteria to the total COD removal (ΔCOD) on an electron basis:

$$CE = \frac{8\int Idt}{FV\Delta COD} \tag{4-1}$$

I is the electric current in the EAD; *F* is the Faraday constant (96485 C/mol); and *V* is the sludge volume (180 mL). The electric energy consumption in the EAD (W_E) is calculated using (Logan et al., 2008):

$$W_E = \int IE_{ap}dt \tag{4-2}$$

The energy recovered as methane gas (W_{CH4}) was also determined by Logan et al. as (Logan et al., 2008):

$$W_{CH4} = n_{CH4}\Delta H_{CH4} \tag{4-3}$$

ΔH_{CH4} is the heat of combustion of methane (890.8 kJ/mol) (Haynes, 2013) and n_{CH4} is the amount of produced methane in moles. The methane production in moles (n_{CH4}) was approximated from ΔCOD as demonstrated in Metcalf & Eddy (2004):

$$n_{CH4} = V\Delta COD\left(\frac{1\ mol - CH_4}{64\ g - COD}\right) \tag{4-4}$$

The conversion factor between mol-CH_4 and g-COD was found from oxidation of methane ($CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$). The energy recovery ($r_E$) is the ratio between W_{CH4} and W_E as:

$$r_E = \frac{W_{CH4}}{W_E} \tag{4-5}$$

4.2.5 Numerical model development

A steady-state version of Anaerobic Digestion Model No. 1 (ADM1) (Batstone et al., 2002) was developed and used to simulate the rate of biosolids destruction, microbial growth and change in organic concentration in both the EAD and control digester (Asztalos and Kim, under review). For each of the 21 included components (Table 4.1), a steady-state mass balance equation was developed using the kinetic rate expressions provided by ADM1 (Table A1 in Appendix A). Using fixed-point iteration, the developed equations were solved simultaneously in Microsoft Excel. Verification of the model was

completed by using an example simulation provided by Rosen & Jeppsson (2006) (Table C1 in Appendix C).

Table 4.1: Influent composition of sludge used for the mathematical model. Influent parameters were selected to match the total COD and fatty acid composition of the influent used in experimentation as well as the typical breakdown found in waste activated sludge.

Model component	Symbol	Influent (mg-COD/L)
Composites	X_c	4600
Particulate Inerts	X_{in}	1300
Carbohydrates	X_{ch}	320
Proteins	X_{pr}	320
Lipids	X_{li}	500
Monosaccharide Degraders	X_{su}	10
Amino Acid Degraders	X_{aa}	10
LCFA Degraders	X_{fa}	10
Valerate and Butyrate Degraders	X_{c4}	30
Propionate Degraders	X_{pro}	30
Acetoclastic Methanogens	X_{ac}	30
Hydrogenotrophic Methanogens	X_{h2}	30
Monosaccharides	S_{su}	300
Amino Acids	S_{aa}	300
Long Chain Fatty Acids	S_{fa}	10
Valerate	S_{va}	13.58
Butyrate	S_{bu}	15.23
Propionate	S_{pro}	4.00
Acetate	S_{ac}	43.51
Hydrogen Gas	S_{h2}	0
Methane Gas	S_{ch4}	0

To account for the MEC reactions in the EAD, the following equations were implemented in the model for acetate removal at the bioanode (Eq. 4-6) and hydrogen gas production at the cathode (Eq. 4-7) (Logan et al., 2008):

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad (4\text{-}6)$$

$$2H^+ + 2e^- \rightarrow H_2 \quad (4\text{-}7)$$

A fixed electric current density governed the rate of the electrode reactions in the model simulation. All simulations were performed at 22°C unless otherwise stated. The ADM1 kinetic parameters for 22°C are summarized in Table 4.2 (Batstone et al., 2002). For simulations at another temperature condition, the Arrhenius equation was used to adjust the kinetic parameters (Grady et al., 2011):

$$k(T) \quad = k(22℃) \quad \theta^{(T-22)} \tag{4-8}$$

Note that k is the kinetic parameter, T is the temperature in °C and θ is the Arrhenius constant.

Table 4.2: Kinetic constants used for mathematical model. Parameters selected were the suggested values for ADM1 (Batstone et al., 2002) and were adjusted for 22°C. The pH for both digesters was fixed at 7. Electric current density for was fixed at 10 A/m^3 for the EAD.

Model parameter	Symbol	Value	Unit	θ
Max. specific disintegration rate	k_{dis}	0.319	d^{-1}	1.035
Microbial decay rate (all)	k_{dec}	0.0127	d^{-1}	1.035
Max. specific hydrolysis rate (all)	k_{hyd}	7.0182	d^{-1}	1
Half-saturation value for sugar utilization	$K_{s,su}$	318.640	mg-COD/L	1.035
Max. specific sugar utilization rate	k_{su}	40.178	d^{-1}	1.017
Half-saturation value for amino acid utilization	$K_{s,aa}$	300	mg-COD/L	1
Max. specific amino acid utilization rate	k_{aa}	40.178	d^{-1}	1.017
Half-saturation coefficient for LCFA utilization	$K_{s,fa}$	400	mg-COD/L	1
Max. specific LCAFA utilization rate	k_{fa}	4.305	d^{-1}	1.026
Half-saturation value for butyrate/valerate utilization	$K_{s,c4}$	127.456	mg-COD/L	1.035
Max. specific butyrate/valerate utilization	k_{c4}	15.366	d^{-1}	1.020
Half-saturation value for propionate utilization	$K_{s,pro}$	48.964	mg-COD/L	1.056
Max. specific propionate utilization	k_{pro}	9.825	d^{-1}	1.022
Half-saturation value for acetoclastic methanogensis	$K_{s,ac}$	95.592	mg-COD/L	1.035
Max. specific acetoclastic methanogenesis rate	k_{ac}	5.098	d^{-1}	1.053
Half-saturation value for hydrogenotrophic methanogenesis	$K_{s,h2}$	0.002	mg-COD/L	1.103
Max. specific hydrogenotrophic methanogenesis rate	k_{h2}	35	d^{-1}	1
Yield of sugar degraders	Y_{su}	0.1	-	
Yield of amino acid degraders	Y_{aa}	0.08	-	
Yield of LCFA degraders	Y_{fa}	0.06	-	
Yield of butyrate/valerate degraders	Y_{c4}	0.06	-	
Yield of propionate degraders	Y_{pro}	0.04	-	
Yield of acetoclastic methanogens	Y_{ac}	0.05	-	
Yield of hydrogenotrophic methanogens	Y_{h2}	0.06	-	
Fraction of inert particulate from composite decomposition	$f_{i,xc}$	0.3	-	
Fraction of carbohydrate from composite decomposition	$f_{ch,xc}$	0.2	-	
Fraction of protein from composite decomposition	$f_{pr,xc}$	0.2	-	
Fraction of lipid from composite decomposition	$f_{li,xc}$	0.3	-	
Fraction of LCFA from lipid decomposition	$f_{fa,li}$	0.95	-	
Fraction of valerate from amino acid decomposition	$f_{va,aa}$	0.23	-	
Fraction of butyrate from sugar decomposition	$f_{bu,su}$	0.13	-	
Fraction of butyrate from amino acid decomposition	$f_{bu,aa}$	0.26	-	
Fraction of propionate from sugar decomposition	$f_{pro,su}$	0.27	-	
Fraction of propionate from amino acid decomposition	$f_{pro,aa}$	0.05	-	
Fraction of acetate from sugar decomposition	$f_{ac,su}$	0.41	-	
Faction of acetate from amino acid decomposition	$f_{ac,aa}$	0.4	-	
Fraction of H_2 gas from sugar decomposition	$f_{h2,su}$	0.19	-	
Fraction of H_2 gas from sugar decomposition	$f_{h2,aa}$	0.06	-	

4.3 Results

4.3.1 VSS and COD removal

The MEC reactions in the EAD expedited the removal of VSS by 5 – 10% under the 7- and 14-day SRT conditions (Figure 4.3). For the 7-day SRT condition, a statistically significant difference was noted (p-value = 0.002). For the 14-day SRT condition, although there was an observed difference in VSS removal between the control digester and EAD it was not found to be statistically significant (p-value = 0.297). A significant difference could not be established between the control digester and EAD for the 10-day SRT condition. It should be noted that the EAD performance in VSS removal was less dependent on the SRT condition with a small increase in the VSS removal from 26 to 28% while the control digester showed a greater variation from 16 to 22% VSS removal when the SRT was increased. The COD removal trend was consistent with that of the VSS removal result (Figure 4.3B). The EAD removed 5 – 10% more total COD compared to the control digester for the 7- and 14-day SRT conditions. The 7-day COD removal results between the two digesters were found to be statistically significantly different (p-value = 0.038). Once again for the 14-day SRT condition, the observed difference between the EAD and control was not found to be statistically significant (p-value = 0.442). The EAD showed no improvement in COD removal over the control digester for the 10-day SRT condition. It should be noted that there is a more gradual increase in the percent COD removal with the increasing SRT for the EAD (30% to 34%) compared to the control digester (20% to 28%). These results emphasize that the total COD removal was less dependent on SRT when MEC reactions were present.

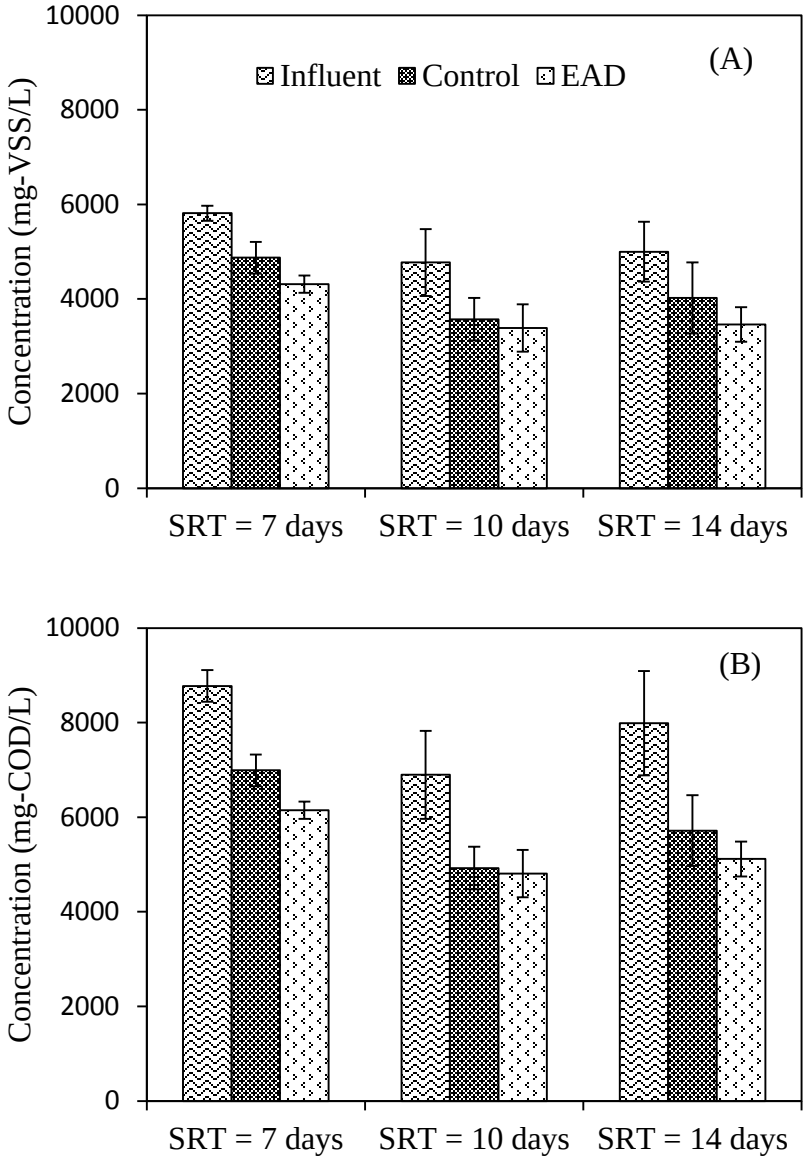


Figure 4.3: (A) VSS concentration and (B) COD concentration in the control and EAD reactors. The error bars indicated the magnitude of the standard deviation (n = 5).

4.3.2 Electric current and organic loading rate

The volume-based electric current density (specific current) in the EAD varied substantially as the sludge composition was not consistent throughout the experiment

(Figure 4.4). However, the current density was generally high between 15 and 25 A/m^3 for the 7-day SRT while it was relatively low usually below 10 A/m^3 for the 10- and 14-day SRT. Even though the sludge composition was not consistent, a clear linear correlation was found between the average current density and the organic loading rate (Figure 4.5A). Exoelectrogenic bacteria in bioelectrochemical systems are known to prefer acetate as an organic substrate to complicated organic compounds, such as sugars and other organic acids (Cheng & Logan, 2007; Catal et al., 2008). Even with the preference, there were no clear correlations between the average electric current density and acetate concentration (Figure 4.5B and 4.5C).

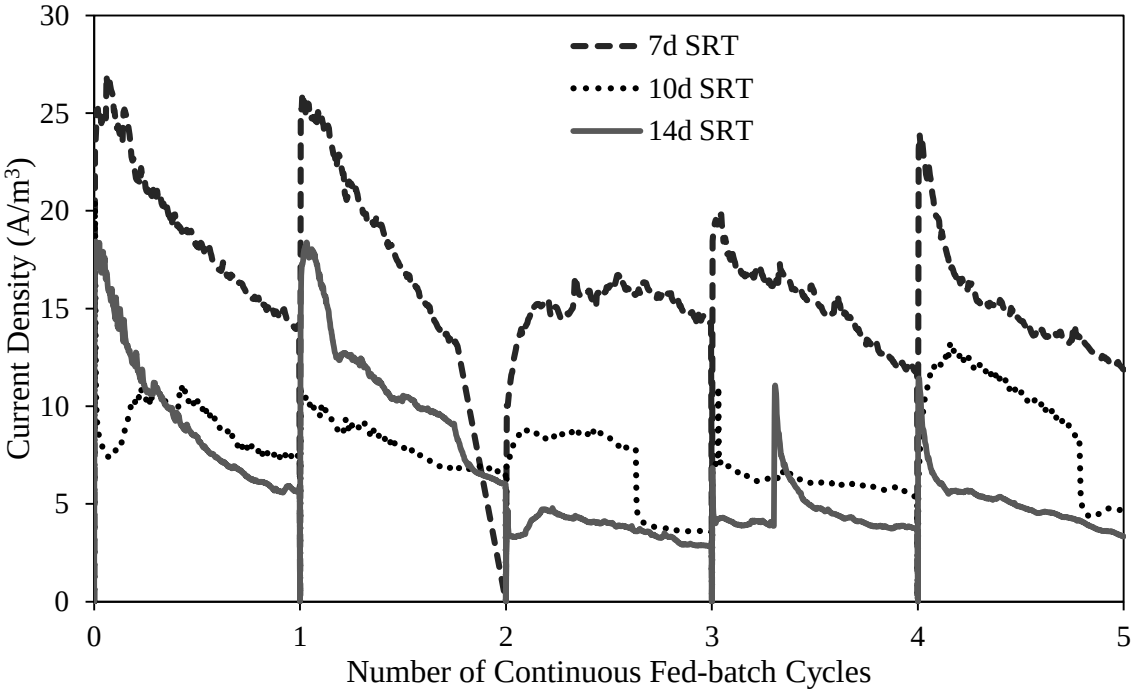


Figure 4.4: Effect of SRT on electric current generation in the EAD. The x-axis (number of continuous fed-batch cycles) was prepared by normalizing time by the length of fed batch cycle; thus, one cycle unit is 7 days (14-day SRT), 5 days (10-day SRT) and 3.5 days (7-day SRT). (Electric current density (specific current) obtained by normalizing electric current by the sludge volume in the EAD, 180 mL).

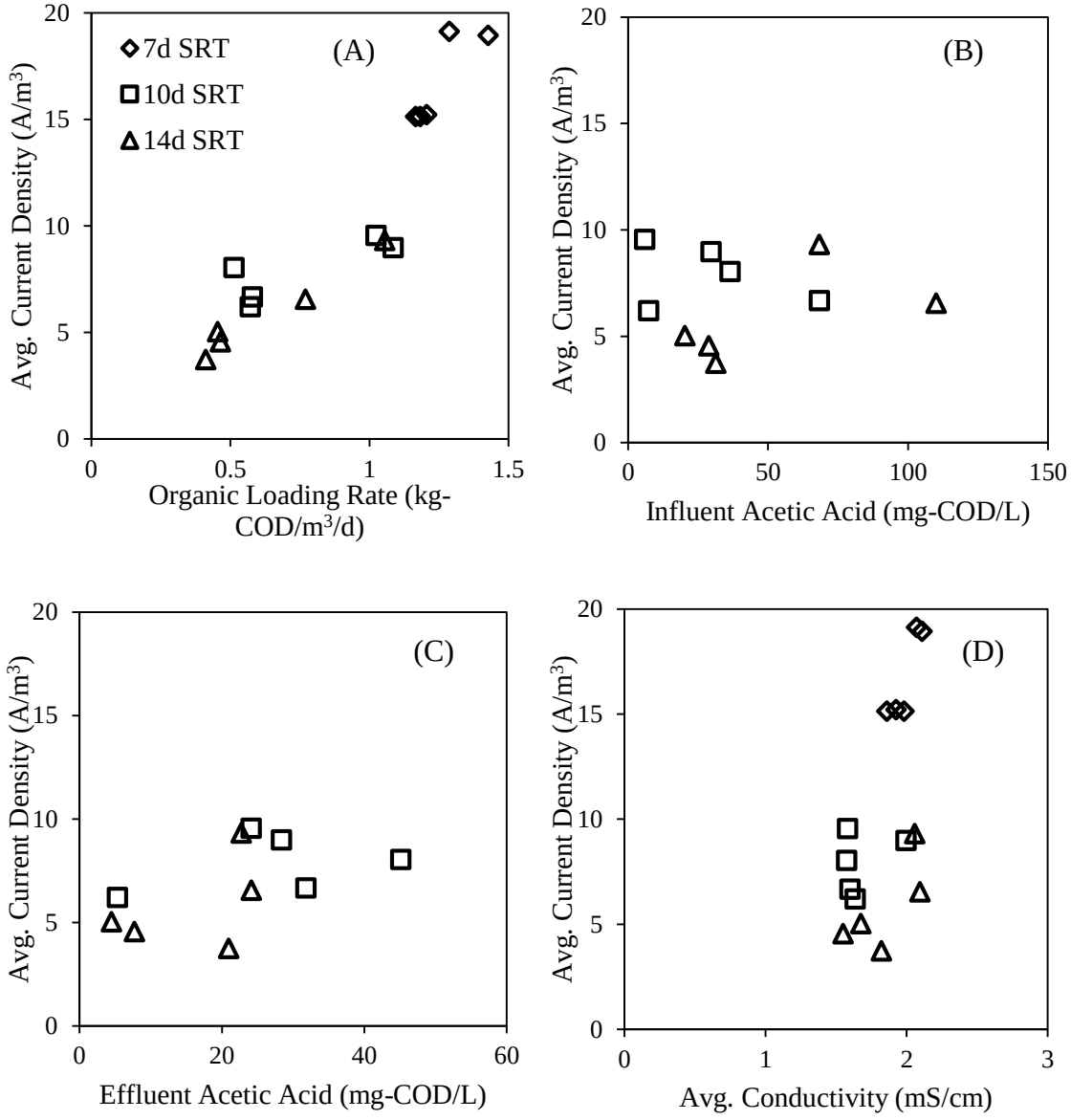


Figure 4.5: Average current density vs. (A) organic loading rate, (B) influent acetic acid concentration, (C) effluent acetic acid concentration, and (D) average conductivity. Acetic acid concentration data is from 10-day and 14-day SRT only.

The conductivity of influent sludge was consistently low 1.31 ± 0.25 mS/cm without any sludge pretreatment. The effluent conductivity in the EAD was almost

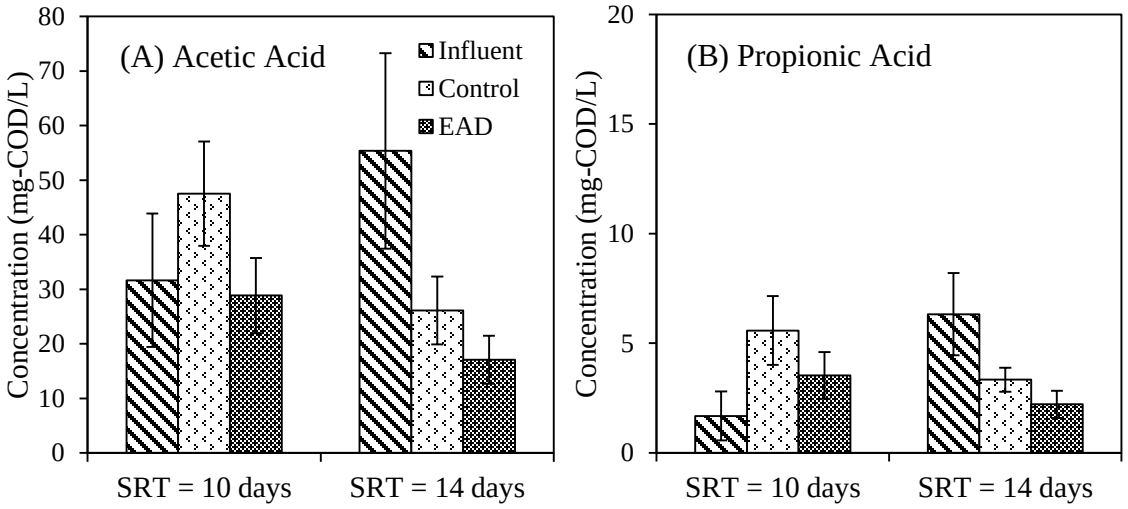
doubled in the EAD to 2.36 ± 0.23 mS/cm without any clear dependency on the SRT condition. The average current density was not also clearly correlated with the average conductivity (Figure 4.5D), indicating that the resistive loss between the bioanode and cathode did not limit the electrode reaction. With the effluent conductivity of 2.36 mS/cm and average distance of 2.0 cm between the bioandes and cathode, the resistive potential loss was 0.011 V (I = 10 A/m^3) and 0.023 V (I = 20 A/m^3), indicating the resistive potential loss was always less than 2% of the E_{ap} of 1.2 V. Thus, the reactor was effectively designed for the low conductivity of the influent.

The Coulombic efficiency (*CE*) in the EAD was 15%, 16% and 13% for 7-, 10- and 14-day SRT, respectively, indicating that the MEC reactions contributed to the total COD removal by 13-16% (Figure D.4 in Appendix D). Also, these *CE* results are relatively independent on the examined SRT conditions, and this independency can be explained by the fact that the magnitude of electric current is linearly proportional to the organic loading rate (Figure 4.5A).

4.3.3 Short-chain fatty acids

The MEC reactions in the EAD not only accelerated acetate consumption, but also improved the removal of other short-chain fatty acids (Figure 4.6). The acetic acid concentration in the EAD was consistently lower by 30 – 40% compared to the control digester (Figure 4.6A). The MEC reactions also enhanced the removal of propionic acid, iso-butyric acid and n-butyric acid (Figure 4.6B, 4.6C and 4.6D). It should be noted that

the variation for the propionic acid results is large enough such that there is also a possibility that the EAD did not enhance the removal. The enhanced removal of iso-butyric acid was most noticeable for the 10-day SRT condition, however the difference between the control digester and EAD is not significantly different for the 14-day SRT condition. The trend in removal results for n-butyric acid was found to be opposite to that of iso-butyric; 10-day results were not significantly different whereas 14-day results were. A discernable trend could not be established between exoelectrogenic activities and the removal of iso-valeric and n-valeric acid. For example, iso-valeric acid removal was not significantly enhanced by MEC reactions (Figure 4.6E), and n-valeric acid concentration was significantly lower in the EAD (2.3 mg-COD/L) compared to the control (6.9 mg-COD/L) only when the digesters were operated under a 10-day SRT (Figure 4.6F).



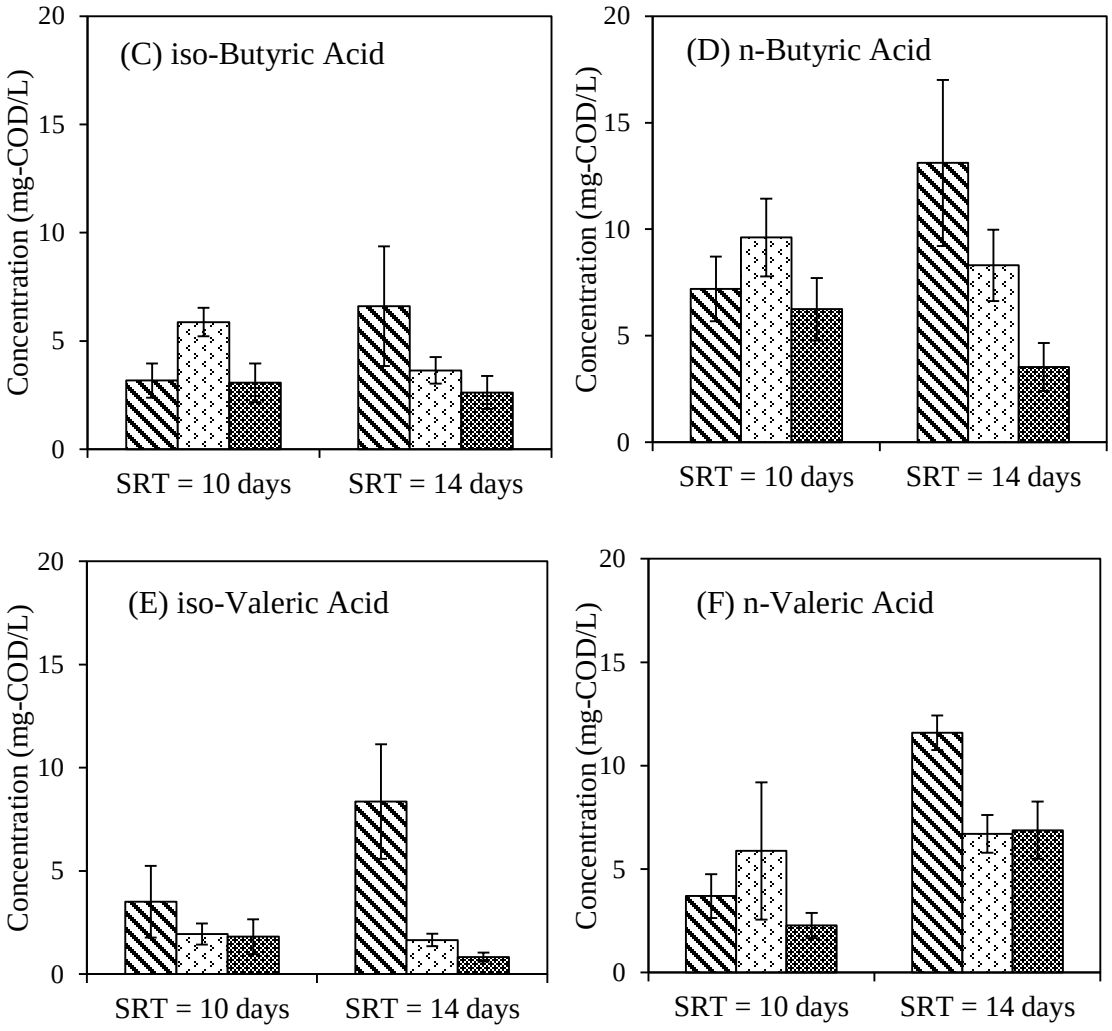


Figure 4.6: Organic acid concentrations. (A) Acetic Acid; (B) Propionic Acid; (C) iso-Butyric Acid; (D) n-Butyric Acid; (E) iso-Valeric Acid; (F) n-Valeric Acid. Data set for 7-day SRT condition was not included as there were not enough data points.

4.3.4 Biogas and energy recovery

Hydrogen gas was not detected in the GC analysis throughout the experiment and the biogas consisted primarily of CH_4 (95%) with a small fraction of CO_2 (5%) (Figure D.3 in Appendix D). In addition, this biogas composition was consistent between the

EAD and control digester. These results are similar to a recent study (Bo et al., 2014); however, the authors of this paper noted that only their MEC digester experienced a change in biogas composition.

The energy recovery (r_E) increased gradually with the increasing SRT condition (326%, 336% and 371% for the 7-, 10- and 14-day SRT, respectively) (Figure D.4 in Appendix D). The relatively high energy recovery displays that the EAD can be operated as a net energy producer while treating wastewater sludge under low temperature conditions.

4.3.5 Simulation results

The mathematical model predicted that the EAD achieves improved total COD removal only for at an SRT between 7 and 12 days (Figure 4.7A). Also, the increasing electric current density from 10 to 20 A/m^3 resulted in the improved total COD removal. The acetoclastic methanogen population (X_{ac}) is expected to be lower in the EAD than that found in the control digester (Figure 4.7B), indicating the bioanode replaces the role of acetoclastic methanogens. The hydrogenotrophic methanogen population (X_{h2}) was consistently higher in the EAD (Figure 4.7B). This difference in the hydrogenotrophic methanogen population (X_{h2}) is a direct result of the enhanced hydrogen gas production at the MEC cathode. Acetate concentration (S_{ac}) was consistently lower in the EAD due to the bioanode reaction accelerating acetate destruction (Figure 4.7C). Hydrogen gas was rapidly consumed and converted to methane gas by the hydrogenotrophic

methanogens in both the EAD and control digester. As a result, hydrogen gas concentration (S_{h2}) was always very low below 0.001 mg-COD/L (Figure 4.7D).

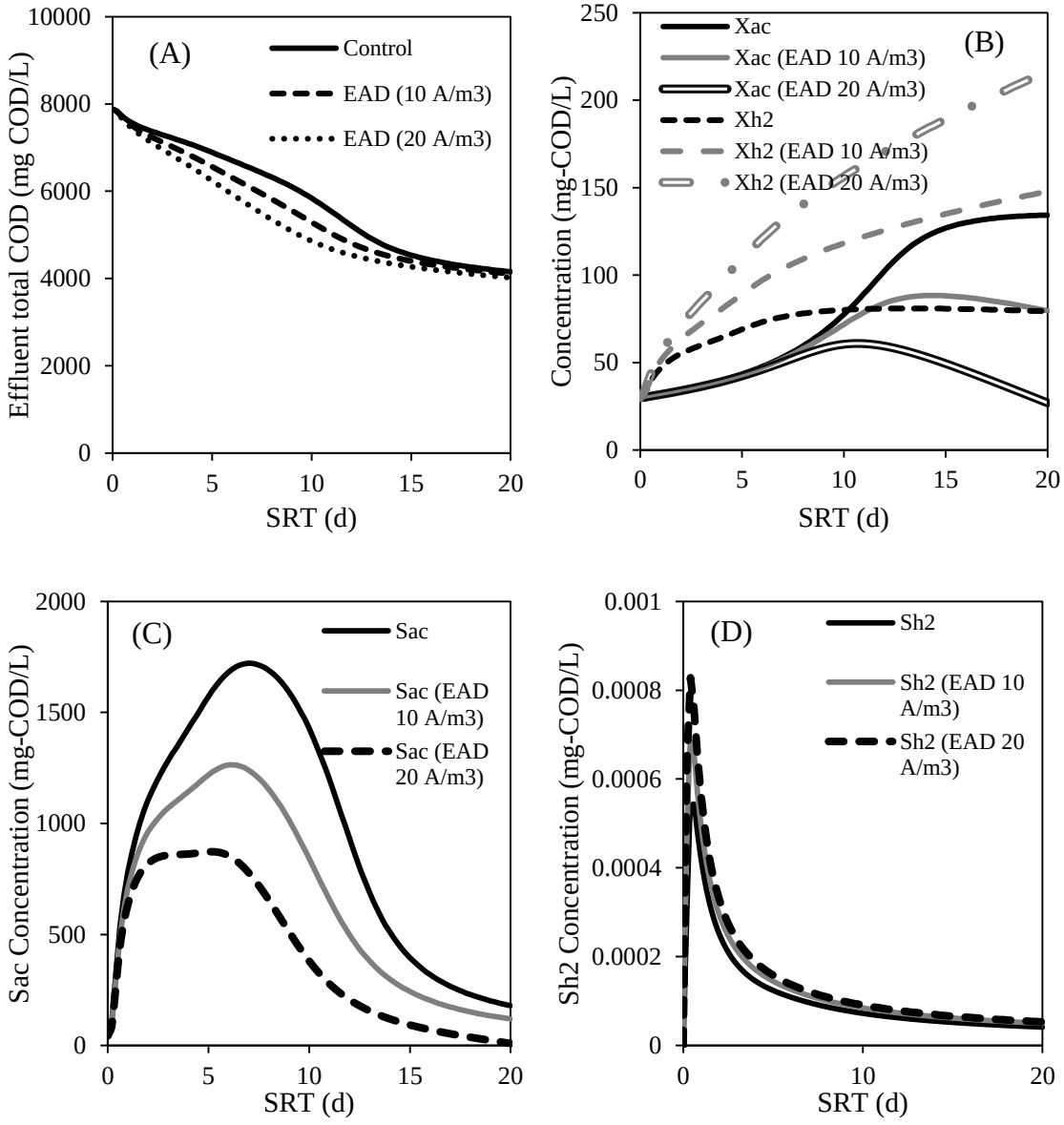


Figure 4.7: Model simulation results. (A) Effluent total COD with varying SRT; (B) change in methanogen population; (C) change in soluble acetic acid concentration; (D) change in soluble hydrogen gas concentration.

4.4 Discussion

4.4.1 MEC reactions enhancing organic acid removal

The bioanode in the EAD is known to directly oxidize acetate (Logan et al., 2008). Both the experimental results (Figure 4.6A) and mathematical model results (Figure 4.7C) show that the bioanode was successful at reducing the acetate (or acetic acid) concentration. An acetate accumulation was observed in the control digester for the 10-day SRT, indicating the rate-limiting role of acetoclastic methanogens for the short SRT condition. Both digesters showed a trend with decreasing acetic acid concentration with increasing SRT (Figure 4.6A). This trend was also found in the simulation results as the acetate concentration (S_{ac}) started to decrease for SRT longer than 7 days (Figure 4.7C).

The enhanced removal of propionic acid, iso-butyric acid and n-butyric acid in the EAD (Figure 4.6B, 4.6C and 4.6D) can be partially explained by their direct oxidation at the bioanode as exoelectrogenic bacteria are known to directly utilize the short-chain fatty acids (Cheng & Logan, 2007). However, this trend becomes less noticeable for n- and iso-valeric acid (Figure 4.6E and 4.6F), indicating that exoelectrogens do not preferably utilize valeric acids with presence of shorter chain fatty acids (such as acetic acid, propionic acid and butyric acid).

The enhanced removal of propionic acid, n-butyric acid and iso-butyric acid can also be induced indirectly via beta-oxidation as previously suggested (Asztalos and Kim, under review). Since acetate is a product of beta-oxidation reactions (Figure 4.1), a lower

concentration of acetate makes these reactions more thermodynamically spontaneous. This low acetate condition consistently found in the EAD due to the MEC reactions created more favorable conditions for microbial growth of beta-oxidizers. However, the lower acetate concentration did not always result in enhanced removal of valeric acid in the EAD. This is most likely because only 31% of valeric acid is converted to acetate (Batstone et al., 2002) whereas 54% is converted to propionic acid with the remainder becoming hydrogen gas. It is predicted that 57% of propionate and 80% of butyrate (Batstone et al., 2002) is converted to acetate, explaining why the MEC reactions had a more significant impact on beta-oxidation of propionic and butyric acids compared to valeric acids.

In the model development, we assumed that short-chain fatty acids except for acetate are not directly oxidized by the bioanode because exoelectrogenic bacteria predominantly utilize acetic acid when it is present with other short chain fatty acids. Thus, propionic acid, butyric acid and valeric acid remained relatively unchanged with the electric current in the mathematical model simulation (not shown). In addition, the yield coefficient for the growth of beta-oxidizing bacteria was also constant (Table 4.2) without considering the positive effect of low acetic acid concentration on their growth. In future work, the contribution of exoelectrogenic bacteria to simultaneous oxidation of short-chain fatty acids should be investigated with a presence of acetic acid. We also recommend that the interaction between exoelectrogenic bacteria and beta-oxidizing

bacteria via acetic acid concentration be included in model development for bioelectrochemical systems treating wastewater sludge.

4.4.2 High purity methane biogas

The biogas composition found from both digesters contained a high fraction of CH_4 gas (95%). In many recent studies, it has been noted that the introduction of MEC reactions do not invoke a change in the overall gas composition (Asztalos and Kim, under review; Sun et al., 2015; Feng et al., 2015). In these previous studies, the biogas composition found was typical to that found from conventional anaerobic digestion systems (i.e. ~65% CH_4 and ~35% CO_2). However, in this study, the collected biogas contained consistently high CH_4 fraction (93% in the EAD and 95% in the control). This high CH_4 content is significantly different compared to our recent study (60% in the EAD and 40% in the control under mesophilic condition, Asztalos and Kim, under review). Psychrophilic conditions are not expected to have a significant impact on the biogas composition as a previous study reported biogas compositions similar to those collected from mesophilic anaerobic digesters (Connaughton et al., 2006). Therefore, the observed high CH_4 content in our experiments cannot be attributed to the low temperature.

Another potential reason for the different CH_4 content from our previous work is the sludge composition as secondary sludge was used in this study while a mixture of primary and secondary sludge was used in our previous work (Asztalos and Kim, under review). Digestion of agricultural and food wastes typically result in a high yield of

methane gas (Zhang et al., 2014; Rincon et al., 2010) because they contain large amounts of lipids and other polymeric substances which stoichiometrically lead to higher CH_4 content. Although no evident explanation was provided, the substantially high CH_4 content of 95% in the biogas implies that the secondary sludge used in this study contained a large amount of complex lipids and polymeric substances compared to primary sludge. Based on this discussion, we suggest that secondary sludge be digested separately from primary sludge to produce high purity CH_4 gas from digestion.

4.4.3 MEC reactions supplementing low temperature digestion

With the induced MEC reactions, the EAD improved COD removal by up to 10% compared to that in the control digester at an SRT of 7 days (Figure 4.3). In order to achieve the same degree of improvement (i.e., additional 10% COD removal) by increasing operation temperature, the control digester would need to be operated at a temperature of ~35°C (Figure 4.8). The total electrical energy input for the EAD per 7 day SRT cycle was approximately 1.0 kJ which is greater than energy required to heat a liquid volume of ~180 mL from 20 to 35°C (0.75 kJ). However, considering an additional energy requirement for maintaining temperature at 35°C for 7 days, an EAD operating at psychrophilic temperatures (~20°C) can substantially save the cost for digester operation with minimized heating energy input while it achieves a similar degree of sludge digestion to a conventional digester operated at mesophilic temperatures (35°C).

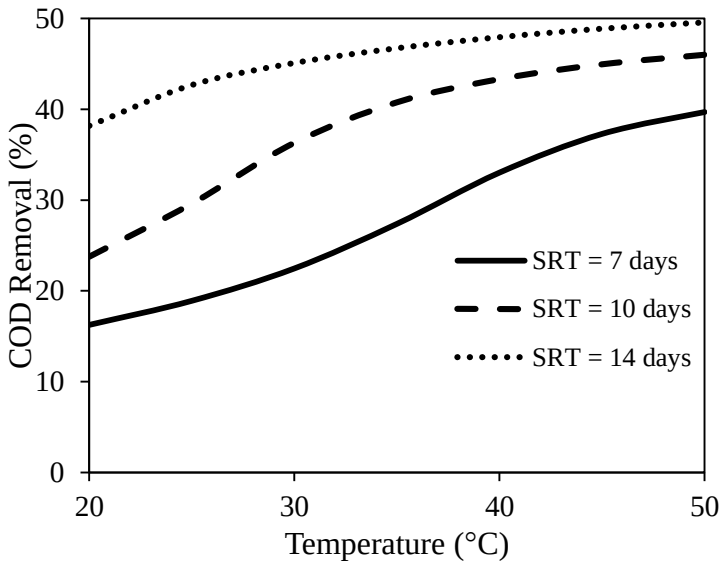


Figure 4.8: Model simulation results for varying temperature and SRT (control digester).

4.5 Conclusions

The electrically-assisted digester (EAD) expedited the VSS and COD removal by 5 – 10% compared to the control digester for the 7- and 14-day SRT condition. The trends in VSS and COD removals indicate that when MEC reactions a present, SRT has less of an effect on the performance of the digester. The MEC reactions in the EAD reduced the steady-state concentration of short chain fatty acids, such as acetic acid, propionic acid, n-butyric acid and iso-butyric acid. Exoelectrogenic bacteria are considered to directly contribute to oxidation of these short-chain fatty acids. In addition, the reduced acetate concentration in the EAD also made beta-oxidation reactions more thermodynamically favorable, indirectly enhancing the degradation of the organic acids. The biogas from both digesters consisted mainly of methane gas (~95%) due to the high concentration of lipids and other complex polymeric substances in the influent. By

comparing the experimental and mathematical simulation results, under psychrophilic temperatures (20°C) the EAD performed similar to a conventional digester operating at mesophilic conditions (35°C). These results suggest that an EAD can substantially reduce heating requirements for efficient digestion.

4.6 Acknowledgements

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5. Conclusions

5.1 Electrically-assisted digestion at mesophilic temperature

When operated at mesophilic temperatures (39°C) the electrically-assisted digester (EAD) was successful at improving VSS and COD removal, removing 55% VSS and 61% COD when operated at a 6-day SRT. These results indicate that the MEC reaction was successful at supplementing the role of acetoclastic methanogens which was substantially limited at a 6-day SRT condition. The mathematical model results were consistent with the experimental results. The improved VSS removal can be indirectly attributed to the reduced acetate concentration (S_{ac}). Since acetate is a product of acidogenic reactions (fermentation and beta-oxidation), the reduced acetate concentration made these reactions thermodynamically spontaneous and thus provided a favorable environment for acidogenic bacteria growth (McCarty, 1975). Since hydrolysis is known to be driven by enzymes excreted by acidogenic bacteria, the rate of VSS removal was increased in the EAD.

The EAD was found to not be effective at improving COD and VSS removal at the 2-day and 14-day SRT condition. When the digesters were operated at a 14-day SRT condition, the acetoclastic methanogen population was sufficiently enriched. As such, the contributions made by the MEC reactions did not improve the overall VSS and COD removal. When operated under a 2-day SRT condition, other biological reactions (e.g., hydrolysis) limit the overall rate of biosolids destruction and thus restrict the additional acetate removal by the MEC reactions. These experimental results demonstrate that the

MEC reactions integrated in conventional anaerobic digesters can improve biosolids destruction for a certain range of the SRT. The model simulation indicated that the effective SRT range was between 3 and 7 days.

5.2 Electrically-assisted digestion at psychrophilic temperature

The EAD improved the removal of VSS and COD by 5 – 10% compared to the control digester for the 7-day and 14-day SRT conditions when operated under psychrophilic temperatures (22°C). In contrast to the control digester, the EAD's performance was found to be less depended on the SRT. The MEC reactions were successful at reducing the concentration of acetic acid. Additionally, the MEC reactions reduced the steady-state concentration of propionic acid, n-butyric acid, and iso-butyric acid. The biogas from both digesters consisted mainly of methane gas (~95%) which was due to high contents of lipids and other complex polymeric substances in the secondary sludge. By comparing the experimental and simulation results, the EAD operated under psychrophilic temperatures performed similarly to a conventional digester operating under mesophilic temperatures. Thus, an EAD can be a potential method for wastewater sludge digestion without the expensive heating requirement.

5.3 Significance

The primary objectives of this research were to:

1) Demonstrate and prove the EAD concept using lab-scale digesters;

2) Evaluate the performance of the digesters by analyzing VSS, COD and short chain fatty acids;
3) Examine the effectiveness of the EAD under mesophilic and psychrophilic conditions;
4) Examine combined sludge (both primary and secondary sludge) and secondary waste activated sludge; and
5) Determine the energy requirement for enhanced digestion.

This research clearly demonstrated that the MEC reactions can be integrated into lab-scale anaerobic digesters to enhance the biosolids destruction performance. The EAD performance was found to be less sensitive to SRT conditions, temperature changes and varying influent compositions than that of the conventional digester. Thus, this research presents a new way to make anaerobic digestion robust and rapid.

5.4 Future work

Since this research was conducted with lab-scale digesters, future work should consider integrating the MEC components into larger anaerobic digesters. Investigation of sludge pretreatment is also necessary. Such pretreatment methods need to be focused on increasing the rate of hydrolysis, making acetoclastic methanogenesis the evident rate-limiting reaction that the MEC reactions can further supplement. Operation of the EAD under thermophilic temperatures (>50°C) can also be another way to substantially accelerated hydrolysis.

The steady-state mathematical model needs additional work to thoroughly include the effect MEC reactions have on the concentration of other soluble organics. In this work, the yield coefficient for the growth of beta-oxidizing bacteria was constant and not affected by low acetic acid concentration. Thus, future work should consider developing more sophisticated models to include the interaction between the growth of beta-oxidizing bacteria and MEC bioanode reaction. It is also recommended that future work include the direct contribution exoelectrogenic bacteria have with the oxidation of various short-chain fatty acids.

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Appendix A: Microbial Kinetics

Table A1: Kinetic rate expressions used in the mathematical model. The equations shown were reproduced using the equations presented for ADM1 (Batstone et al., 2002). A rate expression (R) can be obtained using the following: $\boldsymbol{R_i = \sum_{i,j=1}^{j=19} r_{i,j} \times Rate_{i,j}}$.

	Component → **i**	1	2	3	4	5	6	7	8	9	
j	**Process ↓**	**S_{su}**	**S_{aa}**	**S_{fa}**	**S_{va}**	**S_{bu}**	**S_{pro}**	**S_{ac}**	**S_{h2}**	**S_{ch4}**	**Rate (mg COD/L/d)**
1	Disintegration										$k_{dis}X_c$
2	Hydrolysis of carbohydrates	1									$k_{hyd,ch}X_{ch}$
3	Hydrolysis of proteins		1								$k_{hyd,pr}X_{pr}$
4	Hydrolysis of lipids	$1-f_{fa,li}$		$f_{fa,li}$							$k_{hyd,li}X_{li}$
5	Uptake of sugars	-1				$(1-Y_{su})f_{bu,su}$	$(1-Y_{su})f_{pro,su}$	$(1-Y_{su})f_{ac,su}$	$(1-Y_{su})f_{h2,su}$		$k_{su}\frac{S_{su}}{K_{s,su}+S_{su}}X_{su}I_1$
6	Uptake of amino acids		-1		$(1-Y_{aa})f_{va,aa}$	$(1-Y_{aa})f_{bu,aa}$	$(1-Y_{aa})f_{pro,aa}$	$(1-Y_{aa})f_{ac,aa}$	$(1-Y_{aa})f_{h2,aa}$		$k_{aa}\frac{S_{aa}}{K_{s,aa}+S_{aa}}X_{aa}I_1$
7	Uptake of LCFA			-1				$(1-Y_{fa})0.7$	$(1-Y_{fa})0.3$		$k_{fa}\frac{S_{fa}}{K_{s,fa}+S_{fa}}X_{fa}I_2$
8	Uptake of valerate				-1		$(1-Y_{c4})0.54$	$(1-Y_{c4})0.31$	$(1-Y_{c4})0.15$		$k_{c4}\frac{S_{va}}{K_{s,c4}+S_{va}}X_{c4}\frac{1}{1+{S_{bu}}/{S_{va}}}I_2$
9	Uptake of butyrate					-1		$(1-Y_{c4})0.8$	$(1-Y_{c4})0.2$		$k_{c4}\frac{S_{bu}}{K_{s,c4}+S_{bu}}X_{c4}\frac{1}{1+{S_{va}}/{S_{bu}}}I_2$
10	Uptake of propionate						-1	$(1-Y_{pro})0.57$	$(1-Y_{pro})0.43$		$k_{pro}\frac{S_{pro}}{K_{s.pro}+S_{pro}}X_{pro}I_2$
11	Uptake of acetate							-1		$(1-Y_{ac})$	$k_{ac}\frac{S_{ac}}{K_{s.ac}+S_{ac}}X_{ac}I_3$
12	Uptake of hydrogen								-1	$(1-Y_{h2})$	$k_{h2}\frac{S_{h2}}{K_{s.h2}+S_{h2}}X_{h2}I_1$
13	Decay of X_{su}										$k_{dec,Xsu}X_{su}$
14	Decay of X_{aa}										$k_{dec,Xaa}X_{aa}$
15	Decay of X_{fa}										$k_{dec,Xfa}X_{fa}$
16	Decay of X_{c4}										$k_{dec,Xc4}X_{c4}$
17	Decay of X_{pro}										$k_{dec,Xpro}X_{pro}$
18	Decay of X_{ac}										$k_{dec,Xac}X_{ac}$
19	Decay of X_{h2}										$k_{dec,Xh2}X_{h2}$

Table A1 (Continued)

j	Component → i / Process ↓	10 X_c	11 X_{ch}	12 X_{pr}	13 X_{li}	14 X_{su}	15 X_{aa}	16 X_{fa}	17 X_{c4}	18 X_{pro}	19 X_{ac}	20 X_{h2}	21 X_i	Rate (mg COD/L/d)
1	Disintegration	-1	$f_{ch,xc}$	$f_{pr,xc}$	$f_{li,xc}$								$f_{i,xc}$	$k_{dis}X_c$
2	Hydrolysis of carbohydrates		-1											$k_{hyd,ch}X_{ch}$
3	Hydrolysis of proteins			-1										$k_{hyd,pr}X_{pr}$
4	Hydrolysis of lipids				-1									$k_{hyd,li}X_{li}$
5	Uptake of sugars					Y_{su}								$k_{su}\frac{S_{su}}{K_{s,su}+S_{su}}X_{su}I_1$
6	Uptake of amino acids						Y_{aa}							$k_{aa}\frac{S_{aa}}{K_{s,aa}+S_{aa}}X_{aa}I_1$
7	Uptake of LCFA							Y_{fa}						$k_{fa}\frac{S_{fa}}{K_{s,fa}+S_{fa}}X_{fa}I_2$
8	Uptake of valerate								Y_{c4}					$k_{c4}\frac{S_{va}}{K_{s,c4}+S_{va}}X_{c4}\frac{1}{1+S_{bu}/S_{va}}I_2$
9	Uptake of butyrate								Y_{c4}					$k_{c4}\frac{S_{bu}}{K_{s,c4}+S_{bu}}X_{c4}\frac{1}{1+S_{va}/S_{bu}}I_2$
10	Uptake of propionate									Y_{pro}				$k_{pro}\frac{S_{pro}}{K_{s.pro}+S_{pro}}X_{pro}I_2$
11	Uptake of acetate										Y_{ac}			$k_{ac}\frac{S_{ac}}{K_{s.ac}+S_{ac}}X_{ac}I_3$
12	Uptake of hydrogen											Y_{h2}		$k_{h2}\frac{S_{h2}}{K_{s.h2}+S_{h2}}X_{h2}I_1$
13	Decay of X_{su}	1				-1								$k_{dec,Xsu}X_{su}$
14	Decay of X_{aa}	1					-1							$k_{dec,Xaa}X_{aa}$
15	Decay of X_{fa}	1						-1						$k_{dec,Xfa}X_{fa}$
16	Decay of X_{c4}	1							-1					$k_{dec,Xc4}X_{c4}$
17	Decay of X_{pro}	1								-1				$k_{dec,Xpro}X_{pro}$
18	Decay of X_{ac}	1									-1			$k_{dec,Xac}X_{ac}$
19	Decay of X_{h2}	1										-1		$k_{dec,Xh2}X_{h2}$

$$I_1 = I_{ph}I_{IN,lim}$$

$$I_2 = I_{ph}I_{IN,lim}I_{h2}$$

$$I_{h2} = \frac{1}{1+\frac{S_{h2}}{K_I}}$$

$$I_3 = I_{ph}I_{IN,lim}I_{NH3,Xac}$$

Appendix B: Steady-state Mass Balance Equations in Fixed Point Iteration

B1. Mass balance on composites X_c

$$X_c = \frac{\frac{1}{\theta}X_{c,in} + \begin{bmatrix} k_{dec,Xsu}X_{su} + k_{dec,Xaa}X_{aa} + k_{dec,Xfa}X_{fa} + k_{dec,Xc4}X_{c4} \\ +k_{dec,Xpro}X_{pro} + k_{dec,Xac}X_{ac} + k_{dec,Xh2}X_{h2} \end{bmatrix}}{\left[\frac{1}{\theta} + k_{dis}\right]}$$

B2. Mass balance on particulate inerts X_i

$$X_i = \frac{\frac{1}{\theta}X_{i,in} + f_{i,xc}k_{dis}X_c}{\frac{1}{\theta}}$$

B3. Mass balance on carbohydrates X_{ch}

$$X_{ch} = \frac{\frac{1}{\theta}X_{ch,in} + \left[f_{ch,xc}k_{dis}X_c\right]}{\frac{1}{\theta} + k_{hyd,ch}}$$

B4. Mass balance on proteins X_{pr}

$$X_{pr} = \frac{\frac{1}{\theta}X_{pr,in} + \left[f_{pr,xc}k_{dis}X_c\right]}{\frac{1}{\theta} + k_{hyd,pr}}$$

B5. Mass balance on lipids X_{li}

$$X_{li} = \frac{\frac{1}{\theta}X_{li,in} + \left[f_{li,xc}k_{dis}X_c\right]}{\frac{1}{\theta} + k_{hyd,li}}$$

B6. Mass balance on monosaccharides (sugars) degraders X_{su}

$$X_{su} = \frac{\frac{1}{\theta} X_{su,in} + Y_{su} k_{su} \frac{S_{su}}{K_{s,su} + S_{su}} X_{su} I_1}{\left[\frac{1}{\theta} + k_{dec,Xsu}\right]}$$

B7. Mass balance on amino acid degraders X_{aa}

$$X_{aa} = \frac{\frac{1}{\theta} X_{aa,in} + Y_{aa} k_{aa} \frac{S_{aa}}{K_{s,aa} + S_{aa}} X_{aa} I_1}{\left[\frac{1}{\theta} + k_{dec,Xaa}\right]}$$

B8. Mass balance on long-chain fatty acid (LCFA) degraders X_{fa}

$$X_{fa} = \frac{\frac{1}{\theta} X_{fa,in} + Y_{fa} k_{fa} \frac{S_{fa}}{K_{s,fa} + S_{fa}} X_{fa} I_2}{\left[\frac{1}{\theta} + k_{dec,Xfa}\right]}$$

B9. Mass balance on valerate and butyrate Degraders X_{c4}

$$X_{c4} = \frac{\frac{1}{\theta} X_{c4,in} + Y_{c4} k_{c4} \frac{S_{va}}{K_{s,c4} + S_{va}} X_{c4} \frac{1}{1 + S_{bu}/S_{va}} I_2 + Y_{c4} k_{c4} \frac{S_{bu}}{K_{s,c4} + S_{bu}} X_{c4} \frac{1}{1 + S_{va}/S_{bu}} I_2}{\left[\frac{1}{\theta} + k_{dec,Xc4}\right]}$$

B10. Mass balance on propionate degraders X_{pro}

$$X_{pro} = \frac{\frac{1}{\theta} X_{pro,in} + Y_{pro} k_{pro} \frac{S_{pro}}{K_{s,pro} + S_{pro}} X_{pro} I_2}{\left[\frac{1}{\theta} + k_{dec,Xpro}\right]}$$

B11. Mass balance on acetoclastic methanogens X_{ac}

$$X_{ac} = \frac{\frac{1}{\theta} X_{ac,in} + Y_{ac} k_{ac} \frac{S_{ac}}{K_{s,ac} + S_{ac}} X_{ac} I_3}{\left[\frac{1}{\theta} + k_{dec,Xac}\right]}$$

B12. Mass balance on hydrogenotrophic methanogens X_{h2}

$$X_{h2} = \frac{\frac{1}{\theta} X_{h2,in} + Y_{h2} k_{h2} \frac{S_{h2}}{K_{s,h2} + S_{h2}} X_{h2} I_1}{\left[\frac{1}{\theta} + k_{dec,Xh2}\right]}$$

B13. Mass balance on monosaccharides S_{su}

$$S_{su} = \frac{\frac{1}{\theta} S_{su,in} + \left[k_{hyd,ch} X_{ch} + \left(1 - f_{fa,li}\right) k_{hyd,li} X_{li}\right]}{\frac{1}{\theta} + k_{su} \frac{1}{K_{s,su} + S_{su}} X_{su} I_1}$$

B14. Mass balance on amino acids S_{aa}

$$S_{aa} = \frac{\frac{1}{\theta} S_{aa,in} + \left[k_{hyd,pr} X_{pr}\right]}{\frac{1}{\theta} + k_{aa} \frac{1}{K_{s,aa} + S_{aa}} X_{aa} I_1}$$

B15. Mass balance on LCFA S_{fa}

$$S_{fa} = \frac{\frac{1}{\theta} S_{fa,in} + \left[f_{fa,li} k_{hyd,li} X_{li}\right]}{\frac{1}{\theta} + k_{fa} \frac{1}{K_{s,fa} + S_{fa}} X_{fa} I_2}$$

B16. Mass balance on valerate S_{va}

$$S_{va} = \frac{\frac{1}{\theta} S_{va,in} + \left[(1 - Y_{aa}) f_{va,aa} k_{aa} \frac{S_{aa}}{K_{s,aa} + S_{aa}} X_{aa} I_1\right]}{\frac{1}{\theta} + k_{c4} \frac{1}{K_{s,c4} + S_{va}} X_{c4} \frac{1}{1 + {S_{bu}}/{S_{va}}} I_2}$$

B17. Mass balance on butyrate S_{bu}

$$S_{bu} = \frac{\frac{1}{\theta}S_{bu,in} + \begin{bmatrix} (1-Y_{su})f_{bu,su}k_{su}\frac{S_{su}}{K_{s,su}+S_{su}}X_{su}I_1 \\ +(1-Y_{aa})f_{bu,aa}k_{aa}\frac{S_{aa}}{K_{s,aa}+S_{aa}}X_{aa}I_1 \end{bmatrix}}{\frac{1}{\theta} + k_{c4}\frac{1}{K_{s,c4}+S_{bu}}X_{c4}\frac{1}{1+{S_{va}}/{S_{bu}}}I_2}$$

B18. Mass balance on propionate S_{pro}

$$S_{pro} = \frac{\frac{1}{\theta}S_{pro,in} + \begin{bmatrix} (1-Y_{su})f_{pro,su}k_{su}\frac{S_{su}}{K_{s,su}+S_{su}}X_{su}I_1 \\ +(1-Y_{aa})f_{pro,aa}k_{aa}\frac{S_{aa}}{K_{s,aa}+S_{aa}}X_{aa}I_1 \\ +(1-Y_{c4})0.54k_{c4}\frac{S_{va}}{K_{s,c4}+S_{va}}X_{c4}\frac{1}{1+{S_{bu}}/{S_{va}}}I_2 \end{bmatrix}}{\frac{1}{\theta} + k_{pro}\frac{1}{K_{s.pro}+S_{pro}}X_{pro}I_2}$$

B19. Mass balance on acetate S_{ac} (including the MEC bioanode reaction)

$$S_{ac} = \frac{\frac{1}{\theta}S_{ac,in} + \begin{bmatrix} (1-Y_{su})f_{ac,su}k_{su}\frac{S_{su}}{K_{s,su}+S_{su}}X_{su}I_1 \\ +(1-Y_{aa})f_{ac,aa}k_{aa}\frac{S_{aa}}{K_{s,aa}+S_{aa}}X_{aa}I_1 \\ +(1-Y_{fa})0.7k_{fa}\frac{S_{fa}}{K_{s,fa}+S_{fa}}X_{fa}I_2 \\ +(1-Y_{c4})0.31k_{c4}\frac{S_{va}}{K_{s,c4}+S_{va}}X_{c4}\frac{1}{1+S_{bu}/S_{va}}I_2 \\ +(1-Y_{c4})0.8k_{c4}\frac{S_{bu}}{K_{s,c4}+S_{bu}}X_{c4}\frac{1}{1+S_{va}/S_{bu}}I_2 \\ +(1-Y_{pro})0.57k_{pro}\frac{S_{pro}}{K_{s.pro}+S_{pro}}X_{pro}I_2 \end{bmatrix} - \frac{6.912\times10^8}{F}\frac{I}{V}}{\frac{1}{\theta}+k_{ac}\frac{1}{K_{s.ac}+S_{ac}}X_{ac}I_3}$$

B20. Mass balance on hydrogen gas S_{h2} (Including the MEC cathode reaction)

$$S_{h2} = \frac{\begin{bmatrix} \frac{1}{\theta}S_{h2,in} + (1-Y_{su})f_{h2,su}k_{su}\frac{S_{su}}{K_{s,su}+S_{su}}X_{su}I_1 \\ +(1-Y_{aa})f_{h2,aa}k_{aa}\frac{S_{aa}}{K_{s,aa}+S_{aa}}X_{aa}I_1 \\ +(1-Y_{fa})0.3k_{fa}\frac{S_{fa}}{K_{s,fa}+S_{fa}}X_{fa}I_2 \\ +(1-Y_{c4})0.15k_{c4}\frac{S_{va}}{K_{s,c4}+S_{va}}X_{c4}\frac{1}{1+S_{bu}/S_{va}}I_2 \\ +(1-Y_{c4})0.2k_{c4}\frac{S_{bu}}{K_{s,c4}+S_{bu}}X_{c4}\frac{1}{1+S_{va}/S_{bu}}I_2 \\ +(1-Y_{pro})0.43k_{pro}\frac{S_{pro}}{K_{s.pro}+S_{pro}}X_{pro}I_2 \end{bmatrix} + \frac{6.912\times10^8}{F}\frac{I}{V}}{\frac{1}{\theta}+k_{h2}\frac{1}{K_{s.h2}+S_{h2}}X_{h2}I_1}$$

B21. Mass balance on methane gas S_{ch4}

$$S_{ch4} = \frac{\frac{1}{\theta} S_{ch4,in} + \left[(1 - Y_{ac}) k_{ac} \frac{S_{ac}}{K_{s,ac} + S_{ac}} X_{ac} I_3 + (1 - Y_{h2}) k_{h2} \frac{S_{h2}}{K_{s,h2} + S_{h2}} X_{h2} I_1 \right]}{\frac{1}{\theta}}$$

Appendix C: Model Validation Results

Table C1: Steady-state model results for model. Validation with "Aspects on ADM1 implementation within the BSM2 framework". (Rosen & Jeppsson, 2006) SRT = 20 d; T = 35°C. All kinetic constants used were the same as those used by Rosen & Jeppsson, 2006.

Component	Influent (mg-COD/L)	Effluent (mg-COD/L) This study	Effluent (mg-COD/L) Rosen & Jeppsson, 2006
Xc	2000	308.96	308.70
Xin	25000	25926.9	25617.4
Xch	5000	27.95	27.95
Xpr	20000	102.58	102.57
Xli	5000	29.49	29.48
Xsu	10	427.06	420.17
Xaa	10	1179.20	1179.17
Xfa	10	243.07	243.04
Xc4	10	431.91	431.92
Xpro	10	137.28	137.31
Xac	10	760.78	760.56
Xh2	10	317.00	317.02
Ssu	10	6.98	11.95
Saa	1	5.31	5.317
Sfa	1	98.52	98.62
Sva	1	11.62	11.63
Sbu	1	13.24	13.25
Spro	1	15.77	15.78
Sac	1	190.30	197.63
Sh2	1.0×10^{-5}	2.36×10^{-4}	2.36×10^{-4}

Appendix D: Additional Experimental and Statistical Results

Table D1: Reported p-values for VSS and COD related results found in Chapters 3 and 4. It was assumed that the data was normally distributed and that the data sets were independent. The p-value was calculated from a T-score using a significance level of 0.05.

Chapter 3 (p-value)		
SRT (days)	**VSS Removal**	**COD Removal**
2	0.73705	0.78452
6	0.299848	0.105747
14	0.340913	0.311369
Chapter 4 (p-value)		
SRT (days)	**VSS Concentration**	**COD Concentration**
7	0.002353	0.03848
10	0.595309	0.568096
14	0.297303	0.442235

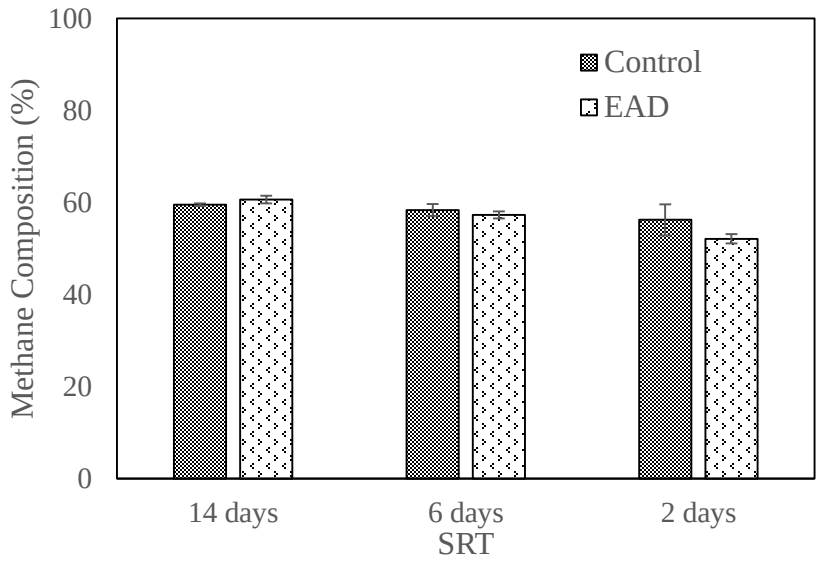

Figure D.1: Methane composition of biogas found from the control digester and EAD from Chapter 3.

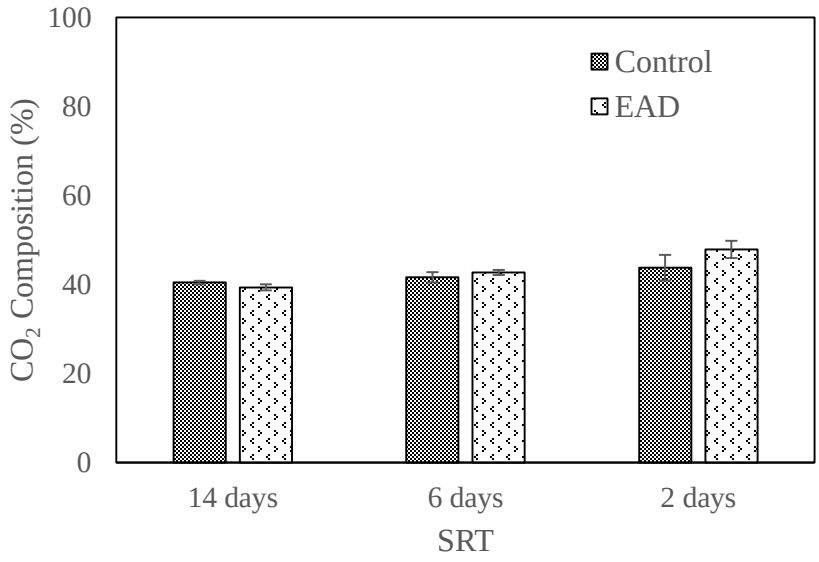

Figure D.2: CO_2 composition of biogas found from the control digester and EAD from Chapter 3.

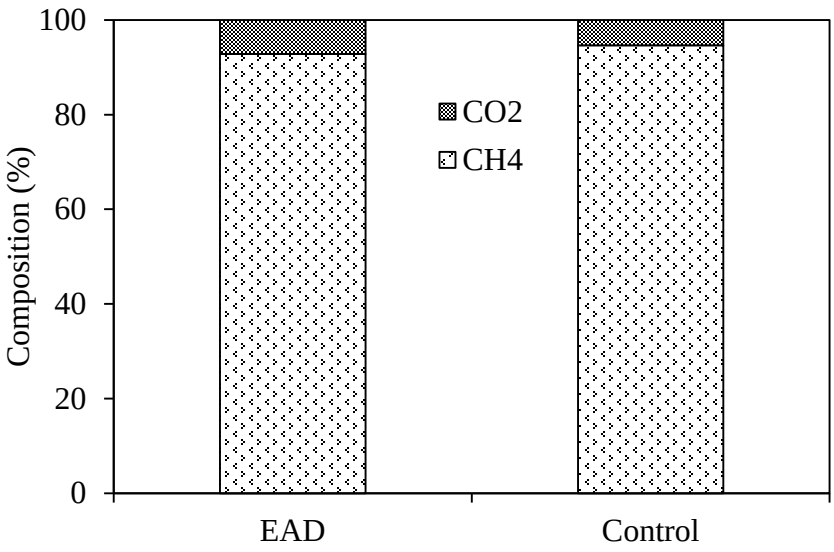

Figure D.3: Biogas composition found in EAD and control when fed secondary waste activated sludge (WAS).

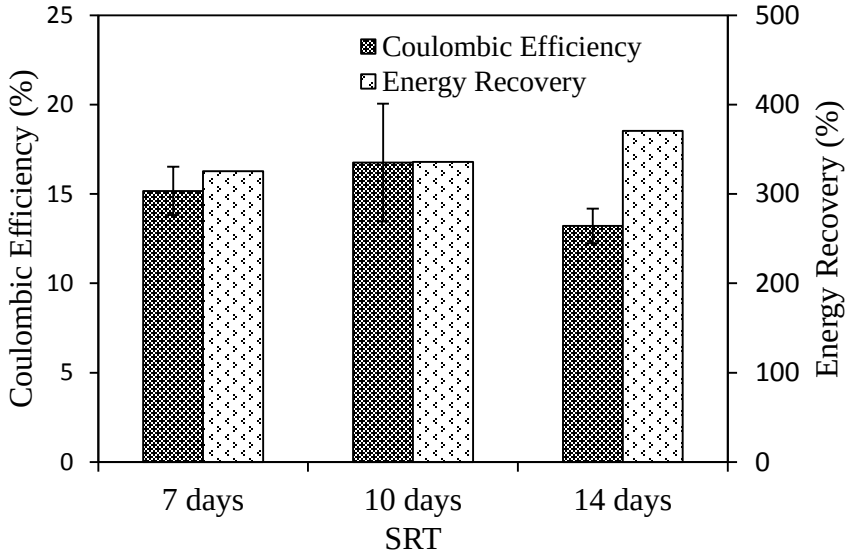

Figure D.4: Coulombic efficiency (CE) and energy recovery (r_E) of EAD under psychrophilic operation.