Low-Temperature Anaerobic Membrane Bioreactor for Energy Recovery from Domestic Wastewater

by

Adam L. Smith

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Doctoral Committee:

Professor Lutgarde Raskin, Co-chair
Professor Steven Skerlos, Co-chair
Professor Nancy Love
Professor Stephen Ragsdale
Professor Willy Verstraete, Ghent University

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Abstract

Anaerobic membrane bioreactor (AnMBR) treatment, which combines the anaerobic microbial conversion of organic compounds into methane-rich biogas with membrane separation of treated wastewater and microbial biomass, has been proposed for direct energy recovery from domestic wastewater. We demonstrated in a bench-scale investigation that AnMBR can achieve 92 ± 5% chemical oxygen demand (COD) removal at 15°C, but that dissolved methane in the permeate represents 40-50% of the total methane produced. If unrecovered, this methane is a lost energy source and results in substantial greenhouse gas emissions. This work motivated an evaluation of the trade-offs between the membrane biofilm's role in treatment and its contribution to fouling. We demonstrated that the development of a biofilm enriched in active syntrophic bacteria and methanogens significantly improved effluent quality, while maintaining acceptable fluxes. However, methanogenesis in the biofilm resulted in substantial levels of dissolved methane in the permeate. The lower temperature limit of AnMBR treatment was explored by sequentially lowering the operating temperature of the system from 15, 12, 9, 6, to 3°C under conditions supporting biofilm treatment. COD removal > 95% was achieved at temperatures as low as 6°C. COD removal fell to 86 ± 4.0% at 3°C and, at this temperature, essentially all COD removal occurred in the biofilm, suggesting that the biofilm was less inhibited by temperature decreases than the suspended biomass. Finally, we evaluated the life cycle environmental and economic impacts of AnMBR technology compared to aerobic treatment systems. AnMBR will not be net energy positive in the foreseeable future without reduction in fouling control energy demands.

Currently, AnMBR is better suited for higher strength domestic wastewater treatment. Further, global warming impacts were over an order of magnitude higher than aerobic systems arising from the direct emission of effluent dissolved methane. Future research is necessary to (1) promote increased biological activity in suspended biomass at low temperatures such that membrane biofilm treatment is reduced and dissolved methane oversaturation avoided, (2) develop low-energy dissolved methane recovery technologies to limit global warming impacts, and (3) establish fouling control strategies that reduce energy demands thereby improving the net energy balance.

Chapter 1. Introduction

1.1 Background

Mounting concerns regarding adverse stressors on our planet (e.g., anthropogenic influences on climate change, biodiversity loss, and nitrogen and phosphorus cycles (Rockström et al. 2009)) are driving an integration of sustainability science (Kates et al. 2001) in engineering the built environment. Wastewater treatment plants are positioned at an important interface between the built and natural environments. Historically, the primary objective in the wastewater treatment field has been to reduce pollutant loading to protect local water quality. However, the integration of sustainability science and environmental engineering is broadening the focus of wastewater treatment from local to regional and even global environmental protection. Wastewater treatment should be re-evaluated with an emphasis on energy demands and environmental impacts such as greenhouse gas emissions, to address an environmental footprint broader than local water quality concerns.

Part of this sustainability-centric mindset is the recognition that wastewater is a valuable resource containing energy, nutrients, and water. Technologies that recover energy could mitigate wastewater treatment plants' burden on the power grid. Recovered nutrients could be sold as fertilizer and serve as a revenue source for utilities while also lessening environmental impacts associated with artificial fertilizer production. Water reuse could provide additional revenue, reduce impacts associated with drinking water production, and alleviate stress on drinking water

reservoirs. By rethinking our approach to wastewater treatment, we can recover these resources and significantly improve the economic and environmental sustainability of water management.

Providing incentives for resource recovery from wastewater depends on multiple factors including location-specific water quality concerns, broader environmental burdens, and freshwater supply. For example, increasingly stringent nitrogen regulations in the Chesapeake Bay Watershed are forcing utilities to implement more advanced wastewater treatment systems for nitrogen removal. Nutrient recovery is now being viewed by utilities in the region as an opportunity to generate revenue while helping to achieve nitrogen removal requirements. Incentive to recover energy from wastewater can be influenced by regional electricity costs, environmental impacts of electricity production (Masanet et al. 2013), availability of alternate renewable energy sources, and other factors. For example, regions that rely heavily on coal for electricity production (e.g., the Midwest) have greater environmental impacts associated with energy use than regions that rely heavily on renewables such as hydroelectric power (e.g., the Pacific Northwest). Further, draught-prone regions such as the Southwest are highly motivated to recover water. In contrast, water plentiful areas such as the Great Lakes region have little incentive to do so. Water use in thermoelectricity production interconnects energy and water recovery and could further incentivize recovery of both from wastewater. These regional concerns will dictate adoption of resource recovery technologies.

Location-specific water quality concerns have traditionally been the primary variable to dictate wastewater treatment plant design (e.g., inclusion of nitrogen and/or phosphorus removal). However, the core of most treatment plants consists of an activated sludge system. Such activated sludge treatment plants protect the aquatic environment in most cases, but limit our ability to recover resources from wastewater, particularly energy and nutrients. For example, organic carbon is mineralized rather than converted into an energy-rich end product (e.g., methane) and ammonia

is converted to nitrogen gas using traditional nitrification/denitrification approaches. Further, these systems have high economic cost with significant energy demands and excessive production of residuals that require further treatment and disposal. For example, wastewater treatment plants account for approximately 3% of the U.S. electric load (EPA Office of Water 2006). Improving the environmental footprint of wastewater treatment requires an approach that reduces costs, energy demands, and environmental impacts of treatment. Rather than focus on optimization of existing activated sludge systems that can only provide limited returns, new technologies that also recover resources are needed.

We need to consider harnessing alternative microbial metabolisms in wastewater treatment to improve the economic and environmental sustainability. However, new approaches to wastewater treatment must be resilient during fluctuating conditions (e.g., changes in wastewater strength, composition, and temperature) to ensure continuous protection of the aquatic environment, economically viable, and avoid creating new environmental problems. Researchers have proposed three leading alternatives: (1) anaerobic treatment to produce biogas (Lettinga et al. 1984), a methane-rich fuel that can be used for electricity production; (2) microbial fuel cells to directly produce electricity (Logan et al. 2006); and (3) photosynthetic microalgae-based treatment for recovery of energy, nutrients, or other algae-derived products (Mallick 2002). Of these alternatives, anaerobic treatment has been implemented to the greatest extent globally due to its ability to recover energy while not requiring energy intensive aeration and generating a fraction of the residuals relative to activated sludge processes (Grady et al. 2011). However, anaerobic processes have only been used widely in high-strength industrial wastewater treatment and sludge digestion, not for domestic wastewater treatment.

Activated sludge processes are traditionally favored over anaerobic treatment for domestic wastewater treatment due to conventional wisdom, that as yet has not been well tested in research: (1) anaerobic reactors must be operated at mesophilic (30-40°C) or thermophilic (50-60°C) temperatures, (2) long solids retention times (SRTs) are difficult to achieve because of poor settleability of anaerobic biomass, and (3) post-treatment is necessary if the effluent is to be discharged directly into the environment. Technologies such as the upflow anaerobic sludge blanket (UASB) reactor attempt to overcome these barriers by employing granular sludge in an upflow reactor configuration to provide liquid/solid separation through gravity sedimentation to extend the SRT (Lettinga et al. 1984). However, UASB treatment is adversely affected by low temperatures (Lew et al. 2011, Turkdogan-Aydinol et al. 2011) with maintenance of well-settling granules particularly challenging at low temperatures (McKeown et al. 2012). Further, UASB requires post-treatment to meet effluent regulations even in warm climates (Chernicharo 2006, Foresti et al. 2006, Langenhoff and Stuckey 2000, Seghezzo et al. 1998). Therefore, the large volumetric flow rates of low-temperature domestic wastewater generated in many parts of the world (Tchobanoglous et al. 2003) make attaining the benefits of anaerobic treatment a considerable challenge.

Anaerobic membrane bioreactors (AnMBRs) combine anaerobic treatment with membranes for complete solid/liquid separation and retention of anaerobic microorganisms to achieve long SRTs. By operating at long SRTs and producing an effluent free of suspended solids, the potential exists to match the effluent quality of activated sludge processes, produce fewer residuals, and directly recover energy. Membrane separation has traditionally been employed in aerobic membrane bioreactors (AeMBRs). AeMBRs have seen widespread implementation in domestic wastewater treatment due to their ability to provide a superior effluent quality relative to activated sludge

processes that rely on gravity sedimentation. Improvements in effluent quality are increasingly more relevant as we are forced to consider water reuse (Daigger et al. 2005, Verstraete et al. 2009) due to population growth and lifestyle choices that have made water demand significantly exceed supply in various regions of the world. The rapid decline in membrane costs during the 1990s (Furukawa 2008, Judd 2010) has also benefitted AeMBR adoption by significantly reducing capital and membrane replacement costs (De Wilde et al. 2007).

Although AeMBRs are commercially viable, they remain energy intensive due to aeration requirements for biological treatment and membrane fouling control and, despite potential water reuse opportunities, fail to recover energy or nutrients. In contrast, treatment technologies such as microbial fuel cells have not been proven commercially viable due to high capital expenses (Xie et al. 2012), issues for scale-up (Logan 2010), poor energy recovery with domestic wastewater (Foley et al. 2010), and inadequate effluent quality (Ahn and Logan 2010). Microalgae treatment is plagued by similar concerns (Pittman et al. 2011) as well as high land use requirement (Fortier and Sturm 2012). AnMBRs can theoretically produce a high quality effluent at low temperatures rich in nutrients for agricultural reuse applications while directly recovering energy from domestic wastewater. These benefits, along with the proven commercial viability of membrane bioreactor technology, make AnMBRs a more attractive approach to domestic wastewater treatment than activated sludge processes, AeMBRs, microbial fuel cells, or microalgae.

The successful development of AnMBR technology requires an increase in fundamental knowledge combined with broad consideration for environmental constraints at multiple scales to contextualize the technology's capabilities, limitations, and appropriate implementation. The overarching goal of this dissertation is to advance AnMBR technology for domestic wastewater treatment at low temperatures using process engineering, microbial ecology, and sustainability

assessment tools. This interdisciplinary approach significantly advances the scientific understanding of AnMBR technology thereby increasing the likelihood of full-scale implementation. Widespread AnMBR implementation has the potential to improve resource recovery from domestic wastewater while minimizing the environmental footprint of treatment.

1.2 Overview of Dissertation

This dissertation specifically focuses on direct energy recovery from domestic wastewater using AnMBR at psychrophilic temperatures. We began with a literature review of the state of knowledge regarding AnMBR technology for domestic wastewater treatment (Chapter 2; (Smith et al. 2012)) and then conducted a preliminary investigation of AnMBR treatment of a synthetic and actual domestic wastewater at 15°C using a bench-scale system (Chapter 3; (Smith et al. 2013)). Observations during this preliminary work motivated an in-depth investigation of the membrane biofilm's role in treatment (Chapter 4; (Smith et al. 2014b)). We then gauged the lower temperature limits of AnMBR domestic wastewater treatment by operating a bench-scale system at lower psychrophilic temperatures:12, 9, 6, and 3°C (Chapter 5; (Smith et al. 2014a)). Molecular analyses including high-throughput sequencing of 16S rRNA gene (rDNA) and 16S rRNA and reverse transcription quantitative PCR (RT-qPCR) targeting expression of the methyl coenzyme-M reductase (*mcrA*) gene in methanogens were used to evaluate microbial community structure and activity in the biofilm and suspended biomass at different operational temperatures. Finally, an assessment framework was developed to compare the life cycle environmental and economic impacts of AnMBR systems and conventional wastewater treatment systems with a focus on establishing operational and design targets AnMBR needs to achieve for the technology to move to full-scale (Chapter 6; (Smith et al. 2014c)). Overarching conclusions and future research directions are provided in Chapter 7.

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Chapter 2. Perspectives on Anaerobic Membrane Bioreactor Treatment of Domestic Wastewater: A Critical Review

2.1 Abstract

Interest in increasing the sustainability of water management is leading to a reevaluation of domestic wastewater (DWW) treatment practices. A central goal is to reduce energy demands and environmental impacts while recovering resources. Anaerobic membrane bioreactors (AnMBRs) have the ability to produce a similar quality effluent to aerobic treatment, while generating useful energy and producing substantially less residuals. This review focuses on operational considerations that require further research to allow implementation of AnMBR DWW treatment. Specific topics include membrane fouling, the lower limits of hydraulic retention time and temperature allowing for adequate treatment, complications with methane recovery, and nutrient removal options. Based on the current literature, future research efforts should focus on increasing the likelihood of net energy recovery through advancements in fouling control and development of efficient methods for dissolved methane recovery. Furthermore, assessing the sustainability of AnMBR treatment requires establishment of a quantitative environmental and economic evaluation framework.

2.2 Introduction

Current domestic wastewater (DWW) treatment schemes are energy intensive, produce large quantities of residuals, and fail to recover the potential resources available in wastewater. In fact, municipal wastewater treatment plants account for approximately 3 percent of the U.S. electrical

Smith, A.L., Stadler, L.B., Love, N.G., Skerlos, S.J. and Raskin, L., 2012. Perspectives on anaerobic membrane bioreactor treatment of domestic wastewater: A critical review. Bioresource Technology 122, 149-159.

energy demand according to the U.S. Environmental Protection Agency's Office of Water (2006). Because of an increased interest in sustainability within water management, DWW treatment practices are being reevaluated with a focus on reducing energy demands and environmental impacts, while recovering resources in the form of water, materials, and energy (Guest et al., 2009). Considering this, it is important to note that the relatively low-strength of DWW (e.g., 5-day biochemical oxygen demand [BOD_5] of 110-350 mg/L in the U.S.) and its production at high per capita flow rates (e.g., 190-460 L/capita*d in the U.S.) (Tchobanoglous et al., 2003) in most of the developed world make sustainable water management particularly challenging.

This focus on sustainable development is driving innovations in anaerobic biotechnology, which has long been considered an option to allow for energy recovery from DWW through the conversion of organic matter to methane-rich biogas. In comparison to aerobic biological DWW treatment, anaerobic processes require less energy input because they do not require aeration, produce a fraction of the residuals, and offer the possibility of operation in energy neutral or even positive configurations due to biogas generation (van Lier and Lettinga, 1999; Zeeman and Lettinga, 1999; Aiyuk et al., 2004; Chu et al., 2005; van Haandel et al., 2006). Conventional wisdom regarding anaerobic treatment assumes, however, that: (1) bioreactors must be heated to mesophilic (30-40^{o}C) or thermophilic (50-60^{o}C) temperatures, (2) long solids retention times (SRTs) are necessary, and (3) post-treatment is required to produce an effluent suitable for direct discharge into the aquatic environment. As a result, anaerobic processes have not been utilized widely for full-scale DWW treatment (Aiyuk et al., 2006). Low DWW temperatures in temperate and cold climates have been considered a barrier for anaerobic treatment because the energy requirements associated with heating large quantities of wastewater outweigh the energy recovery potential (Lettinga et al., 2001; Martin et al., 2011). Therefore, low-temperature, ambient or

psychrophilic (<20^{o}C), treatment essentially is the only economically feasible option for anaerobic DWW treatment in temperate and cold climates. Furthermore, high-rate treatment with short hydraulic retention times (HRTs) is necessary to treat the large volumes of dilute DWW, while long SRTs are essential to maintain the slow growing anaerobic microbial populations in the treatment systems. At low temperatures, biomass growth is greatly reduced, which increases the need for a long SRT and necessitates the elimination of even minor sludge washout (Lettinga et al., 2001). Two review papers (O'Flaherty et al., 2006; van Haandel et al., 2006) independently concluded that no microbial barriers exist to anaerobic treatment of DWW, even at low temperatures, provided the system is operated at long SRTs. The well-established upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) reactor configurations largely meet the requirements necessary for high-rate anaerobic treatment (Seghezzo et al., 1998; Rebac et al., 1999; Aiyuk et al., 2004). Anaerobic membrane bioreactors (AnMBRs), by coupling membrane filtration with anaerobic treatment, provide an alternative strategy for DWW treatment at low temperatures with the potential for a higher quality effluent.

AnMBRs can provide the same benefits as aerobic membrane bioreactors (AeMBRs), but may do so with reduced energy requirements. AeMBRs have gained considerable popularity in the past decade for the treatment of both high and low strength wastewater as membrane costs have decreased dramatically (Furukawa, 2008). For instance, AeMBRs have been installed in over 200 countries with 4,400 total installations by the top three suppliers (Kubota, Mitsubishi Rayon, and Zenon (now GE)) as of 2009 (Judd, 2010). Furthermore, the MBR industry is predicted to have a mean growth rate of approximately 12% from 2000 to 2013 (Judd, 2010). This is largely because AeMBRs have the ability to provide superior effluent quality when compared to conventional aerobic treatment that relies on gravity sedimentation, can reduce the footprint of operation, and

have potential in water reuse schemes (Daigger et al., 2005). However, AeMBRs remain energy intensive due to aeration requirements. In addition, membrane fouling continues to be a primary challenge to implementing any MBR system, aerobic or anaerobic, because of its direct effect on capital and operating costs. Consequently, many studies have been conducted to better understand fouling and to assess fouling control strategies in AeMBRs as reviewed by Le-Clech et al. (2006). Significantly less work has been done on fouling in AnMBRs, particularly in applications of low-strength wastewater treatment (Bérubé et al., 2006). Despite this, research on AnMBRs has increased substantially over the past decade because of the interest in reducing energy demands. Two review papers have already appeared on AnMBR treatment of a variety of waste streams. Liao et al. (2006) reviewed AnMBR technology for a wide range of high and low-strength wastewaters including DWW. Bérubé et al. (2006) focused on membrane fouling when considering AnMBRs for low-strength wastewater treatment. However, these reviews did not address operational concerns beyond membrane fouling for AnMBR low-strength wastewater treatment and a substantial amount of AnMBR research has been conducted since they were published.

The objective of the current review is to comprehensively discuss the available literature on AnMBRs for DWW treatment and identify the main research areas that need further attention. Interest in AnMBRs for DWW treatment has grown rapidly during the past few years, as evidenced by a surge of research publications on the topic since 2010 (Baek et al., 2010; Gao et al., 2010; Ho and Sung, 2010; Dagnew et al., 2011; Gimenez et al., 2011; Huang et al., 2011; Kim et al., 2011; Martinez-Sosa et al., 2011; Salazar-Pelaez et al., 2011). Much of the reviewed literature and studies published so far have focused on proof of concept and membrane fouling. However, a broader understanding of AnMBR technology in the context of DWW treatment is needed for successful

full-scale implementation. Building on the review paper by Bérubé et al. (2006), which focused on membrane fouling, the current review discusses recent advancements in fouling control, but examines in greater detail other operational concerns that need to be resolved to allow full-scale implementation of AnMBRs for DWW treatment. For instance, the lower limits of HRT and temperature allowing for adequate treatment performance have yet to be established. The complex relationships among HRT, SRT, treatment performance, and membrane fouling are also poorly defined in the current literature. Furthermore, methane solubility, especially at low temperatures, complicates methane recovery. In addition, anaerobic treatment lacks the capacity for substantial nutrient removal, which is an important consideration when direct discharge of treated effluents in nutrient sensitive watersheds is necessary. Thus, coupling AnMBR treatment with downstream treatment is necessary to remove (and ideally recover) nutrients available in DWW. Such post-treatment, if possible, should retain the excellent AnMBR effluent quality with respect to suspended solids. Beyond nutrient removal, the removal of trace contaminants by AnMBR treatment has yet to be evaluated. It is clear that AnMBR research must extend beyond membrane fouling to best determine the circumstances under which AnMBR DWW treatment is practical and economically feasible. Therefore, this review covers recent advancements made in membrane fouling control, the effects of HRT and SRT on treatment performance and fouling, the role of the membrane biofilm in treatment, implications of temperature on AnMBR performance, complications with methane recovery, nutrient removal limitations, the fate of trace contaminants in AnMBR treatment, and finally, pilot-scale studies.

2.3 Selection of Reactor Configuration and Membrane Pore Size, Material, and Configuration

Simply defined, an AnMBR is an anaerobic bioreactor coupled with membrane filtration. The membrane filtration component can exist in three configurations: external cross-flow, internal

submerged, or external submerged (Liao et al., 2006). In an external cross-flow configuration, the membrane unit is separate from the bioreactor and the membranes operate under pressure to produce permeate. Suspended anaerobic biomass maintained in the bioreactor is pumped into the membrane unit creating a positive pressure that leads to permeate production. The rejected biomass or retentate is returned to the bioreactor. In an internal submerged membrane configuration, membranes are submerged directly into the suspended biomass in the bioreactor and permeate is produced by exerting a vacuum on the membrane. Alternatively, membranes may be located in an external chamber separate from the main bioreactor, but are still submerged in suspended biomass and are operated under vacuum. In such an external submerged configuration, suspended biomass from the bioreactor is pumped to the external chamber, while retentate is returned to the main bioreactor. This configuration facilitates membrane cleaning and replacement by allowing isolation of the membrane unit in an external chamber. This separation enables anaerobic conditions to be maintained in the main bioreactor during membrane cleaning or replacement.

Regardless of membrane configuration, the anaerobic bioreactor is most commonly a continuously stirred tank reactor (CSTR). Alternatives to a CSTR have also been proposed, such as UASB (Aiyuk et al., 2004; Ho and Sung, 2009), EGSB (Chu et al., 2005), and fluidized bed (Kim et al., 2011) reactors coupled with membrane filtration. These reactor designs allow for considerable biomass retention in the bioreactor, which potentially limits membrane fouling by reducing the amount of biomass in contact with the membranes (Liao et al., 2006). However, biomass growth on the membrane surface, colloidal solids, soluble microbial products (SMP), and extracellular polymeric substances (EPS) are also important contributors to membrane fouling (Bérubé et al., 2006). Therefore, bioreactor designs that limit membrane-biomass contact are not guaranteed to reduce fouling.

The selection of membrane pore size, material, and configuration are important design decisions. Microfiltration and ultrafiltration membranes are most commonly used in MBRs. In addition, there is growing interest in using dynamic or secondary membranes, which rely on the formation of a cake layer for biomass retention rather than on an actual membrane, in both aerobic (Chu and Li, 2006) and anaerobic applications (Zhang et al., 2010). Organic and inorganic membranes have been applied in AnMBR DWW treatment and it has been shown this choice of material can impact the type and extent of membrane fouling, as well as the associated costs (Bérubé et al., 2006). Finally, flat-sheet (Hu and Stuckey, 2007; Huang et al., 2011), tubular (Baek and Pagilla, 2006; Ho and Sung, 2009; Salazar-Pelaez et al., 2011), and hollow fiber (Wen et al., 1999; Chu et al., 2005; Lew et al., 2009; Dagnew et al., 2011; Gimenez et al., 2011; Kim et al., 2011) membranes have been studied for AnMBR DWW treatment. Table 1 presents various operational parameters and treatment performance results obtained in bench-scale AnMBR studies for DWW treatment (studies with simulated and actual DWW are included). A broad range of configurations, membrane materials, operational temperatures, and fouling control strategies have been researched.

Table 2-1. Operational parameters and treatment performance results obtained in published bench-scale AnMBR studies for DWW treatment.

Study	Average Influent Strength [mg/L TCOD[a]]	Temp [°C]	Bioreactor Configuration	Membrane Information	Fouling Control	SRT [d]	HRT [h]	Average Effluent [mg/L TCOD[a]/% removal]
Wen et al. (1999)	100-2600[b]	12-25	UASB with submerged membrane	0.03 µm polyethylene submerged hollow fiber	Periodic cleaning with 5% NaOCl	150	6	19/97
							4	12/97
Chu et al. (2005)	383-849[c]	25	EGSB with submerged membrane	0.1 µm polyethylene submerged hollow fiber	Backflushing and relaxation; Periodic cleaning with 0.03% NaOCl	145	3.5-5.7	93-96
		20						87-92
		15						85-86
		11						76-81
Hu and Stuckey (2006)	460[c]	35	Submerged AnMBR	0.4 µm submerged hollow fiber / 0.4 µm polyethylene chloride submerged flat sheet	Biogas sparging	∞	48	23/95 / 25/95
							24	29/94 / 32/93
							12	38/92 / 32/93
							6	40/91 / 40/91
							3	44/90 / 43/91
Baek and Pagilla (2006)	84 [SCOD][b]	32	Completely mixed anaerobic bioreactor	0.1 µm PVDF[d] external tubular	Cross-flow; weekly cleaning with 0.1% w/w NaOH and disinfectant	∞	48	25/58
							24	37/55
							16	37/56
							12	24/68
Saddoud et al. (2007)	685[b]	37	Jet flow anaerobic reactor	100 kDa external	Cross-flow	∞	15-60	87/88
Ho and Sung (2009)	500[c]	25	Completely mixed anaerobic reactor	1 µm PTFE[e] external tubular	Cross-flow; periodic cleaning with NaOCl	90-360	6-12	<40/>92
Lew et al. (2009)	540[b]	25	Completely mixed anaerobic reactor	0.2 µm external hollow fiber	Periodic backflushing; chemical cleaning with 0.1 M NaOH, 1% H_2O_2, and 1% HCl	∞	4.5-12	65/88
Ho and Sung (2010)	500[c]	25	Completely mixed anaerobic reactor	1 µm PTFE[e] external tubular	Periodic backflushing	∞	9	25/95
		15						75/85
Gao et al. (2010)	500[c]	30	Upflow anaerobic reactor	100 kDa external coated PVDF[d] and 30 kDa external polyetherimide	Cross-flow	50	24	<20/>96
Huang et al. (2011)	550[c]	25-30	Completely mixed anaerobic reactor	0.45 µm PES[f] flat sheet	Biogas sparging	30, 60, ∞	8-12	<17/>97
Salazar-Peláez et al. (2011)	350[c]	----	UASB with external membrane	100 kDa external PVDF[d] tubular	Cross-flow; NaOCl cleaning every 6 hours	∞	4-12	70/80
Kim et al. (2011)	513[c]	35	Two-stage fluidized bed/ membrane bioreactor	0.1 µm PVDF[d] hollow fiber	GAC fluidization; periodic backflushing and/or NaOCl/NaOH cleaning	∞	4.2-5.9	7/99
Smith et al. (2011)	440[c]	15	Submerged AnMBR	0.2 µm PES[f] flat sheet	Biogas sparging and backflushing	300	16	36/92

[a]TCOD = total COD
[b]actual DWW; [c]simulated DWW
[d]PVDF = polyvinylidene fluoride ; [e]PTFE = polytetrafluoroethylene; [f]PES = polyethersulfone

2.4 Membrane Fouling Control

Membrane fouling continues to be a substantial challenge in advancing AnMBR technology considering membrane material costs and energy demands associated with fouling prevention. Fouling results from the accumulation of inorganic and organic foulants internally in the membrane pores and externally on the membrane surface, which reduce flux, increase TMP, and potentially necessitate chemical cleaning or membrane replacement. The primary foulants of interest in AnMBR systems include suspended biomass, colloidal solids, SMP, EPS, attached cells, and inorganic precipitates such as struvite.

Membrane fouling has been controlled through various strategies, which are linked to the membrane configuration. In external cross-flow configurations, a high cross-flow velocity is maintained to limit inorganic and organic foulant buildup on the membrane. In submerged configurations, fouling control is typically accomplished through biogas sparging, backflushing, and/or membrane relaxation. A consensus has yet to be determined on which strategy is most effective per energy input. For instance, Martin et al. (2011) highlighted the high variability in biogas sparging intensity and thus energy demand for fouling control used in submerged AnMBR studies. When comparing AeMBR and AnMBR studies, lower permeate fluxes are typically observed in AnMBRs potentially as a result of less flocculation and thus increased concentrations of fine particulates and colloidal solids at the membrane surface (Liao et al., 2006; Martin et al., 2011). However, direct comparison studies between AeMBRs and AnMBRs for DWW treatment have indicated similar fouling potential (Achilli et al., 2011) or less propensity for fouling in AnMBRs (Baek and Pagilla, 2006).

Fouling control represents the most intensive energy demand associated with AnMBR treatment, and therefore, reducing this demand is central to maximizing the potential energy recovery.

Considering the low organic strength of DWW and correspondingly low potential biogas generation, minimizing energy demands associated with fouling control is likely necessary to achieve energy neutral or positive operation. To this end, Hu and Stuckey (2007) first proposed powdered or granular activated carbon (PAC or GAC) addition to submerged AnMBRs to reduce membrane fouling in conjunction with biogas sparging. Their results suggest that PAC and GAC addition increase membrane flux and enable operation under lower TMP as compared to a control AnMBR in which only biogas sparging was used. However, they did not evaluate the effect of reduced biogas sparging intensity in the presence of PAC or GAC. The PAC or GAC is not used for adsorption and therefore would not need to be regenerated or replaced during operation, however, the initial costs and potential life cycle environmental impacts of the activated carbon must still be considered. More recently, Kim et al. (2011) proposed the use of fluidized GAC through liquid recirculation without biogas sparging for fouling control. Their results show fouling may be controlled with substantially less energy input than biogas sparging requires, however, the long-term effects on the membrane material have yet to be established. This is particularly important as both studies used organic membranes and it has been suggested that aggressive fouling control through the use of PAC, GAC, or other media in contact with the membrane may be better suited for more abrasion resistant inorganic membranes, despite their higher life cycle costs (Ghyoot and Verstraete, 1997). Examining the long-term impact of these aggressive fouling control measures on organic membranes is an important area of research that has received little attention.

2.5 Effects of HRT and SRT on Treatment Performance and Membrane Fouling

HRT and SRT are important operational parameters that impact treatment performance and affect membrane fouling in an AnMBR. In the context of DWW treatment, a low HRT is desirable to

reduce AnMBR size and the overall footprint of operation, whereas a high SRT may be required to achieve the necessary treatment performance under the constraints of discharge limits especially for lower temperatures (O'Flaherty et al., 2006). However, increasing the SRT, while keeping the HRT constant, increases the suspended biomass concentration potentially leading to decreased permeate flux (Bérubé et al., 2006; Liao et al., 2006; Huang et al., 2011). Furthermore, increasing the SRT may result in higher SMP and EPS production (Huang et al., 2011), which in-turn play a role in membrane fouling. Therefore, a tradeoff could exist between controlling HRT and SRT for membrane fouling mitigation and obtaining the necessary treatment performance.

The dependence of AnMBR treatment performance on HRT has been evaluated in various studies (Table 2-1; Figure 2-1). Hu and Stuckey (2006) observed a marginal decrease in COD removal (approximately 5% overall) when they lowered the HRT from 48 hours to 24, 12, 6, and 3 hours during treatment of simulated DWW at mesophilic temperature (35°C). Even at a 3-hour HRT, COD removal greater than 90% was achieved. Comparing HRTs of 3.5, 4.6, and 5.7 hours, Chu et al. (2005) did not observe a correlation between treatment performance and HRT at temperatures greater than 15°C. Likewise, Huang et al. (2011) found that treatment performance was independent of HRT when comparing HRTs of 8, 10, and 12 hours in an AnMBR treating a simulated DWW at 25-30°C. Several other studies similarly concluded that HRT had little effect on AnMBR permeate quality (Ho and Sung, 2009; Lew et al., 2009; Baek et al., 2010). Ho and Sung (2009), however, observed an accumulation of soluble COD in an AnMBR operating at 25°C when the HRT was reduced from 12 to 6 hours despite stable permeate COD concentrations. Another study in which HRT was decreased from 12 to 8 and then 4 hours observed an increase in permeate COD at the lowest HRT (temperature was not reported in this study), while there was no significant difference between the permeate COD values obtained for HRTs of 12 and 8 hours

(Salazar-Pelaez et al., 2011). Salazar-Pelaez et al. (2011) also observed an increase in retentate EPS and SMP concentrations at the lowest HRT, which resulted in increased membrane fouling. The authors recommended a lower limit be placed on HRT due to fouling concerns. Furthermore, Huang et al. (2011) noted that combining a short HRT with a long SRT inevitably leads to increases in suspended biomass concentrations, which positively correlates with membrane fouling rates. Taken together, these studies suggest that adequate AnMBR treatment performance may be obtained at relatively short HRTs even at low temperatures, but that a lower limit on HRT may exist primarily due to concerns with membrane fouling.

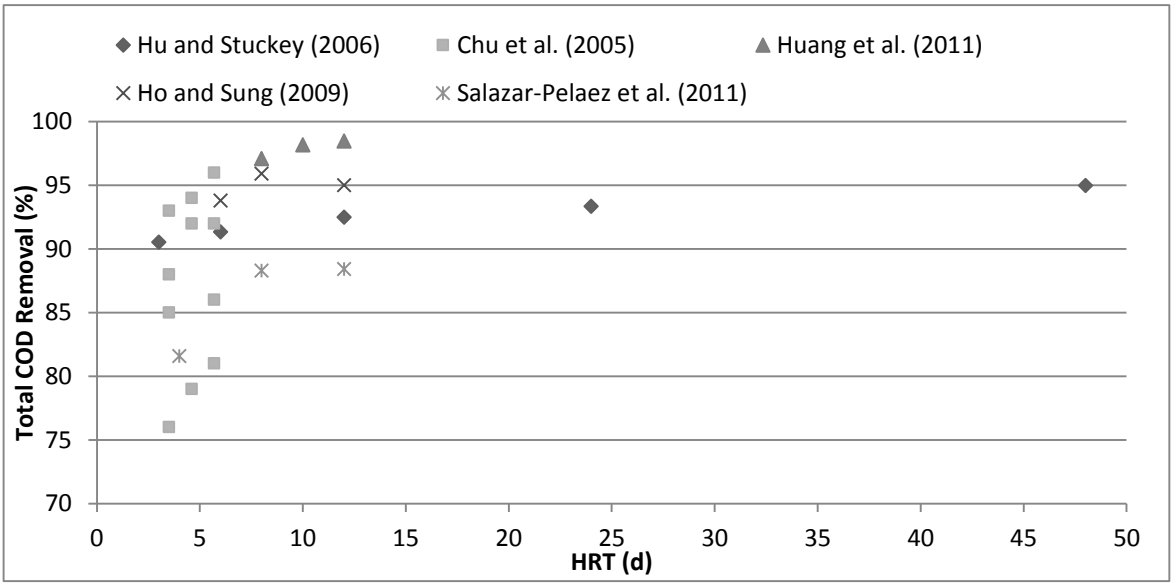

Figure 2-1. Total COD removal as a function of HRT observed in Chu et al. (2005), Hu and Stuckey (2006), Ho and Sung (2009), Huang et al. (2011), and Salazar-Pelaez et al. (2011).

Membrane separation enables absolute retention of biomass and thus complete control of SRT. Because of this, SRT is an easily controllable operational parameter affecting both treatment performance and membrane fouling. Baek et al. (2010) operated a bench-scale AnMBR and reduced the SRT through biomass wasting in five steps from 213 to 40 days. The decrease in SRT did not impact treatment performance or membrane fouling. Conversely, Huang et al. (2011)

compared performance during operation at SRTs of 30 and 60 days and for a period without biomass wasting and observed better treatment performance at longer SRTs but at the cost of increased membrane fouling resulting from higher suspended biomass concentrations and SMP production. However, a negative correlation between EPS concentrations and SRT was found, which was linked to smaller median particle sizes in the suspended biomass as a function of reduced flocculation in the presence of lower concentrations of EPS. The authors speculated that the decrease in median particle size associated with the increase in SRT likely accelerated membrane fouling. Baek at al. (2010) also observed a decrease in EPS concentrations at higher SRTs but noted that concentrations detected were considerably lower than literature values for AeMBRs possibly indicating relatively less propensity for EPS fouling in AnMBRs. Conversely, other AnMBR studies have pointed to EPS as a major contributor to direct membrane fouling (Chu et al., 2005; Gao et al., 2010). Therefore, EPS may act to reduce membrane fouling by increasing suspended biomass particle size, whereas EPS may directly contribute to membrane fouling when present in excess or when generated directly on the membrane surface by the biofilm or cake layer. These observations suggest that a certain SRT may exist to limit EPS-mediated membrane fouling. However, the role of EPS quantity and characteristics in fouling as a function of SRT as well as other operational variables is not well understood in AnMBRs and controlling SRT is further complicated by its interrelatedness with treatment performance.

2.6 Role of the Membrane Biofilm beyond Fouling

The biofilm or cake layer that develops on the membrane surface plays a role in membrane fouling, but may also contribute to soluble COD removal and thus final permeate quality in an AnMBR. The latter has received limited attention in the literature on AnMBRs for DWW treatment. Mechanisms of soluble COD removal across the membrane may include microbial activity,

adsorption, size exclusion, and charge exclusion. Several AnMBR studies have shown substantial differences between the soluble COD concentrations in the bioreactor and in the permeate (Chu et al., 2005; Hu and Stuckey, 2006; Ho and Sung, 2009; Baek et al., 2010; Ho and Sung, 2010; Smith et al., 2011). In addition, Ho and Sung (2010) observed an increase in soluble COD removal across the membrane surface with decreasing temperatures. Some researchers have compared soluble COD removal in the bioreactor and soluble COD removal across the membrane and have referred to these as, respectively, "biological" and "physical" soluble COD removals (Ng et al., 2000; Baek and Pagilla, 2006; Ho and Sung, 2009). These definitions are misleading as it is improbable that biological activity does not occur in the membrane biofilm. However, it is important to understand the significance of biological soluble COD removal by the biofilm in total COD removal and relative to other potential non-biological soluble COD removal mechanisms. Ho and Sung (2010) compared specific methanogenic activities in suspended and attached biomass and found that the attached biomass was indeed biologically active although it was far lower in activity than the suspended biomass. Conversely, Vyrides and Stuckey (2011) compared biological activity of attached and suspended biomass from an AnMBR treating high-salinity wastewater and found that the attached biomass was considerably more active under both high and low salinity conditions. The authors speculated that increases in biological activity result from lower mass-transfer limitations in the biofilm. Furthermore, Vyrides and Stuckey (2009) observed an increase in dissolved organic carbon (DOC) removal when they reduced the frequency of biogas sparging from continuous to intervals of 10 min every 15 min during treatment of high-salinity wastewater. The decrease in biogas sparging increased TMP indicating a thicker biofilm developed on the membrane, which likely caused an increase in adsorption and eventual biodegradation of high molecular weight compounds. Smith et al. (2011) operated two membrane units in parallel in an

AnMBR subjected to biogas sparging. One membrane unit was backflushed at a regular interval, while the other one was not backflushed. The differences in fouling control led to a higher amount of fouling in the non-backflushed membrane unit, as indicated by higher TMPs and by visual observations. A positive correlation was observed between membrane fouling and soluble COD removal across the membrane. These results suggest that a tradeoff may exist between increased fouling and increased soluble COD removal across the membrane.

2.7 Temperature Implications on Treatment Performance

Untreated DWW in the U.S. varies in temperature from approximately 3 to 27°C, with an average of about 16°C (Tchobanoglous et al., 2003). Given the relatively low average temperature of DWW, heating of DWW would be necessary in the U.S. and many regions in the world for most of the year if mesophilic treatment were required. Martin et al. (2011) concluded that influent COD concentrations higher than 4-5 g/L, an order of magnitude greater than those present in typical DWW (Tchobanoglous et al., 2003), would be necessary to generate enough biogas to heat a bioreactor to mesophilic temperatures. Therefore, operation at ambient temperatures is essential for economical implementation of AnMBRs for DWW treatment.

Hydrolysis of particulate organics is generally considered to be the rate-limiting step in anaerobic digestion (Lee and Rittmann, 2011) and is of special importance in DWW treatment as particulate organics represent a large fraction of the total COD. Hydrolysis rates decline with temperature (Lettinga et al., 2001), requiring longer SRTs for hydrolysis to occur at psychrophilic temperatures. This creates a limitation for most anaerobic treatment systems as the relatively poor settleability of anaerobic biomass makes it difficult to retain all biomass in systems that rely on gravity separation. Even minor sludge washout in such systems could reduce the SRT to below the limit necessary for acceptable treatment performance (Lettinga et al., 2001). Because membranes enable

complete retention of particulates, hydrolysis rates may still be sufficiently high in an AnMBR even at psychrophilic temperatures resulting in acceptable treatment performance. Thus, other pathways may be more critical or rate-limiting in psychrophilic AnMBR operation. Ho and Sung (2009) cited acetogenesis as the rate-limiting step in AnMBRs operating at 25°C based on an increase in bioreactor soluble COD, but a lack of volatile fatty acid (VFA) accumulation. Rebac et al. (1999) investigated the effects of temperature on the kinetics of fatty acid degradation using psychrophilically-grown (10°C) mesophilic seed sludge. They found that, although low temperatures negatively affected degradation rates, specific methanogenic activities at mesophilic temperatures using the psychrophilically-grown biomass were higher than the specific methanogenic activities of mesophilically-grown biomass, indicating that psychrophilic conditions do not inhibit development of methanogenic microbial communities. Overall, low temperatures may reduce maximum specific growth and substrate utilization rates of microorganisms but can also lead to an increase in net biomass yield (O'Flaherty et al., 2006). Furthermore, most biological reactions pertinent to anaerobic digestion such as hydrolysis and various fermentations are less energetically favorable at low temperatures. On the other hand, several reactions are more exergonic at low temperatures because of the increased solubility of hydrogen, including hydrogenotrophic sulfate reduction, hydrogenotrophic methanogenesis, and homoacetogenesis (Lettinga et al., 2001). This implies that hydrogenotrophic methanogenesis may be more important than aceticlastic methanogenesis at low temperatures. Indeed, McKeown et al. (2009) found increased hydrogenotrophic methanogenic activity at psychrophilic temperatures during a long-term study (1,243 days) on anaerobic treatment of acidified wastewater. While the temperature of operation certainly impacts various pathways in anaerobic metabolism, there is no evidence that

low temperatures are inhibitory to process performance under the appropriate operational conditions.

Despite the lack of microbial barriers at low temperatures, only a few studies have assessed AnMBR performance for DWW treatment at psychrophilic temperatures (Table 2-1). Even fewer studies have evaluated performance as a function of varying DWW temperature within the low-temperature range. An early study in which a bench-scale UASB system was coupled with membrane filtration treating DWW found that high COD removals (averaging 97%) could be attained at temperatures ranging from 12 to 25°C for HRTs of 4-6 hours (Wen et al., 1999). A dependence on temperature was observed in the UASB with COD removals dropping below 70% at temperatures below 15°C. More importantly, however, was the finding that total system COD removal (UASB and membrane filtration) was only slightly affected by temperature, and remained greater than 88% at the lowest operational temperature. This result highlights the possible role membrane filtration has in performance stability across temperature fluctuations. In another study, an EGSB coupled with microfiltration treating a simulated DWW was initially operated at 25°C and subsequently operated at 20, 15, and 11°C. The temperature was finally increased stepwise back to 25°C (Chu et al., 2005). COD removal decreased slightly from >90% at 25°C with decreases in temperature to 15°C, but sharply declined to 78% when the temperature was further reduced to 11°C. This low COD removal may have been related to the relatively short HRTs used, i.e., 3.5 to 5.7 hours. Indeed, changes in HRT significantly affected COD removal when the system was operated at 11°C indicating that adequate treatment performance may be obtained at this temperature at longer HRTs. Ho and Sung compared AnMBR performance treating a simulated DWW using parallel reactors operated at 25 and 15°C and observed COD removals greater than 95 and 85%, respectively (Ho and Sung, 2010). Although these data suggest some performance

dependence on temperature, both reactors were inoculated with sludge from a mesophilic anaerobic digester and an AnMBR previously operated at 25°C and thus higher performance at 25°C could have been expected especially considering the relatively short operational period of 112 days. VFA profiles for the AnMBR operated at 15°C show a long acclimation phase of approximately 60 days after which permeate VFAs remained low suggesting that performance of the two reactors may have converged over a longer operational period. Smith et al. (2011) showed COD removals greater than 90% could be attained in an AnMBR operated at 15°C.

Low temperatures may impact the choice of an appropriate inoculum for seeding an AnMBR. Kashyap et al. (2003) and O'Flaherty et al. (2006) suggested that psychrophiles from natural habitats be considered for potential use in psychrophilic anaerobic treatment processes citing the large number of psychrophilic methanogens and acetogens isolated from terrestrial ecosystems. In fact, a psychrophilic methanogen was recently isolated from the Zoige Wetland of the Tibetan Plateau that is active at temperature as low as 0°C (Zhang et al., 2008). Xing et al. (2010) investigated the use of psychrophilic inocula from natural habitats (lake sediments) in anaerobic digestion at 15°C and determined that inoculation with psychrophilic biomass is feasible. Conversely, Rebac et al. (1999) concluded that psychrotolerant mesophiles were adequate for psychrophilic anaerobic treatment and that true psychrophiles are not required. Smith et al. (2011) seeded an AnMBR with inocula from two mesophilic and one psychrophilic environment and compared the microbial community structure of suspended biomass and membrane biofilm samples taken after 275 days of operation at 15°C. The AnMBR microbial communities most closely resembled the mesophilic inocula rather than the psychrophilic inoculum suggesting that psychrotolerant mesophiles dominated in their system (Smith et al., 2011). Nonetheless, AnMBR inoculation with truly psychrophilic microbial communities may be critical for process

performance and stability at even lower temperatures. O'Flaherty et al. (2006) commented that better characterization of psychrophiles in anaerobic treatment may require longer term operation (>1,000 days) at psychrophilic conditions to allow enrichment of psychrophilic microorganisms relative to psychrotolerant mesophiles. It should be noted that, to the best of our knowledge, no studies have assessed anaerobic treatment performance of psychrophiles at elevated temperatures. This is of particular importance in AnMBR DWW treatment as most temperate climates experience seasonal temperature variation that lead to fluctuations in DWW temperature between approximately 5 and 25°C. Thus, it is possible that performance may deteriorate at higher temperatures if the AnMBR is seeded with only psychrophilic biomass. A better approach may be to seed the AnMBR with a diverse consortium of mesophilic and psychrophilic microbial communities to maintain performance across seasonal temperature variation.

2.8 Complications with Methane Solubility and Recovery

Anaerobic treatment enables energy recovery from DWW as long as methane can be easily collected. Capturing methane is also important to mitigate direct greenhouse gas emissions as methane has a global warming potential 25 times that of carbon dioxide (IPCC, 2007). Because of this, methane should not be emitted to the atmosphere during AnMBR operation. Methane in the gas phase can be easily collected, but dissolved methane is more difficult to capture. Specifically, methane is approximately 1.5 times more soluble at 15°C compared to 35°C, for a typical biogas methane content of 70%. Because of the relatively low strength of DWW, dissolved methane leaving the treatment process in the liquid phase represents a substantial portion of the total methane generated. Consequently, recovery of dissolved methane is key to approaching energy-neutral AnMBR DWW treatment.

Few AnMBR studies have addressed methane solubility (Hu and Stuckey, 2006; Dagnew et al., 2011; Gimenez et al., 2011) and even fewer have quantified dissolved methane (Kim et al., 2011; Smith et al., 2011). Kim et al. (2011) reported that 30% of the methane generated left their system through the liquid phase during operation at 35°C. Smith et al. (2011) observed that approximately 50% of the methane generated remained in the liquid phase during operation at 15°C, highlighting the important role temperature has in methane solubility and, therefore, direct biogas methane recovery. In a recent study by Bandara et al. (2011), dissolved methane in the effluent of a bench-scale UASB reactor treating a simulated wastewater operated at 35, 25, and 15°C was quantified and recovered through the use of a degassing membrane, a non-porous membrane only permeable to gases. As expected, an increase in the dissolved methane concentration was observed with a decrease in temperature. Dissolved methane concentrations in the UASB effluent were on average 15.8, 20.5, and 26.0 mg/L (converted from concentrations provided in COD based units) at temperatures of 35, 25, and 15°C, respectively. Comparing these concentrations using concentrations derived using Henry's Law based on the biogas methane content reported suggests a high level of methane oversaturation in the UASB effluent. A number of other studies using anaerobic bioreactors without membrane separation for low-strength wastewater treatment also found methane oversaturation (e.g., Singh et al., 1996; Hartley and Lant, 2006). Pauss et al. (1990) provided a theoretical and experimental evaluation of methane oversaturation for a number of anaerobic bioreactor configurations without membrane separation and cited mass transfer limitations as the cause of the observed oversaturation. The methane oversaturation reported in these non-MBR studies may have resulted from poor gas-liquid phase equilibrium. Utilizing biogas sparging for fouling control in an AnMBR would be expected to result in methane equilibrium by limiting the effect of mass transfer limitations. However, methane oversaturation

was also observed in an AnMBR study in which biogas sparging was used (Smith et al., 2011). Since substantial soluble COD removal took place across the biofilm in this study, it was hypothesized that methane oversaturation in the permeate was, at least in part, due to methane generation via biological activity in the biofilm. The presence of TMP across the biofilm likely forces methane generated by methanogens in the biofilm into the permeate stream, regardless of methane saturation. Taken together, the results of several studies indicate methane oversaturation should be expected in AnMBR permeate.

Several methane removal processes have been proposed to capture dissolved methane, including stripping of AnMBR effluent through post-treatment aeration (Hartley and Lant, 2006; McCarty et al., 2011), methane recovery using a degassing membrane (Bandara et al., 2011), and the use of a down-flow hanging sponge (DHS) reactor (Hatamoto et al., 2010). Methane stripping with air is commonly employed on landfill leachate to limit methane release from the liquid to the gas phase in sewer systems. Energy demands associated with methane stripping with air are estimated to be less than 0.05 kWh/m^3 of AnMBR permeate (McCarty et al., 2011). Energy recovery from the resulting mixture of methane and air has not yet been attempted. Foreseeable complications with this practice include the dilution of methane with air and potential explosion hazards resulting from a methane and oxygen rich off-gas. Furthermore, the efficiency of this practice for removing dissolved methane from AnMBR effluent is not well established. The use of degassing membranes represents a more controlled approach by which methane is recovered from AnMBR effluent but not diluted with air. Bandara et al. (2011) observed high recoveries of dissolved methane using degassing membranes with higher efficiencies for lower temperature as a result of increased methane solubility at lower temperatures. However, the degassing technology used was energy intensive; energy requirements (0.042 kW) far outweighed the amount of energy embedded in the

recovered methane (0.00014 kW). Therefore, although this technology is worth further investigation, energy requirements must be substantially reduced for economic feasibility. Finally, biological methane oxidation through the use of a DHS reactor has been evaluated (Hatamoto et al., 2010). Using this system, up to 95% of the dissolved methane in the effluent was oxidized by methanotrophs. However, because dissolved methane was oxidized, methane could not be recovered for energy generation using this approach. This technology shows promise for drastically reducing potential greenhouse gas emissions from AnMBR effluent, but at the cost of energy requirements to operate the DHS and lost energy potential in the methane oxidized. Overall, dissolved methane recovery or oxidation is possible through a number of methods although each with substantial drawbacks. Addressing the issue of dissolved methane perhaps represents the greatest barrier to AnMBR implementation.

2.9 Nutrient Removal Limitations

Another major barrier to full-scale adoption of AnMBR DWW treatment is the lack of direct nutrient removal capability. Some nutrient removal takes place as a result of biomass growth, but is limited due to the low biomass yields typical for anaerobic microbes. In addition, ammonium and phosphate concentrations increase as a result of ammonification and phosphate release under anaerobic conditions. Challenges exist in coupling AnMBRs with conventional biological nutrient removal treatment technologies due to the low COD:N and COD:P ratios typical of AnMBR effluents. Biological nitrogen and phosphorus removal processes require sufficient amounts of organic electron donor to fuel denitrification and enhanced biological phosphorus removal. Chernicharo (2006) suggests treating only a fraction of the waste anaerobically (50 – 70%) and using the remaining fraction to support denitrification in downstream biological nitrogen removal. Aerobic or partial aerobic treatment common to many biological nutrient removal systems detracts

from the energy savings gained by using anaerobic treatment. In addition, dissolved methane present in AnMBR effluents will be stripped during aerobic treatment, contributing to greenhouse gas emissions. The challenges of capturing or oxidizing methane dissolved in AnMBR effluents and achieving nutrient removal are inextricably linked.

An attractive option for biological nitrogen removal downstream of AnMBR DWW treatment may be anaerobic ammonium oxidation (anammox), a process in which ammonium and nitrite are converted to nitrogen gas by anammox bacteria (Van De Graaf et al., 1996). It has received increasing attention as a cost-effective nitrogen removal strategy in comparison to traditional nitrification/denitrification approaches (Schmidt et al., 2003). Because it is a strictly autotrophic process and ammonium serves as the electron donor, no additional carbon source/electron donor is required to fuel denitrification. Additional benefits of anammox include limited sludge production, low energy input, and almost complete nitrogen removal (some nitrate is produced) (Gao and Tao, 2011). Full-scale anammox treatment has been applied successfully in Europe, Japan, and China where it is being used to treat high-ammonium waste streams, such as industrial wastewaters and anaerobic digestates (Gao and Tao, 2011). However, control of the partial nitritation process (i.e., partial oxidation of ammonium to nitrite) can be challenging, startup times are typically long due to the slow-growth of anammox bacteria, and mesophilic temperatures are thought to be necessary. Furthermore, little research has been conducted on anammox treatment of waste streams with relatively low ammonium concentrations such as DWW and, to the best of our knowledge, no papers have reported results from studies that couple AnMBR treatment with downstream anammox treatment. Regardless, the anammox process has been proposed for mainstream DWW nitrogen removal (Kartal et al., 2010; O'Shaughnessy et al., 2011). A downstream anammox system that utilizes a biofilm process would enable nitrogen removal while

maintaining the high quality of AnMBR effluent with regards to suspended solids. With additional research and process optimization, it may represent a viable nitrogen removal option for use in combination with AnMBR DWW treatment.

Physical/chemical nutrient removal processes are also promising, but can be significantly more energy intensive than biological treatment. Several studies have examined physical/chemical treatments coupled with anaerobic treatment to achieve nutrient removal. Aiyuk et al. (2004) proposed pretreatment to remove suspended solids and phosphorus through flocculation/coagulation, and ammonia removal through post-treatment zeolite adsorption in applications of anaerobic DWW treatment. Overall, 94% phosphorus and 99% nitrogen removals were achieved in their study, indicating that this approach may be applicable for achieving sufficient nutrient removal in AnMBR DWW treatment. Struvite (magnesium ammonium phosphate) precipitation represents another means of nutrient removal with potential use in AnMBR DWW treatment processes (de-Bashan and Bashan, 2004). A benefit of this approach is that recovered struvite is saleable as a fertilizer and struvite recovery limits potential pipe scaling and membrane fouling. A disadvantage of this approach is that magnesium must be added to encourage struvite formation because its levels are usually limited in DWW. Furthermore, although struvite precipitation typically removes all the phosphorus, the stoichiometry of the process means that for medium-strength U.S. DWW only 12.5% of ammonium will be removed through struvite precipitation and that residual ammonium will remain (Tchobanoglous et al., 2003). Johir et al. (2011) proposed using an ion-exchange/adsorption process downstream of an AeMBR for nutrient recovery. The study found that a pruolite ion-exchange resin achieved phosphate and nitrate removal efficiencies of 85% and 95%, respectively. Ion-exchange membrane bioreactors, similar to those demonstrated by Matos et al. (2009) to remove nitrate in marine

systems, may also be coupled with AnMBRs to achieve nutrient removal. Alternatively, ion-exchange resins that are specific for ammonium and phosphate removal could be used. In either ion exchange process, the nutrients can be recovered during regeneration, but drawbacks include large capital and chemical regeneration costs (Miladinovic and Weatherley, 2008).

The nutrients in AnMBR effluent can be harnessed and recycled if the effluent is used for irrigation purposes (McCarty et al., 2011). Offsetting the environmental impacts associated with artificial fertilizer use could thus be an added benefit to AnMBR DWW treatment. Challenges regarding the transport of AnMBR effluent for irrigation or locating treatment facilities in close proximity to agricultural areas certainly exist; however, this solution maximizes resource recovery from DWW.

2.10 Trace Contaminant Fate Considerations

As AnMBRs move towards full-scale implementation for the treatment of DWW, the fate of trace contaminants, such as pharmaceuticals and personal care products (PPCPs), during AnMBR treatment requires further attention. Trace contaminants are widely detected in aquatic environments (Kolpin et al., 2002) and many are present in DWW treatment plant effluents (Rosal et al., 2010). Despite the fact that DWW treatment plants represent an important first line of defense against the proliferation of these emerging contaminants in the environment, the DWW treatment plant design process typically does not consider trace contaminant removal. Nevertheless, partial to complete removal does occur in traditional DWW treatment systems for some compounds (Metcalfe et al., 2003), but levels of removal for a given compound can vary widely depending upon the process configuration (Fent et al., 2006). Few studies have focused on the fate of trace contaminants found in DWW during anaerobic treatment and, to the best of our knowledge, none have studied pharmaceutical and PPCP removal during AnMBR treatment.

Carballa et al. (2007) studied the fate of PPCPs during anaerobic digestion of sewage sludge, but not during anaerobic DWW treatment, and found varying degrees of removal depending on the specific compound. Microbial aerobic (or oxic) degradation pathways for substituted and unsubstituted aromatic rings, chemical structures common to many PPCPs, have been studied extensively (e.g. Harayama et al., 1992). However, reductive pathways utilized during anaerobic treatment are not well understood and are an area in need of further research.

As we consider the potential role of AnMBR processes on trace contaminant fate in resource recovery systems, the applicability of existing knowledge about the anaerobic fate of xenobiotic compounds to trace contaminant fate needs to be evaluated. Most studies on microbial degradation in anaerobic environments work with high concentrations of the target compounds (mg/L - μg/L range), concentrations that are far from environmentally relevant (typically in the μg/L - ng/L range for DWW). This choice may dramatically impact values obtained for degradation kinetics. A variety of anaerobic microbes, including denitrifiers, iron-reducers, sulfate-reducers, methanogens, and anoxygenic phototrophs, are capable of degrading aromatic compounds (Tierney et al., 2010). A better understanding of the microbes involved and enzymes used in degrading trace contaminants in anaerobic environments will help inform the development of fate pathways in AnMBRs. In addition, transformation products of anaerobically degraded trace contaminants and their ecotoxicity or public health risks are largely unknown and uncharacterized. As pharmaceuticals and PPCPs evolve and take new forms, such as with the emergence of nanoparticles in medicine (Wagner et al., 2006), their behavior and biodegradability may also change. Understanding the fate of trace contaminants in AnMBRs and post-treatment processes, which utilize different redox environments than conventional treatment systems, is necessary to ensure a safe effluent that limits trace contaminant pollution.

2.11 Pilot-scale Studies

The performance of AnMBRs treating DWW has been assessed in three recent pilot-scale studies by Giménez et al. (2011), Dagnew et al. (2011), and Martinez-Sosa et al. (2011) (Table 2). Each one of these studies indicates that treatment performance similar to that observed during bench-scale research may be obtained at a larger scale. Furthermore, they report that membrane fouling may be avoided in the long-term. Giménez et al. (2011), however, highlighted that high sulfate concentrations in DWW severely reduce the potential methane generation and energy recovery of AnMBR systems. Considering additional complications with sulfide corrosion and the need for biogas scrubbing, AnMBR treatment of sulfate-rich DWW should be avoided.

Giménez et al. (2011) operated a pilot-scale facility fed with pre-treated DWW at a 70 day SRT, an HRT ranging from 20 to 6 hours, and a temperature of 33°C. The pilot consisted of an anaerobic reactor connected to two membrane tanks with 0.05 µm hollow fiber membranes. The total liquid volume of the system was 2,500 L. The pilot also included a rotofilter for pre-treatment screening, an equalization tank, and a degasification vessel installed between the membrane tanks and permeate pump. Biogas sparging, relaxation, and backflushing were employed for membrane fouling control. Biogas sparging was also utilized in the anaerobic reactor to enhance mixing. The total and soluble COD concentrations in the influent averaged 445 ± 95 mg/L and 73 ± 25 mg/L, respectively. Sulfate concentrations were particularly high, averaging 297 ± 54 mg/L, an order of magnitude higher than average sulfate concentrations reported for DWW (Tchobanoglous et al., 2003). During the study, COD removal averaged 87% during stable operation resulting in a permeate COD of 77 mg/L. The high levels of sulfate in the influent greatly impacted biogas production as methanogens and sulfate reducers compete for substrates. Theoretically, 0.67 mg/L of COD is consumed per 1 mg/L of sulfate reduced, therefore, assuming complete sulfate reduction

occurred, approximately 45% of the influent COD was consumed for sulfate reduction rather than for methanogenesis. The authors noted that sulfate removal was below 50% during startup but quickly increased to near complete removal. This increase in sulfate removal also correlated with an increase in the relative abundance of sulfate reducing bacteria. Despite substantial production of sulfides during operation, the methane content in the biogas averaged 55%. Giménez et al. (2011) discussed methane solubility, but did not quantify dissolved methane. In addition, the effectiveness of the degasification vessel was not discussed. No irreversible fouling was observed during the study indicating that the combination of relaxation, backflushing, and biogas sparging was effective at preventing fouling while operating at a sub-critical flux of 10 L/m^2h (LMH). According to the authors, the pilot is currently being operated at 20°C to assess the impact of lower temperature on treatment performance.

Dagnew et al. (2011) operated a 630 L pilot-scale AnMBR for DWW treatment with a configuration similar to the one described by Giménez et al. (2011). The system was operated at an HRT of 8.5 hours, an SRT of 80-100 days, a temperature of 22°C, and was fed screened DWW. Membrane relaxation and biogas sparging were used to control fouling, while operating at a sub-critical flux of 17 LMH. In addition, the membranes were chemically cleaned on a weekly basis. During the study, 79 and 85% COD and BOD_5 removals were observed, respectively. Permeate COD and BOD_5 concentrations averaged 47 and 14 mg/L, respectively, indicating good treatment performance. Essentially no membrane fouling was detected based on TMP at the flux used (17 LMH) even though the flux was relatively high in comparison to other studies. However, this performance may have resulted from the unnecessarily aggressive chemical cleaning schedule. Membrane cleaning was done weekly rather than based on feedback from membrane performance.

Martinez-Sosa et al. (2011) operated a pilot-scale AnMBR with a total volume of 350 L for DWW treatment. Consistent with the other pilot studies, their system consisted of an external submerged reactor configuration with hollow fiber membranes. The pilot was operated for 100 days over which the temperature was reduced from 35°C to 28°C on day 69, and then to 20°C on day 79. Membrane fouling was controlled using biogas sparging, membrane relaxation, and periodic backwashing. The reactor was operated at a sub-critical flux of 7 LMH and an HRT of 19.2 hours during the entire operational period. Suspended biomass was only removed from the AnMBR for sampling purposes and therefore the system had an SRT of approximately 680 days. DWW was used as the influent, however, it was substantially supplemented with glucose to increase the average total COD from 398 mg/L to 630 mg/L. Regardless of temperature, COD removals remained approximately 90% except for some brief performance perturbations when the reactor temperature was reduced. The high COD removal may be misleading, however, since glucose, an easily biodegradable substrate, was added to the influent. An analysis of VFAs in the reactor and permeate suggested VFA degradation by the membrane biofilm, supporting the results of bench-scale studies discussed above.

Table 2-2. Operational parameters and treatment performance results obtained in published pilot -scale AnMBR studies for DWW treatment.

Study	Average Influent Strength [mg/L TCOD[a]]	Temp [°C]	Bioreactor Configuration	Membrane Information	Fouling Control	SRT [d]	HRT [h]	Average Effluent [mg/L TCOD[a]/% removal]
Giménez et al. (2011)	445[b]	33	Completely mixed anaerobic reactor (pilot-scale)	0.05 µm hollow fiber	Biogas sparging	70	6-21	77/83
Dagnew et al. (2011)	224[b]	22	Completely mixed anaerobic reactor (pilot-scale)	ZeeWeed™ hollow fiber	Biogas sparging; relaxation; weekly chemical cleaning	80-100	8.5	47/79
Martinez-Sosa et al. (2011)	630[c]	35	Completely mixed anaerobic reactor (pilot-scale)	38 nm PES[d] flat sheet	Biogas sparging; relaxation; backflushing	680	19.2	<80/90
		28						
		20						

[a]TCOD = total COD
[b]actual DWW; [c]actual DWW supplemented with glucose
[d]PES = polyethersulfone
™General Electric Company

2.12 Future Research Needs

This review paper has shown that many of the inherent benefits of anaerobic treatment may be obtained through AnMBR DWW treatment, while generating an effluent that is comparable in quality to effluent obtained through aerobic treatment. The majority of studies indicated adequate DWW treatment performance at a wide range of operational parameters including low temperatures and HRTs comparable to aerobic treatment. In addition, advancements in fouling control offer the potential to reduce energy requirements. The potential of AnMBR treatment of DWW has been assessed in several recent pilot-scale studies. However, additional fundamental research, pilot-scale investigations, as well as quantitative environmental and economic evaluations are needed before widespread full-scale AnMBR implementation will take place. For instance, more membrane fouling research is needed to enable operation at higher fluxes under the constraints of low energy requirements for fouling control. There is also limited research on the effects of different membrane materials and larger pore sizes (e.g., dynamic membranes) which may enable operation at higher fluxes. Furthermore, the lower limits of AnMBR treatment in terms of temperature have not yet been fully established. Implications of low temperatures on microbial pathways, microbial community structure, and the appropriate inoculum in AnMBR DWW treatment also requires further research. Additionally, the relationships among HRT, SRT, treatment performance, and membrane fouling in AnMBRs are complex and poorly defined in the current literature. The role of the membrane biofilm in treatment also warrants more research along with efforts to evaluate and characterize the fate of trace contaminants in AnMBR treatment. Moreover, nutrient recovery/removal processes such as struvite precipitation and anammox should be evaluated in conjunction with AnMBRs. Ultimately, the recovery or handling of dissolved methane represents the most challenging barrier to AnMBR implementation. Advancements must

be made to sustainably recover or oxidize discharged dissolved methane before AnMBR technology can be implemented on a larger scale. In general, future research on AnMBR DWW treatment must be performed at low temperatures considering DWW in cold and temperate climates is relatively cold and it is not feasible to heat DWW to mesophilic temperatures. We recommend that future research efforts specifically focus on advancements in membrane fouling that reduce energy demands, efficient methods for dissolved methane handling, and establishment of a quantitative environmental and economic evaluation of the technology based on controllable design variables.

2.13 Conclusions

AnMBRs have the ability to produce effluents similar in quality to those generated during aerobic treatment, while recovering energy and producing substantially less residuals. The majority of studies at the bench-scale and pilot-scale indicated adequate treatment performance at HRTs comparable to those used in aerobic treatment and at low temperatures. However, a number of operational concerns exist that require further research before AnMBR DWW treatment can reach full-scale implementation. Specifically, future research efforts should focus on advancements in membrane fouling that reduce energy demands, efficient methods for dissolved methane handling, and establishment of a quantitative environmental and economic evaluation framework.

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Chapter 3. Psychrophilic Anaerobic Membrane Bioreactor Treatment of Domestic Wastewater

3.1 Abstract

A bench-scale anaerobic membrane bioreactor (AnMBR) equipped with submerged flat-sheet microfiltration membranes was operated at psychrophilic temperature (15°C) treating simulated and actual domestic wastewater (DWW). Chemical oxygen demand (COD) removal during simulated DWW operation averaged 92 ± 5% corresponding to an average permeate COD of 36 ± 21 mg/L. Dissolved methane in the permeate stream represented a substantial fraction (40-50%) of the total methane generated by the system due to methane solubility at psychrophilic temperatures and oversaturation relative to Henry's Law. During actual DWW operation, COD removal averaged 69 ± 10%. The permeate COD and 5-day biochemical oxygen demand (BOD_5) averaged 76 ± 10 mg/L and 24 ± 3 mg/L, respectively, indicating compliance with the U.S. EPA's standard for secondary effluent (30 mg/L BOD_5). Membrane fouling was managed using biogas sparging and permeate backflushing and a flux greater than 7 LMH was maintained for 30 days. Comparative fouling experiments suggested that the combination of the two fouling control measures was more effective than either fouling prevention method alone. A UniFrac based comparison of bacterial and archaeal microbial communities in the AnMBR and three different inocula using pyrosequencing targeting 16S rRNA genes suggested that mesophilic inocula are suitable for seeding psychrophilic AnMBRs treating low strength wastewater. Overall, the research described relatively stable COD removal, acceptable flux, and the ability to seed a psychrophilic

Smith, A.L., Skerlos, S.J. and Raskin, L., 2013. Psychrophilic anaerobic membrane bioreactor treatment of domestic wastewater. Water Research 47(4), 1655-1665.

AnMBR with mesophilic inocula, indicating future potential for the technology in practice, particularly in cold and temperate climates where DWW temperatures are low during part of the year.

3.2 Introduction

Because of the recent emphasis on sustainability in the water quality industry, various studies are exploring how domestic wastewater (DWW) treatment can be accomplished in an energy neutral or even energy positive fashion (Guest et al. 2009, McCarty et al. 2011). Current DWW treatment plants often recover energy in the form of methane-rich biogas produced during anaerobic digestion of primary sludge and biomass generated during conventional aerobic treatment. However, approximately 45% of the total biodegradable chemical oxygen demand (COD) in DWW is lost through oxidation to carbon dioxide (McCarty et al. 2011), and thus constitutes a lost resource. Furthermore, the energy requirements of aerobic treatment are typically much greater than the energy recoverable via anaerobic sludge digestion (Foley et al. 2010). To improve energy recovery, reduce costs, and minimize environmental impacts, mainstream anaerobic processes are being considered as replacements for conventional aerobic DWW treatment (van Haandel et al. 2006). In comparison to conventional aerobic treatment schemes with anaerobic sludge digestion, anaerobic mainstream DWW treatment has the potential to convert all biodegradable COD present in DWW to methane, generate substantially less residuals due to the much lower biomass yield of anaerobic microbes, and eliminate aeration requirements.

Although water conservation and source separation have the potential to change DWW characteristics and flow rates, DWW in the U.S. and in many other developed countries is still relatively low strength (average 5-day biochemical oxygen demand [BOD_5] varies from 110-350 mg/L in the U.S.) and is generated at high per capita flow rates (average production rate varies

from 190-460 L/(capita•d) in the U.S.) (Pons et al. 2004, Tchobanoglous et al. 2003). In addition, DWW temperatures are relatively low (average of 16°C in the U.S.) and vary seasonally (Pons et al. 2004, Tchobanoglous et al. 2003). Given the common perception that anaerobic bioreactors must be heated to mesophilic (30-40^{o}C) or thermophilic (50-60^{o}C) temperatures to operate efficiently, it is not surprising that aerobic processes have been favored over anaerobic systems for the treatment of high volume and relatively cold DWW. Heating high volumes of DWW would not be economically feasible, especially since the potential energy recovery from low-strength DWW on a per volume basis is low (Lettinga et al. 2001, Martin et al. 2011). As a result, anaerobic treatment has not been used for mainstream DWW treatment except in regions with hot climates, which naturally benefit from elevated DWW temperatures (Aiyuk et al. 2006). In most temperate climates, efficient treatment at low temperatures would need to be demonstrated before widespread implementation of anaerobic DWW treatment could be considered.

The need to treat high volumetric flow rates of DWW necessitates treatment at short hydraulic retention times (HRTs) to keep capital costs and footprints of treatment systems sufficiently low. At the same time, the low growth rates of anaerobic microbes require long solids retention times (SRTs) to ensure adequate treatment. These opposing constraints call for a decoupling of HRT and SRT in anaerobic systems. This decoupling becomes even more important at low temperatures for which biomass growth rates are especially low and any sludge washout must be avoided (Lettinga et al. 2001). Consequently, further development of anaerobic technologies capable of adequately treating DWW at high volumetric loading rates and low temperatures is a prerequisite to materializing the potential benefits of mainstream anaerobic treatment of DWW.

AnMBRs have recently emerged as a potential technology for high-rate anaerobic treatment by combining anaerobic biological treatment with membrane filtration. This leads to nearly absolute

biomass retention and allows for operation at high SRTs, and thus low temperatures, with the potential to generate a high quality effluent (permeate). A number of studies have been published assessing AnMBR performance for the treatment of simulated and actual DWW (Baek et al. 2010, Chu et al. 2005, Dagnew et al. 2011, Gao et al. 2010, Gimenez et al. 2011, Ho and Sung 2010, Ho and Sung 2009, Hu and Stuckey 2006, Huang et al. 2011, Kim et al. 2011, Lew et al. 2009, Martinez-Sosa et al. 2012, Martinez-Sosa et al. 2011, Salazar-Pelaez et al. 2011, Wen et al. 1999) as reviewed recently by Smith et al. (2012). However, only a few studies have evaluated AnMBR performance at psychrophilic temperatures of 15^{o}C and below. Specifically, Chu et al. (2005) and Ho and Sung (2010) observed average COD removals of 85-86% at 15°C, and Wen et al. (1999) reported an average COD removal of 88% at 12°C.

Several approaches have been applied to counteract membrane fouling in AnMBRs, such as backflushing (Chu et al. 2005, Ho and Sung 2010, Lew et al. 2009) and biogas sparging (Dagnew et al. 2011, Gimenez et al. 2011, Hu and Stuckey 2006, Huang et al. 2011, Martinez-Sosa et al. 2011). Using biogas sparging and backflushing concurrently has been observed to be more effective than either control method alone in aerobic MBRs (Lu et al. 2005), but the effectiveness of this combined approach versus the use of only biogas sparging has not been directly compared for AnMBRs. In addition, the impact of methane solubility on AnMBR energy recovery has not been adequately addressed (Dagnew et al. 2011, Gimenez et al. 2011, Hu and Stuckey 2006, Kim et al. 2011). Finally, the implications of psychrophilic operation on the anaerobic microbial communities and appropriate inoculum choices for AnMBRs have received limited attention in the literature. Molecular methods, such as clone library based microbial community analyses, have been used only in one study so far (Gao et al. 2010) and high-throughput DNA sequencing methods

have yet to be employed to examine microbial community structure and considerations regarding appropriate inocula.

This study addresses the aforementioned gaps in the AnMBR literature by assessing the long-term performance of a bench-scale AnMBR treating simulated and actual DWW at psychrophilic temperatures. Pyrosequencing targeting 16S rRNA genes was used to assess the implications of low-temperature AnMBR treatment on the archaeal and bacterial community structures in the suspended biomass and in the biofilm. Pyrosequencing was also used to evaluate the selection of inocula seeds for psychrophilic AnMBR treatment.

3.3 Materials and Methods

3.3.1 AnMBR Configuration

The bench-scale AnMBR used in this study (Figure 3-1) had a liquid volume of 5 L (total volume of 7 L) and contained two submerged membrane housings (manufactured by eMachineShop, Mahway, NJ). Each membrane housing incorporated two separate flat-sheet microfiltration polyethersulfone membranes (GE Osmonics, Greenville, SC) with a pore size of 0.2 µm and a total effective membrane area of 0.0387 m^2 (7.74 m^2/m^3). Because of the two separate membrane housings, two permeate streams, designated P1 and P2, were generated during operation. Intermittent mixing (1 minute every 30 minutes) was provided by magnetic impeller (Applikon Biotechnology, Foster City, CA). Influent and permeate were pumped by peristaltic Masterflex L/S pumps (Cole-Parmer, Vernon Hills, IL). The bioreactor was equipped with a water jacket connected to a Polystat 6-L recirculating water bath (Cole-Parmer, Vernon Hills, IL) for temperature control. Pressures in the system were measured using pressure transducers (Omega Engineering, Stamford, CT) located in the headspace and on each permeate

line. The bioreactor contained a level sensor and temperature probe (Applikon Biotechnology, Foster City, CA). The bioreactor headspace was connected to a biogas collection system with a 1-L Tedlar gas bag and mini diaphragm pump (KNF Neuberger, Trenton, NJ), which recirculated headspace biogas and dispersed it directly below each membrane through a horizontally placed sparging tube designed for fouling control. The bench-scale AnMBR was connected to a computer, which operated a control program (written in C++) and LabVIEW (National Instruments, Austin, TX) data acquisition software. The control program was responsible for operation of all pumps, biogas recirculation, and mixing. The LabVIEW application continuously monitored and recorded temperature, pressures, and feed flow rate.

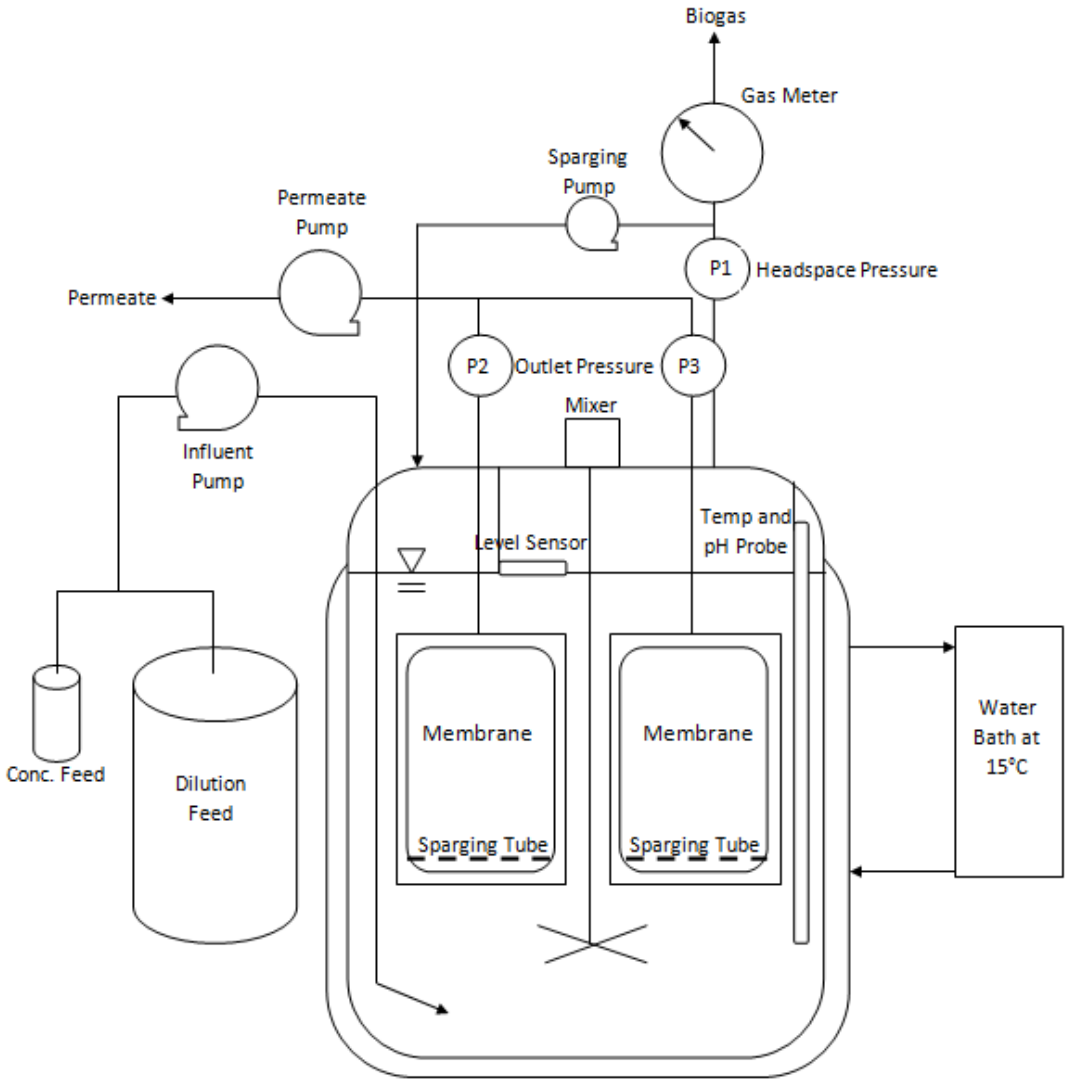

Figure 3-1. Schematic of bench-scale AnMBR.

3.3.2 Inoculation and Operational Parameters

The bench-scale AnMBR was inoculated with seed sludge from three sources: a mesophilic (35.5°C) upflow anaerobic sludge blanket (UASB) reactor (Anheuser-Busch, St. Louis, MO), a mesophilic (32°C) DWW treatment plant anaerobic sludge digester (Northfield Wastewater

Treatment Plant, Whitmore Lake, MI), and a psychrophilic (0-23°C; yearly temperature range was estimated based on a few data points) anaerobic lagoon used for the treatment of DWW (Maybee, MI). The system was inoculated with a total volatile suspended solids (VSS) concentration of 6,000 mg/L, consisting of 2,500 mg/L VSS of the UASB sludge, 2,500 mg/L VSS of the anaerobic digester sludge, and 1,000 mg/L VSS of the anaerobic lagoon sludge.

During the first operational period of 351 days, the bench-scale AnMBR was fed a synthetic wastewater that simulated DWW. The synthetic DWW was prepared as a concentrated solution adapted from the SYNTHES recipe presented by Aiyuk and Verstraete (2004) (Table S3-1, Appendix A). The original SYNTHES recipe had some divergences from reported medium strength U.S. DWW composition (Tchobanoglous et al. 2003), including elevated concentrations of phosphorus, nitrogen, and alkalinity. These concentrations were modified in the adapted recipe to formulate a DWW feed representative of medium strength U.S. DWW. The concentrated feed was prepared biweekly, acidified with hydrochloric acid to a pH of 3.5, and refrigerated at 4°C to prevent biodegradation. After dilution with a basic buffer solution containing 3.57 mM sodium bicarbonate, 0.126 mM magnesium phosphate, 0.110 mM potassium phosphate, and 0.605 mM sodium hydroxide through in-line mixing, the synthetic feed had average measured total and soluble COD (SCOD) concentrations of 440 mg/L and 290 mg/L, respectively.

The reactor temperature was maintained at 15.0 ± 0.1°C throughout the study. The initial organic loading rate (OLR) during synthetic wastewater operation was 660 mg COD/(L•d), which corresponded to a hydraulic retention time (HRT) of 16 hours. The target membrane flux to achieve this HRT was 8 L/(m^2•h). At times, this target HRT was not reached due to reduced pump efficiency, resulting in a lower OLR. The OLR thus varied between approximately 440 and 660

mg COD/(L•d) and the HRT varied between 16 and 24 hours. Biomass was only removed from the AnMBR for sampling purposes, which resulted in an SRT of approximately 300 days.

Biogas sparging and permeate backflushing were employed to prevent membrane fouling. Biogas sparging was operated continuously at a flow rate of 4.67 L/min evenly distributed across the four membrane surfaces (specific gas demand of 7.24 $m^3/(m^2 \cdot h)$; superficial gas velocity of 13.9 m/h). Permeate backflushing was initialized by reversing the flow of the permeate pumps while keeping the flow rate constant (5.21 mL/min). During the first 185 days of operation, backflushing was performed for 30 seconds every 30 minutes. From days 186 through 351, backflushing was carried out for four minutes every four hours to increase the duration of backflush events without decreasing permeate production, except as described below. In replicate experiments designed to study the contribution of backflushing to fouling prevention, membrane P1 was backflushed for four minutes every four hours and membrane P2 was not backflushed. These experiments were carried out from days 231 through 269 and days 320 through 351.

During the second operational period, the bench-scale AnMBR was operated using actual DWW collected from the Dundee Wastewater Treatment Plant (Dundee, MI). A batch of primary influent was collected immediately after preliminary treatment (mechanical screen and grit removal) twice a week and stored at 4°C. For consistency, wastewater was collected at approximately the same time on each collection day. Fresh membranes were installed at the start of the second operational period. Both membrane housings were backflushed for four minutes every four hours. All other operational variables remained as described above except the OLR (170-393 mg COD/(L•d)) which was lower relative to the first operational phase. During the first 50 days of this second operational period, unstable performance was observed and likely resulted from high and variable sulfate concentrations in the influent (160 ± 100 mg/L). These elevated and fluctuating sulfate

concentrations were determined to be caused by the influent collection time coinciding with a once daily industrial facility wastewater discharge in close proximity to the Dundee Wastewater Treatment Plant. Unstable performance may have resulted from inhibitory compounds present in this industrial discharge. The influent collection time was changed and this resulted in lower and less variable influent sulfate concentrations for the next 40 days of AnMBR operation (65 ± 33 mg/L). Data are reported for these 40 days only.

3.3.3 Chemical Assays and Sampling

BOD_5, COD, alkalinity, total suspended solids (TSS) and VSS were determined using procedures outlined in Standard Methods (2005). Soluble COD was determined by filtering samples through a 0.2 µm filter to be consistent with the physical removal capacity of the membrane (same pore size). BOD_5 was analyzed by the Ann Arbor Drinking Water Treatment Plant (Ann Arbor, MI) on day 269 of the synthetic DWW run and on a weekly basis by the Dundee Wastewater Treatment Plant (Dundee, MI) during operation with actual DWW.

Concentrations of volatile fatty acids (VFAs) (formic acid, acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid) were determined by high-performance liquid chromatography (HPLC). The HPLC (1100 Series, Hewlett Packard, Palo Alto, CA) was equipped with a UV detector, an autosampler, and a vacuum degasser. A 5 mM sulfuric acid eluent solution was passed through an Aminex HP87-H column at 60°C. Sulfate concentrations were measured using an ion chromatography system (Dionex, Sunnyvale, CA) with a Dionex DX 100 conductivity detector. Chromatographic separation was achieved using a Dionex AS-14 column (Dionex, Sunnyvale, CA). Anions were eluted through the column with a mixture of ACS reagent grade 1 mM bicarbonate and 3.5 mM carbonate at a flow rate of 1 mL/min.

Biogas methane content was measured with a gas chromatograph (Gow-Mac, Bethlehem, PA) coupled with a thermal conductivity detector (TCD). Measurement of dissolved methane in the permeate was accomplished as previously described (Rudd et al. 1974). Briefly, 30 mL of permeate was collected in a syringe containing 30 mL nitrogen gas. The syringe was shaken by hand for 1 minute to strip dissolved methane into the gas phase, which was used for gas chromatography analysis. Theoretical methane production was calculated assuming 350 L of methane was generated per kg of COD removed (Grady et al. 2011) and by considering the influent COD unavailable for methane generation due to sulfate reduction. Biomass yield was not taken into account in the calculation as it was assumed to be very low (see below). Biogas production was measured by collecting gas in a 1-L Tedlar bag and quantifying the production daily using a wet-type gas meter (Actaris Metering Systems, Dordrecht, The Netherlands).

3.3.4 EPS Extraction and Quantification

Extracellular polymeric substances (EPS) were extracted by a cationic exchange resin method (Frolund et al. 1996) from biofilm samples removed from the AnMBR (with the membrane attached). Biofilm samples were removed from the AnMBR on days 276 and 320 of the first operational period and cut into 4x6 cm sections using a sterile scalpel. Additional biofilm sections were cut to determine volatile solids (VS) according to Standard Methods (2005). Biofilm samples for EPS extraction were immediately stored at -80°C prior to extraction. Duplicate EPS extractions were performed for each membrane. EPS extraction was also performed on a fresh PES membrane section as a negative control. Proteins and carbohydrates in extracted EPS were quantified according to the Bradford assay (Bradford and Williams 1976) and the Dubois method (Dubois et al. 1956), respectively.

3.3.5 Microbial Analysis

Inocula biomass samples were individually stored at -80°C upon AnMBR startup until further processing. Suspended and biofilm biomass samples were collected from the AnMBR 275 days after startup and stored at -80°C. DNA extractions were completed using a phenol chloroform extraction method (Urakawa et al. 2010). Additional DNA purification was done using the Wizard DNA Clean-Up System (Promega, Madison, WI) according to manufacturer's instructions. The V3, V4, and V5 variable regions of the 16S ribosomal RNA (rRNA) gene were targeted with bacterial pyrosequencing primers Bact-338F/Bact-909R and archaeal pyrosequencing primers Arch-340F/Arch-915 (Pinto and Raskin 2012). A minimum of two uniquely barcoded primer pairs were used for amplification of each sample to provide replication in sequencing results. PCR products were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA). DNA was quantified using a spectrophotometer (NanoDrop, Wilmington, DE). After PCR purification and DNA quantification, bacterial and archaeal amplicons were separately pooled by equal mass (for each uniquely barcoded primer pair) and subsequently concentrated through PCR purification using the QIAquick PCR purification kit. Concentrated bacterial and archaeal amplicons were pooled at 40% bacterial amplicon mass and 60% archaeal amplicon mass. The resulting amplicon pool was concentrated through PCR purification using the QIAquick PCR purification kit and run on a 1% agarose gel. Gel extraction was performed using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) according to manufacturer's instruction. An additional PCR purification was done prior to submitting the amplicon pool to Engencore (University of South Carolina, Columbia, SC) for pyrosequencing of $1/8^{th}$ pico-titer plate (Pinto and Raskin 2012). The pooled amplicons generated approximately 20,000 reads and after quality screening 12,368 sequences remained (Table S3-2). The resulting sequences were classified using the Ribosomal Database Project (Maidak et al. 1997) and further analyzed with Mothur (Schloss et al. 2009) for operational

taxonomic unit (OTU)-based clustering (average neighbor algorithm at 3% cutoff), principle co-ordinate analyses, and determination of weighted UniFrac distances (Liu et al. 2007).

3.4 Results and Discussion

3.4.1 Reactor Performance

To assess long-term treatment performance at a psychrophilic temperature of 15ºC, the bench-scale AnMBR was operated for 351 days treating simulated DWW. COD removal during this period averaged 92 ± 5% corresponding to an average permeate COD concentration of 36 ± 21 mg/L (Figure 3-2). This level of COD removal was higher than the previously reported COD removal of approximately 85% for this temperature (Chu et al. 2005, Ho and Sung 2010). The greater COD removal in the current study may have resulted from differences in membrane configuration (hollow fiber (Chu et al. 2005) and tubular (Ho and Sung 2010) versus flat-sheet in the current study) and/or other differences. A study directly comparing the impact of different membrane configurations on COD removal in an AnMBR has yet to be done. Influent and permeate BOD_5 values were measured on day 269 of operation and averaged 227 and 18 mg/L, respectively (92% removal). The permeate COD concentration on this sampling day averaged 43 mg/L, slightly higher than the average permeate COD for the 351 days of operation. VFAs in the permeate were largely comprised of acetate (average concentration 18 ± 16 mg/L), with propionate present in lower concentrations (average concentration 4 ± 4 mg/L). Permeate concentrations of other VFAs, such as formate, isobutyrate, butyrate, isovalerate, and valerate, averaged ≤ 1 mg/L. The total VFA concentration in the permeate averaged 22 ± 20 mg/L as acetate whereas the total VFA concentration in the reactor averaged 28 ± 22 mg/L as acetate. Periodic spikes in permeate COD corresponded with spikes in permeate VFA concentrations, which typically occurred immediately after membrane replacement and unavoidable exposure of the system to oxygen.

During the operational period of 351 days, bioreactor VSS gradually increased from 6,000 mg/L to 10,600 mg/L, which corresponds to a yield < 0.10 g VSS/g COD removed (the yield calculation takes into account biomass removal through sampling and membrane replacement).

Consistent differences in bioreactor and permeate soluble COD concentrations (Figure 3-2) indicated substantial soluble COD removal across the membrane. This removal averaged 21 ± 8% of the total COD removal. It should be noted that differences in the physical removal capacity of the filters used in sample processing relative to the AnMBR membranes despite having the same pore size could have influenced this observation. However, other AnMBR studies have observed a similar phenomenon at a range of operational temperatures (Baek et al. 2010, Chu et al. 2005, Ho and Sung 2009, Hu and Stuckey 2006, Huang et al. 2011). Furthermore, Ho and Sung (2010) noticed an increase in membrane-mediated soluble COD removal with a decrease in operational temperature, but an explanation was not provided for why this temperature dependence may have occurred. The mechanism of soluble COD removal across the membrane may be related to microbial activity, size or charge exclusion, and/or adsorption. In two studies, specific methanogenic activity (SMA) experiments were performed with biofilm biomass, which suggested microbial activity in the membrane biofilm contributed to soluble COD removal across the membrane (Ho and Sung 2010, Vyrides and Stuckey 2011). The relative contribution of biological activity compared to other potential mechanisms has yet to be studied in detail. Regardless of mechanism, the removal of soluble COD across the biofilm is an important factor in achieving a high quality effluent during AnMBR treatment.

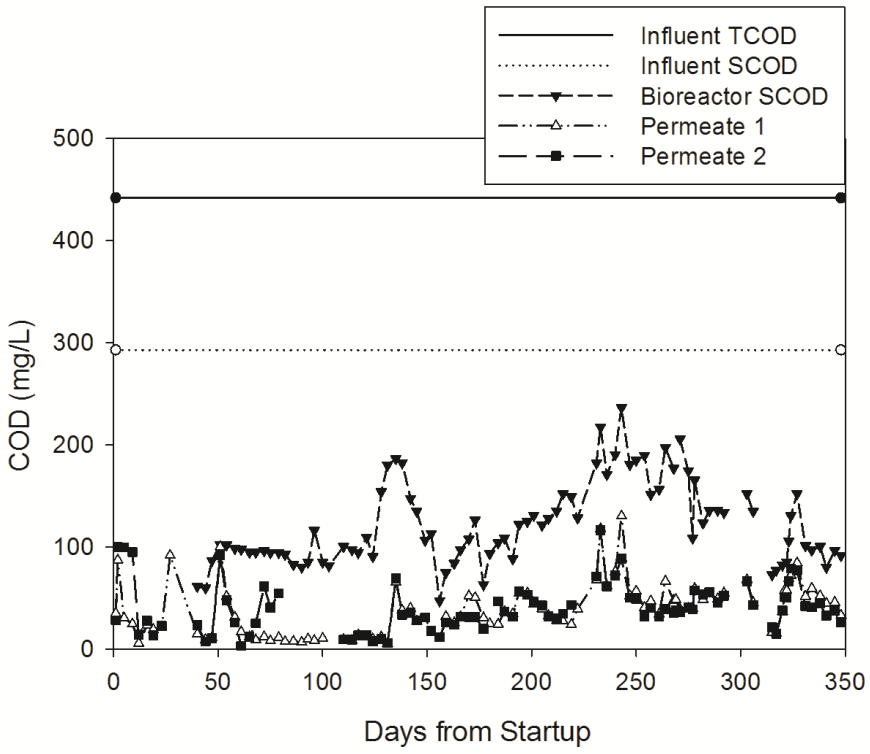

Figure 3-2. Average measured COD concentration in influent (total and soluble COD), bioreactor (soluble COD), and Permeate 1 (P1) and Permeate 2 (P2) (total COD).

Approximately 40-50% of the total methane generated in the AnMBR was dissolved in the permeate and was thus discharged with the permeate rather than collected in the headspace (Figure 3-3). The relatively high fraction of methane lost through the permeate is in part due to methane's increased solubility at psychrophilic temperatures. However, substantial methane oversaturation, approximately 1.5 times that predicted by Henry's law, was also responsible for this high methane loss through the permeate (Henry's law constant of 34,300 atm was used for the operational temperature; Tchobanoglous et al. 2003). Methane oversaturation has been observed in several non-membrane anaerobic bioreactor studies (Hartley and Lant 2006, Pauss et al. 1990, Singh et al. 1996), which cited mass-transfer limitations as the likely cause. Conversely, Giménez et al. (2012) did not observe methane oversaturation when operating an AnMBR and contributed this observation to the use of biogas sparging creating equilibrium between the gas and liquid-phases.

In the current study, the use of biogas sparging likely reduced mass-transfer limitations compared to conventional anaerobic bioreactors although methane oversaturation was still observed. It is possible that the pressure differential across the membrane plays a role in increasing permeate dissolved methane concentrations to the point of oversaturation. Further, methanogenic activity in the biofilm results in methane generation near the membrane surface and may contribute to permeate methane oversaturation, especially in combination with a pressure differential across the membrane. The oversaturated methane quantified in the permeate theoretically corresponds to 56% of the soluble COD removal that occurred across the membrane. It should be noted that the dissolved methane in the permeate was not included in the measured permeate COD, assuming our analyses results were consistent with those reported by Hartley and Lant (2006). Because methane has a global warming potential 25 times that of carbon dioxide (IPCC 2007), management of permeate dissolved methane is necessary to limit greenhouse gas emissions (Smith et al., 2012). Furthermore, permeate dissolved methane represents a considerable fraction of the total energy available in DWW and its recovery may be necessary for energy neutral operation. The magnitude of potential direct greenhouse gas emissions from an AnMBR or other mainstream anaerobic treatment process is a direct result of the high volume of effluent containing dissolved methane generated and is only marginally increased by a lower operational temperature. Therefore, management of effluent dissolved methane is critical to limit greenhouse gas emissions from mainstream anaerobic processes regardless of temperature.

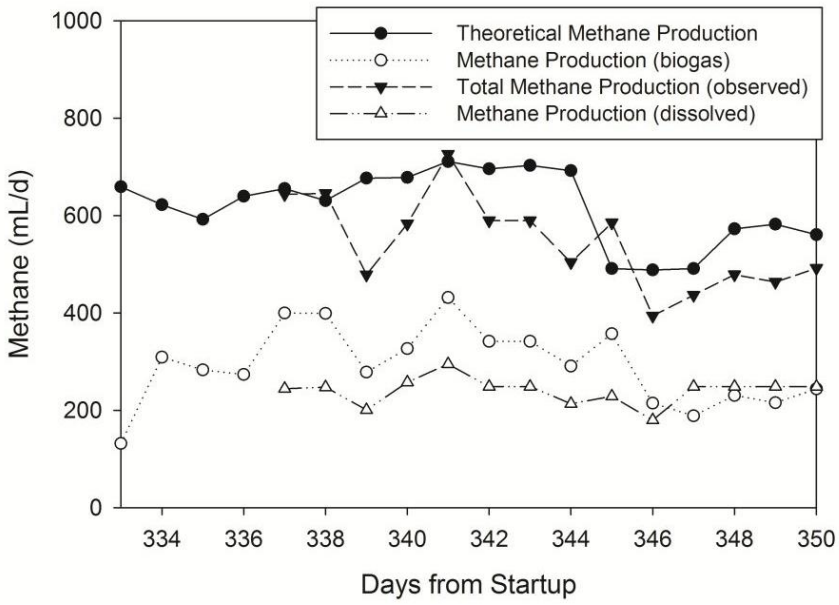

Figure 3-3. Methane production in the system (headspace, dissolved, and total observed) during 20 days of AnMBR operation compared to theoretical methane production. Theoretical methane production was calculated assuming 350 L of methane was generated per kg of COD removed (Grady et al. 2011) and by considering the influent COD unavailable for methane generation due to sulfate reduction.

The COD removal during operation with actual DWW, 69 ± 10%, was substantially lower than during treatment of simulated DWW, 92 ± 5%. This lower COD removal was partly a result of the lower strength of the actual DWW compared to the simulated DWW (259 ± 82 mg/L versus 440 ± 68 mg/L, respectively). However, permeate COD was also higher for the actual DWW, averaging 76 ± 10 mg/L, versus 36 ± 21 mg/L for the simulated DWW. Despite lower COD removal, permeate BOD_5 values averaged 25 ± 3 mg/L during operation with actual DWW. Nearly complete sulfate reduction was observed with permeate sulfate concentrations averaging 2.3 ± 2.1 mg/L (96% reduction). Sulfate reduction theoretically consumed 23% of the total COD removed. Biogas production was limited by sulfate reduction, the wastewater's low strength, the high methane solubility at the low operational temperature, and methane oversaturation in the permeate. No measureable biogas production was observed at influent COD < 225 mg/L.

The effluent quality in this study suggests that U.S. EPA's standards for secondary effluent (<30 mg/L BOD_5, <30 mg/L TSS, 5-9 pH) can be met during low-temperature AnMBR treatment. However, it is important to note that AnMBR treatment does not remove nutrients and therefore additional treatment may be required in watersheds where nutrient effluent limits are in place. Conversely, the nutrient richness of the AnMBR effluent may be considered an asset in locations where reuse of the effluent for agricultural irrigation is feasible. The relatively high quality of AnMBR effluent, especially when compared to other high-rate anaerobic treatment processes (Khan et al. 2011), offers the potential for agricultural reuse without post-treatment.

3.4.2 Comparative Membrane Fouling Experiment and Biofilm EPS Quantification

To assess the role of biogas sparging and permeate backflushing in short- and long-term membrane fouling, comparative experiments were performed using the parallel membrane housings in the AnMBR. In a first type of experiment, permeate backflushing was practiced for only one membrane housing (P1), while biogas sparging was employed continuously on both membrane housings. Fresh membranes were installed at the beginning of the experiment. Over the course of the experiment (days 320-351), P1 did not show evidence of membrane fouling as the transmembrane pressure (TMP) and flux remained constant throughout the experiment (Figure 3-4). However, P2 TMP increased to -45 kPa during the first 6 days of operation and then remained constant for the remainder of the experiment. P2 flux declined to approximately 3.5 L/m^2*h over the first 15 days of operation and did not change thereafter. During this fouling experiment, the difference in permeate COD concentrations between P1 and P2 averaged 10 ± 4 mg/L ($p < 0.05$). The more fouled membranes, P2, generated a higher quality permeate. This observation indicates that a correlation exists between membrane fouling and permeate quality. This experiment was reproduced (days 231-269) with a similar outcome. In a second type of experiment, biogas

sparging was discontinued for both membrane housings, while permeate backflushing was continued to assess the role of biogas sparging in comparison to backflushing in membrane fouling control. Discontinuation of biogas sparging resulted in abrupt membrane fouling evidenced by a substantial increase in TMP (30-40 kPa) over the course of several hours. Taken together, these two types of experiments suggest that backflushing is necessary to avoid long-term membrane fouling, whereas biogas sparging is a pre-requisite to having an operational AnMBR system. Furthermore, the combination of both fouling control measures enables better control of long-term fouling than when either is used individually.

Membranes with different levels of fouling were removed from the AnMBR and subjected to EPS extraction. Three of the four membranes were more fouled based on visual observation and higher TMP prior to membrane removal (-65 to -80 kPa), whereas one of the membranes was less fouled (TMP was -10 kPa). The membranes exhibiting greater fouling contained higher concentrations of EPS, measured as protein and carbohydrate mass per total organic mass (volatile solids) associated with the membrane, than less fouled membranes (Figure 3-5). EPS may be a factor in the greater soluble COD removal observed with more fouling. However, fouled membranes also had considerably more attached biomass: 10 g VS/m^2 for the less fouled membrane and an average of 135 ± 69 g VS/m^2 for the fouled membranes, which corresponded to 8.7 ± 1.0% of the system's total VSS. Therefore, it is not possible to ascertain the relative contributions of EPS versus attached biomass on membrane fouling and soluble COD removal based on these data. Several AnMBR studies have considered EPS as a major contributor to membrane fouling (Chu et al. 2005, Gao et al. 2010) but its correlation with soluble COD removal in AnMBRs has not been assessed. EPS may increase adsorption of soluble organics or may correlate with increases in biofilm microbial

activity as observed in aerobic filters (Gao et al. 2008). These potential mechanisms may be a factor in the greater soluble COD removal observed by membranes with more fouling.

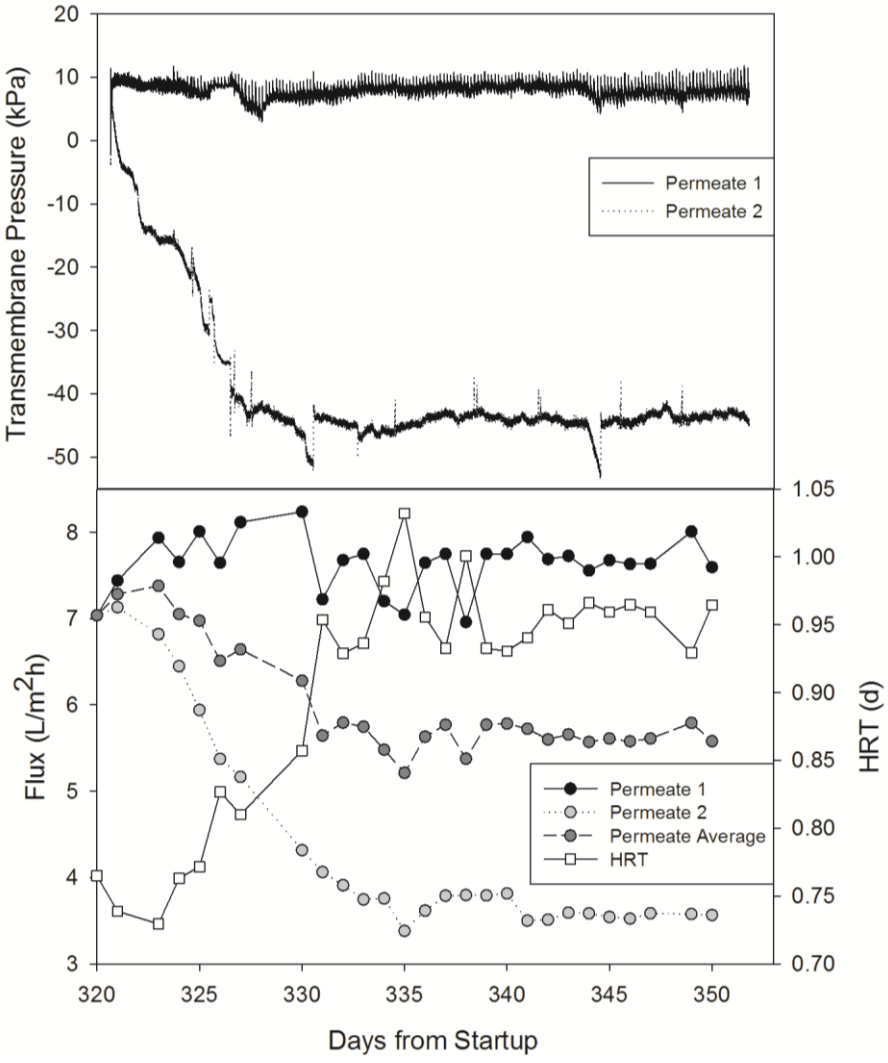

Figure 3-4. Transmembrane pressure for Permeate 1 (P1) and Permeate 2 (P2) over time (top). Flux for Permeate 1 and Permeate 2 and HRT over time (bottom). During this operational period, days 320 to 351, Permeate 1 was backflushed for 4 minutes every 4 hours operation while Permeate 2 was not backflushed.

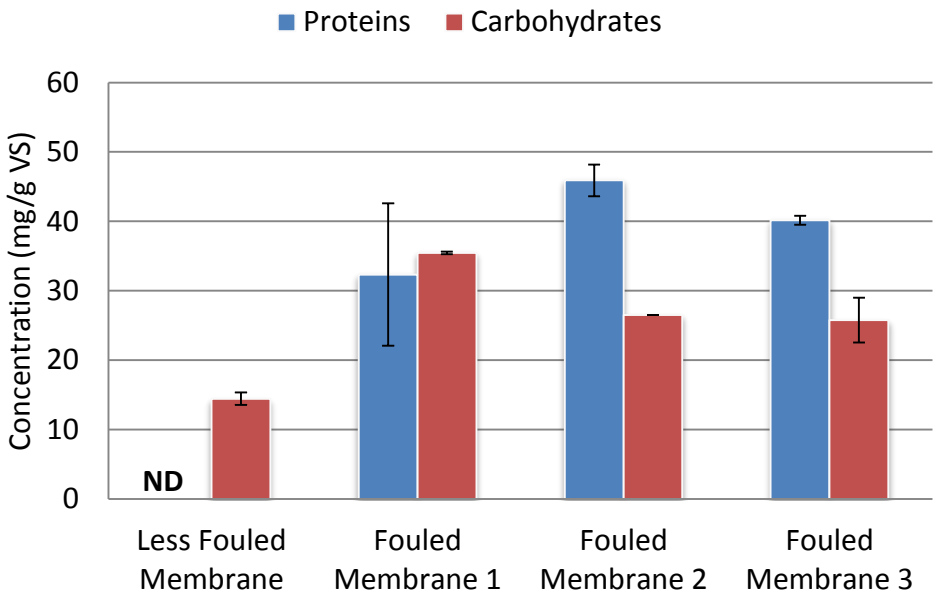

Figure 3-5. Concentration of proteins and carbohydrates in extracted EPS from membrane samples removed from the AnMBR. Less fouled membrane and fouled membrane 1 were removed from the AnMBR on day 276. Fouled membrane 2 and 3 were removed from the AnMBR on day 320. Error bars represent the standard deviation of duplicate EPS extractions and triplicate protein/carbohydrate measurements.

3.4.3 Microbial Community Analysis

Analyzing the archaeal microbial communities in the biofilm and suspended biomass 275 days after AnMBR startup indicated *Methanosaeta* was the dominant genus in each sample representing 61.2 ± 5.1% and 66.7 ± 1.3% relative abundance (average and standard deviation obtained by using sequencing data generated using three different uniquely barcoded primer sets for each DNA extract), respectively, indicating that aceticlastic methanogens were abundant in the system (Figure 3-6). Most hydrogenotrophic methanogens in the biofilm and suspended biomass belonged to the genera *Methanobacterium* and *Methanospirillum*. The relative abundance of these genera was considerably different in the biofilm and suspended biomass communities: *Methanobacterium* constituted 10.7 ± 2.2% and 21.5 ± 2.2% of the relative abundance in the biofilm and suspended biomass samples, respectively. In contrast, *Methanospirillum* represented 19.6 ± 3.0% and 8.2 ±

1.1% relative abundance in the biofilm and suspended biomass samples, respectively. Kinetic values observed by Schauer et al. (1982) and Karadagli and Rittman (2005) suggest that *Methanospirillum* spp. have a higher substrate affinity than *Methanobacterium* spp., whereas their maximum specific growth rates are similar. Substrate concentrations are likely lower in the biofilm in comparison to suspended biomass creating conditions in the biofilm favorable to methanogens with higher substrate affinity. However, these kinetic parameters were not determined at psychrophilic temperatures and may not be appropriate to describe the present study. Alternatively, the propensity of *Methanospirillum* to grow in filaments (Beveridge et al. 1991) may have resulted in the observed higher relative abundance of *Methanospirillum* over *Methanobacterium* in the biofilm.

The dominance of aceticlastic methanogens in the system indicates that low temperatures may not offer a considerable energetic advantage to hydrogenotrophic methanogens as suggested by Lettinga et al. (2001) or alternatively that any energetic advantage is not great enough to be reflected in relative abundance. It should be noted that both aceticlastic and hydrogenotrophic methanogens have low biomass yields: reported yield values range from 0.01 to 0.07 g biomass COD/g COD (Batstone et al. 2001, Conklin et al. 2006). Even though the long SRT and low OLR of this study potentially created conditions in which hydrogenotrophic methanogens could have been more active than their aceticlastic counterparts such differences are more difficult to detect with DNA-based methods for slow growing microbes with low biomass yields even over relatively long time periods. More research using RNA-based methods such as RT-qPCR (reverse transcriptase quantitative polymerase chain reaction) is necessary to better understand the effect of psychrophilic temperatures on methanogenic pathways.

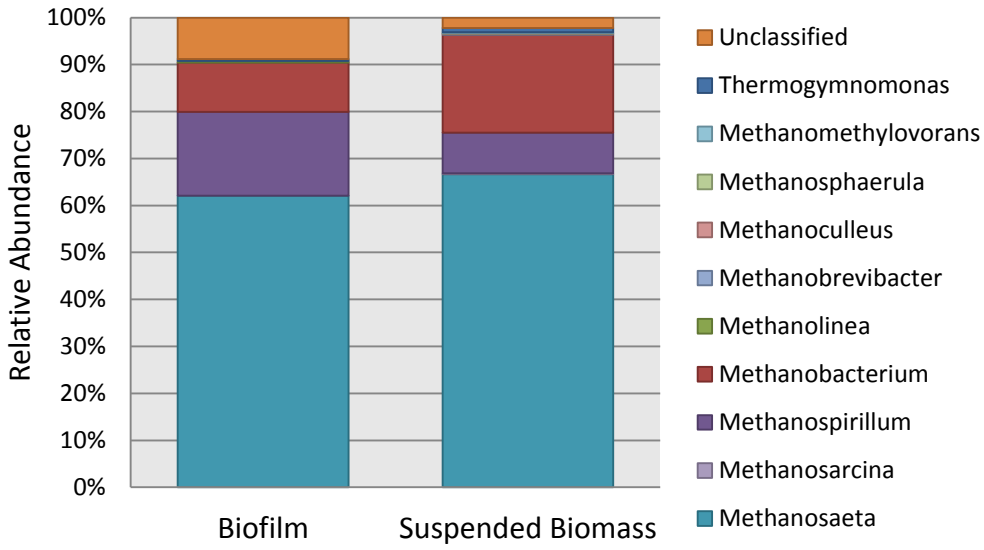

Figure 3-6. Classification at the genus level of the archaeal communities in the biofilm and suspended biomass samples taken 275 days after startup.

Bacteroidetes were the dominant bacterial phylum in the biofilm and suspended biomass samples (Figure 3-7). This contrasts with the work by Gao et al. (2010) in which *Bacteroidetes* were observed in the suspended biomass but not in the fouling layer of an AnMBR operated for treatment of a synthetic domestic wastewater at a temperature of 30°C. In addition to *Bacteroidetes*, *Proteobacteria* and *Firmicutes* showed a high relative abundance in both AnMBR biomass samples. Known syntrophic bacteria belonging to the genera *Smithella* and *Syntrophorhabdus* were found in both the suspended biomass and biofilm at relatively low abundances (<1%) indicating the presence of syntrophic interactions between hydrogenotrophic methanogens and syntrophs. The 'semi-syntrophic' class *Anaerolineae* of the bacterial phylum *Chloroflexi* (Narihiro et al. 2012) were also detected at 0.8% and 1.8% relative abundance in the biofilm and suspended biomass, respectively. Gao et al. (2010) speculated that candidate division OP11 specifically contributed to membrane fouling as this phylum was abundant (37-63% of bacteria) in the fouling layer in their study. Candidate division OP11 was detected in only the

biofilm biomass in our study but at very low abundance (<0.1%) and therefore likely did not play a role in membrane fouling in the current study. Different operational parameters such as temperature may have caused this apparent inconsistency. A comparison between the biofilm and suspended biomass bacterial communities OTUs indicated a total of 193 and 145 OTUs in the biofilm and suspended biomass, respectively, with 84 OTUs shared.

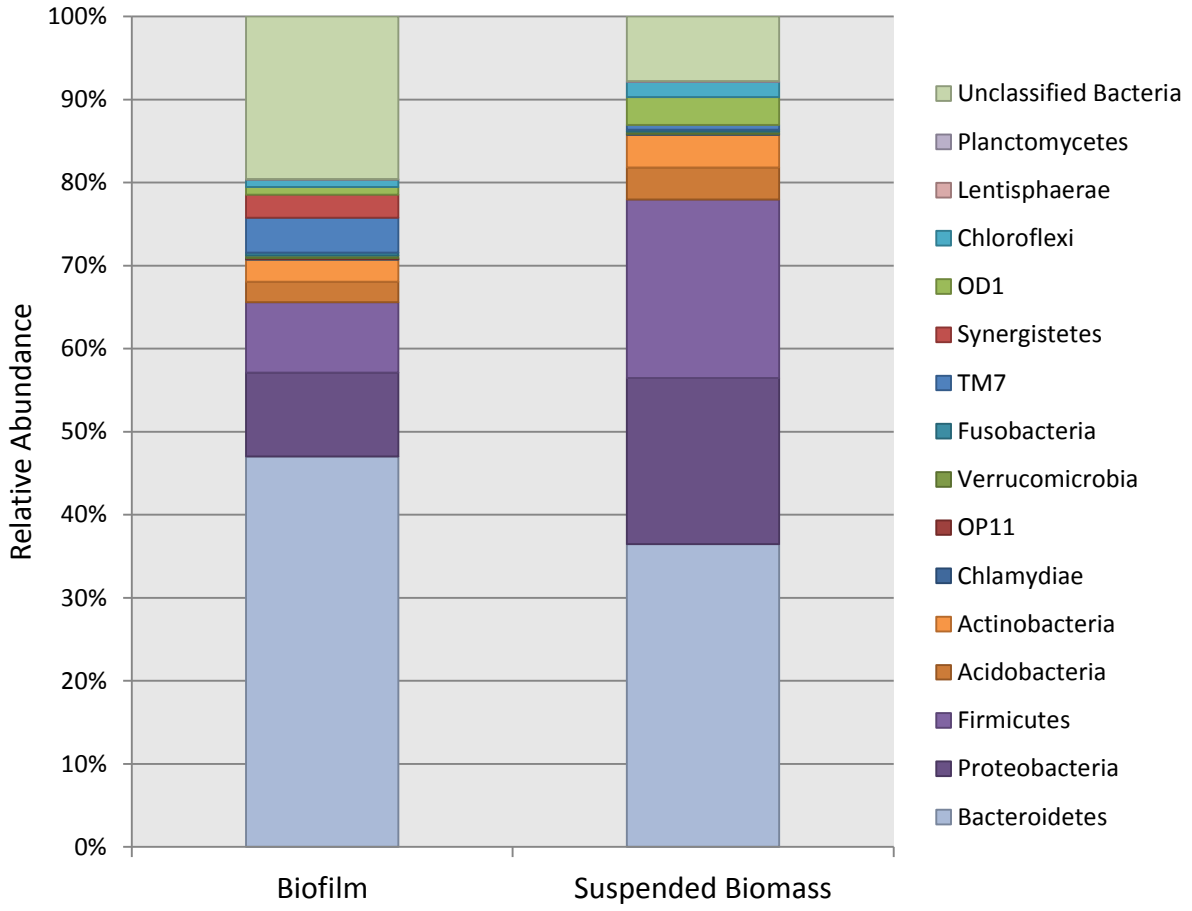

Figure 3-7. Classification at the phylum level of the bacterial communities in the biofilm and suspended biomass samples taken 275 days after startup.

Comparing the bacterial and archaeal communities in the AnMBR and the inocula indicated that the AnMBR communities showed the highest level of similarity with the mesophilic inocula (Figure 3-8). The bacterial communities in the AnMBR biofilm and suspended biomass were most

similar to each other, and showed a high degree of similarity to the mesophilic anaerobic digester inoculum. The AnMBR archaeal communities were most similar to the mesophilic UASB inoculum.

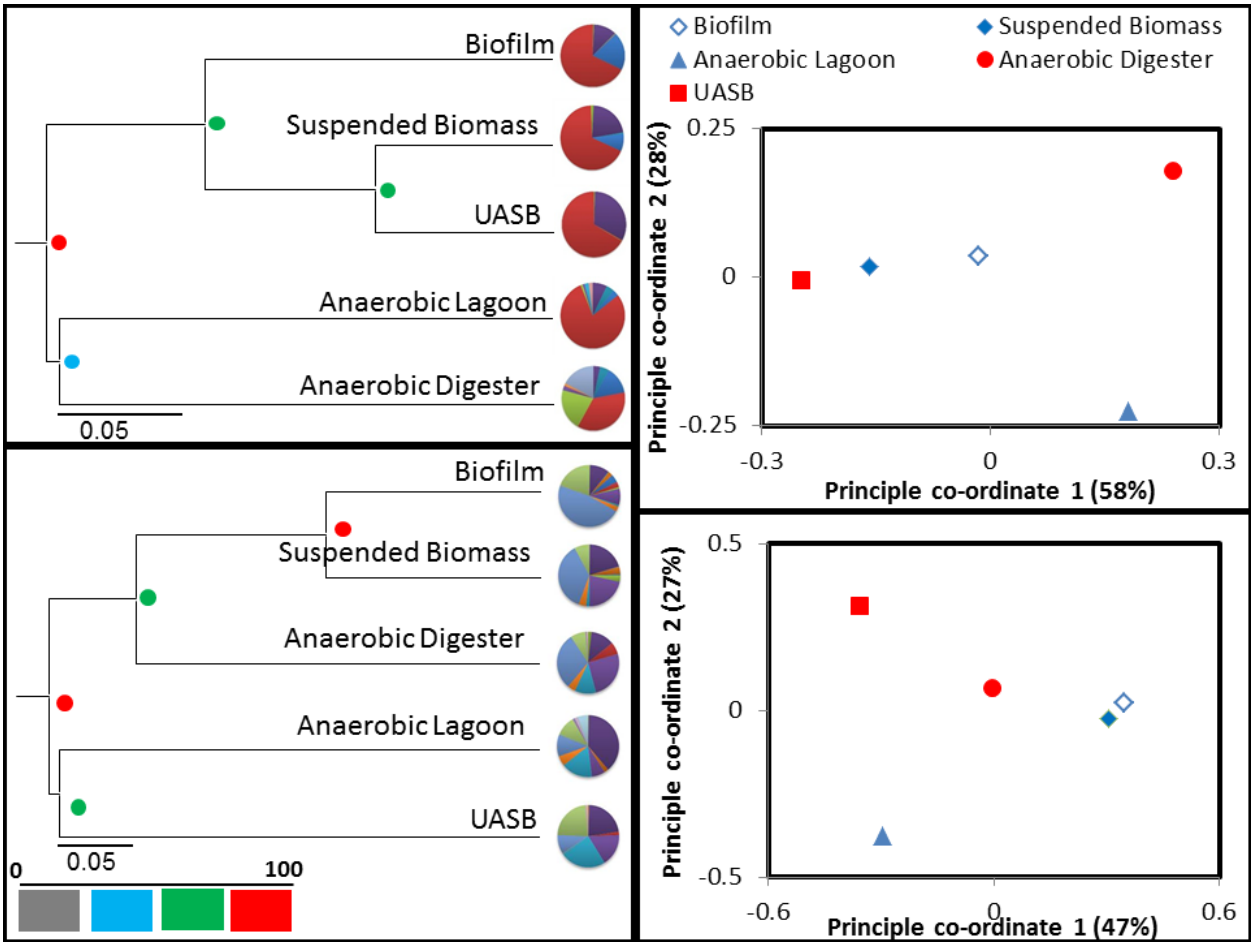

Figure 3-8. Comparison of the AnMBR communities in biofilm and suspended biomass samples (275 days after startup) with the three inocula using dendrograms of the weighted UniFrac distance metric (archaea top left; bacteria bottom left) and principle co-ordinate analyses (archaea top right; bacteria bottom right). Jackknife support for each node of the dendrogram is indicated by the colored circle. For a visual comparison, pie charts next to the sample name represent their respective community structures at the phylum level and genus level for bacteria and archaea, respectively.

The analysis of microbial communities in the AnMBR and the inocula suggest that mesophilic psychrotolerant populations were most abundant in the AnMBR as indicated by the more similar

bacterial and archaeal community structures of the AnMBR biomass samples and the mesophilic inocula (anaerobic digester and UASB, respectively). These results indicate that seeding the AnMBR with both mesophilic inocula may have been helpful for attaining the treatment performance observed. Seeding with the psychrophilic inoculum (anaerobic lagoon) appeared to have been less important in establishing the AnMBR bacterial and archaeal communities. However, it is important to note that sequences classified within the phylum *Acidobacteria* were detected in the AnMBR biofilm and suspended biomass (2.5% and 3.8% relative abundance, respectively) as well as in the psychrophilic inoculum, but were not detected in either mesophilic inoculum. Of the sequences classified within this phylum, 93% and 94% in the biofilm and suspended biomass classified with class *Holophagae*, respectively, strict anaerobes that ferment aromatic compounds (Hugenholtz et al. 1998). A benefit of the observation that mesophilic psychrotolerant populations appeared to dominate in the AnMBR is the possibility that their activity increases with an increase in temperature. If so, a rise in temperature would immediately result in an increase in treatment performance as the microbial community structure would not need to change substantially. This finding is positive from a practical perspective as seasonal variations will not necessitate engineered shifts in microbial community structure (e.g., reinoculation). However, the ability of psychrotolerant mesophilic communities to adapt to even lower temperatures (<15°C), which may occur during winter months in temperate and cold climates, and still provide adequate treatment deserves further study. It is also unknown whether or not a different psychrophilic inoculum would have benefited treatment performance in this study. Therefore, additional research is necessary to elucidate an AnMBR inoculation protocol that ensures both optimal treatment and stabile performance across seasonal temperature variations.

3.5 Conclusions

A bench-scale AnMBR was operated to treat simulated and actual DWW for at a psychrophilic temperature of 15^{o}C. The following conclusions were made based on observations during the study:

- A high quality effluent was generated during AnMBR treatment at psychrophilic temperature (92 ± 4% COD removal and 36 ± 21 mg/L average permeate COD during simulated DWW operation; 24 ± 3 mg/L average permeate BOD_5 during actual DWW operation).
- Dissolved methane in the permeate represented a substantial portion of the total methane generated in the system (approximately 40-50% of total methane generated over time).
- Membrane fouling was successfully managed using biogas sparging and permeate backflushing. Comparative fouling experiments suggested that the combination of the two fouling control measures was important.
- Pyrosequencing of the AnMBR and inocula microbial communities demonstrated that mesophilic inocula are suitable for psychrophilic AnMBR seeding.

Collectively, these conclusions indicate AnMBRs represent a strong candidate technology for innovation within DWW treatment with the ability to produce similar quality effluents relative to aerobic treatment, while concurrently recovering useful energy and producing considerably less residuals. However, full-scale implementation of the technology will require further research to overcome the existing operational concerns. Future research efforts should specifically focus on a better understanding of membrane fouling, the biofilm's role in treatment, and development of efficient dissolved methane recovery processes.

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Chapter 4. Improving Anaerobic Membrane Bioreactor Treatment of Wastewater through Membrane Biofilm Development

4.1 Abstract

Membrane biofilm development was evaluated to improve domestic wastewater treatment of low-temperature anaerobic membrane bioreactor (AnMBR). Three levels of membrane fouling indicated by transmembrane pressure (TMP) were compared using a bench-scale system at constant flux equipped with replicate membrane housings, separate permeate collection, and independent biogas sparging control. High fouling reduced permeate chemical oxygen demand (COD) >50 mg/L, but resulted in a permeate dissolved methane concentration 2-3 times the predicted concentration by Henry's Law at saturation. Restoring fouled membranes to a TMP near zero by increasing biogas sparging did not impair biofilm treatment, suggesting that the biologically active biofilm was tightly adhered to the membrane surface and distinct from the foulant layer that contributed to high TMP. High dissolved methane oversaturation persisted in the absence of high TMP implying that methanogenesis in the biofilm, rather than high TMP or a combination of the two, was the primary driving force in methane oversaturation. RNA-based 16S rRNA sequencing, reverse transcription quantitative PCR (RT-qPCR) targeting the methyl coenzyme-M reductase (*mcrA*) gene, and performance observations indicated that a specialized microbial community enriched in highly active methanogens and syntrophic bacteria developed in the biofilm. The results describe a potentially attractive operational strategy to improve COD

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removal of low-temperature AnMBR by supporting syntrophy and methanogenesis in the membrane biofilm.

4.2 Introduction

Anaerobic membrane bioreactor (AnMBR) treatment allows for the direct recovery of energy from wastewater in the form of methane-rich biogas. In AnMBRs, methane is produced during the anaerobic microbial degradation of the organic compounds present in wastewater in a bioreactor containing microbial biomass in suspension. This suspended biomass is separated from the treated wastewater using membrane filtration to produce a particle free wastewater effluent (permeate). The recent recognition of the potential benefits of AnMBR treatment of domestic wastewater compared to conventional activated sludge treatment has resulted in a surge in AnMBR research activity (e.g., (Ma et al. 2013, Smith et al. 2013, Yoo et al. 2012)) and pilot-scale evaluations (Dagnew et al. 2011, Gimenez et al. 2011, Martinez-Sosa et al. 2011, Robles et al. 2013, Shin et al. 2014). As the pumping energy demand needed for membrane filtration increases during the development of a membrane fouling layer, membrane fouling has received considerable attention in AnMBR research (Chen et al. 2012, Gao et al. 2010, Huang et al. 2011, Kola et al. 2014, Yang et al. 2011). The consensus in the water quality engineering field has been to operate membrane-filtration systems, including AnMBRs, with minimal membrane fouling (Yang et al. 2006), which is accomplished using gas sparging, backflushing, and chemical cleaning. As a result, almost no research has been performed on the potential benefits of membrane fouling. The membrane fouling layer contains considerable microbial biomass and can thus be considered a membrane biofilm, which has the potential to improve effluent quality by providing additional biodegradation not accomplished by the suspended biomass.

Anaerobic microbial communities in a membrane biofilm could have an advantage over suspended microbial communities because of reduced mass-transfer limitations. Mass-transfer phenomena likely have a substantial effect on substrate utilization when substrate concentrations are low such as during domestic wastewater treatment (Gonzalez-Gil et al. 2001), at low temperatures (Wu et al. 1995), and when mass-transport is influenced by advective forces such as liquid flow through a membrane biofilm. In addition, biofilms may support lower intercellular distances between methanogens and their syntrophic partners and thus provide enhanced microbial activity relative to the suspended biomass activity. Upflow anaerobic sludge blanket (UASB) reactors rely on this enhanced activity through the formation of granules (dense aggregates of microbes), which are essentially spherical biofilms (Saravanan and Sreekrishnan 2006). UASB reactors are designed and operated to allow anaerobic microbes to form these granules allowing for low intercellular distances (Tiwari et al. 2006). In AnMBRs, membrane filtration provides complete biomass retention and therefore does not offer conditions amenable to granule formation. However, a membrane biofilm potentially represents an environment conducive to efficient syntrophic interactions and a high degree of biological activity similar to the environment in UASB granules.

This study elucidated the contribution of the membrane biofilm in AnMBR treatment of domestic wastewater using a bench-scale AnMBR operated at 15°C equipped with three submerged membrane housings with independent permeate collection and biogas sparging control. The three membrane housings were operated to allow for three different levels of membrane fouling and membrane biofilm development. Illumina sequencing of 16S rRNA genes (rDNA) and 16S rRNA and reverse transcription-quantitative PCR (RT-qPCR) targeting the *mcrA* gene transcripts were applied to compare microbial community structure and activity dynamics in the suspended biomass and in the membrane biofilms.

4.3 Materials and Methods

4.3.1 AnMBR operation and chemical assays

A bench-scale AnMBR described previously (Smith et al. 2013) was redesigned to incorporate three submerged flat-sheet membrane housings incorporating microfiltration polyethersulfone membranes (GE Osmonics, Greenville, SC) with a pore size of 0.2 µm and a total effective membrane area of 924 cm^2. The system was operated at 15°C with a synthetic domestic wastewater containing particulate and soluble organic compounds, nutrients, and trace elements to simulate the chemical oxygen demand (COD) and other constituents of domestic wastewater (Smith et al. 2013). The three membrane housings, designated P1, P2, and P3, each generated a separate permeate stream during operation. Three mini diaphragm pumps (KNF Neuberger, Trenton, NJ) recirculated headspace biogas and dispersed it directly below each membrane housing by horizontally placed sparging tubes for fouling control. Biogas sparging flow rates were independently controlled for each membrane housing using in-line flow meters. The liquid volume of the reactor was 4 L. The AnMBR was inoculated with sludge from a mesophilic (32°C) wastewater treatment plant anaerobic sludge digester (Northfield Wastewater Treatment Plant, Whitmore Lake, MI) at an initial volatile suspended solids (VSS) concentration of approximately 8,000 mg/L.

The system was operated at a target hydraulic retention time (HRT) of 16 h, which corresponds to an organic loading rate (OLR) of 670 mg COD/L•d. Biomass was only removed from the AnMBR for sampling purposes, which resulted in a solids retention time (SRT) of approximately 300 days. From days 1 through 99 (Phase 1), a membrane flux of 2.7 $L/m^2/h$ (LMH) was targeted for each membrane housing. This relatively low membrane flux ensured operation with minimal membrane fouling could be maintained without chemical cleaning and provided greater operational control.

The high biogas sparging flow rate selected for Phase 1 (3.0 L biogas/min for each membrane housing or 5.8 m^3 biogas /h/m^2 membrane surface area) helped prevent the formation of a membrane biofilm. Backflushing was performed for 30 s every 10 min of bioreactor operation. Biogas sparging flow rates were reduced from days 100-138 (Phase 2) for P2 and P3 to allow for different levels of membrane biofilm development for each membrane housing, resulting in the need to operate with different transmembrane pressures (TMPs) to maintain similar fluxes. Low fouling (LF), medium fouling (MF), and high fouling (HF) were targeted for P1, P2, and P3, respectively (Figure 4-1). Due to pump slippage at high TMP, the flux for P1 was increased as necessary to maintain an HRT of 16 h. Data from days 139-151 are not presented due to a brief exposure of the system to oxygen during biofilm sampling on day 138 (described below), which resulted in poor system performance. On day 152, biogas sparging flow rates were increased for P2 and P3 to reduce fouling and restore the TMP to near zero. All membranes were operated at a TMP near zero by day 154 and continued in that operational regime until day 161 (Phase 3). From days 162-172 (Phase 4), the biogas sparging flow rate was reduced for P2 to target a high level of fouling comparable to P3 during Phase 2.

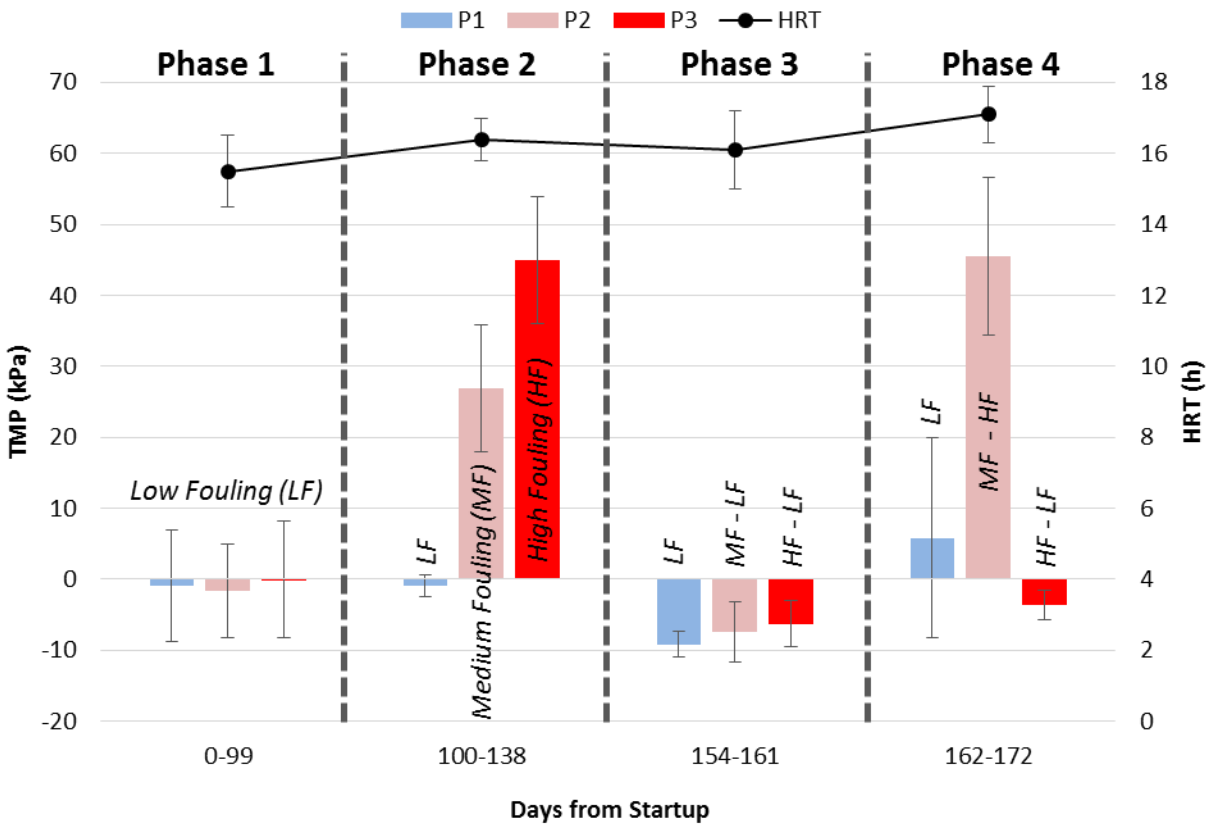

Figure 4-1. Average transmembrane pressure (TMP) for each of the membranes P1, P2, and P3 (left y axis) and bioreactor HRT (right y axis) from days 0 to 172. This time period is divided in four phases defined by operating regime. Data from days 139-153 are not reported due to poor AnMBR performance. Error bars for HRT represent the standard deviation of daily flow rate measurements. Error bars for TMP represent the standard deviation of pressure data recorded every minute of operation.

COD, total suspended solids (TSS), and VSS were determined using procedures outlined in Standard Methods (APHA 2005). Soluble COD was determined after filtering samples through a 0.2 µm filter to be consistent with the physical removal of suspended material in the AnMBR by membrane filtration (same pore size). Concentrations of volatile fatty acids (VFAs) (formate, acetate, propionate, butyrate, and valerate) and sulfate were determined with an ion chromatograph (ICS-1600, Dionex, Sunnyvale, CA) equipped with a conductivity detector, autosampler, and

reagent free eluent generator to produce a KOH gradient. Eluent was passed through a Dionex AS-11HC column at 60°C at a flow rate of 0.30 mL/min.

Biogas methane content was measured with a gas chromatograph (Gow-Mac, Bethlehem, PA) coupled with a thermal conductivity detector (TCD). Measurement of dissolved methane in the permeate was accomplished as previously described (Rudd et al. 1974). Briefly, 30 mL of permeate were collected in a syringe containing 30 mL nitrogen gas. The syringe was shaken by hand for 1 minute to strip dissolved methane into the gas phase. The gas phase was collected from the syringe and sampled for gas chromatography. Biogas production was measured by collecting gas in a 1-L Tedlar bag and quantifying the production daily using a wet-type gas meter (Actaris Metering Systems, Dordrecht, The Netherlands).

Permeate sampling was performed approximately daily for measurements of COD, VFAs, and sulfate, and every two to four days for dissolved methane content. Influent and bioreactor content were collected every two to four days to measure COD and VFAs, and weekly for TSS and VSS. Biogas content was determined every two to four days. Samples for soluble COD, VFA, and sulfate analyses were immediately filtered through a 0.2 μm-filter after sampling and preserved using concentrated sulfuric acid (COD) or 1 M sodium hydroxide (VFAs and sulfate) and stored at 4°C for up to 10 days.

4.3.2 Nucleic acids extraction

Suspended biomass samples from the AnMBR were taken on days 0, 26, 52, 76, 100, and 138 of operation, pelletized by centrifugation at 5,000 x *g* for 5 min at 4°C, and immediately stored at -80°C after decanting the supernatant. Biofilm biomass samples were gently scraped from the membrane surface of P1 (LF), P2 (MF), and P3 (HF) on day 138 using sterile lazy-l spreaders, pelletized, decanted, and immediately stored at -80°C. P1 (LF) had limited biofilm biomass, which

was only loosely associated with the membrane consistent with the low fouling condition. Biomass samples for RNA extraction were prepared similarly except for the addition of RNAlater (Qiagen, Valencia, California) prior to storage. DNA extraction from pelletized biomass was performed by three 2-min bead beating steps (Mini-Beadbeater-96, BioSpec Products, Bartlesville, OK) with 0.1 mm diameter silicon beads in lysis buffer, proteinase K digestion, and automated extraction using the Maxwell 16 Blood LEV kit according to manufacturer's instruction (Promega, Madison, WI). RNA extraction was performed by three 2-min bead beating steps with 0.1 mm diameter silicon beads in 1-thiolyglycerol homogenization buffer and automated extraction using the Maxwell 16 simplyRNA tissue kit according to manufacturer's instructions except that 10 µL of DNase 1 (instead of 5 µL) was used. DNA and RNA quality and quantity were assessed via spectrophotometry (Nanodrop 1000, Thermo Fisher Scientific, Wilmington, DE) and the Quantifluor dsDNA and RNA systems (Promega, Madison, WI), respectively, with a fluorospectrometer (Nanodrop 3000, Thermo Fisher Scientific, Wilmington, DE). Select RNA extracts were further analyzed for quality via automated electrophoresis using the Experion RNA analysis kit (Bio-Rad, Hercules, CA).

4.3.3 RT-qPCR

An *in silico* analysis of primers targeting the *mcrA* gene (Juottonen et al. 2006, Steinberg and Regan 2009, 2008, Zeleke et al. 2013) was performed in MEGA (Tamura et al. 2013) using partial *mcrA* sequences available in GenBank (NCBI, Bethesda, MD) and back-translated full-length *mcrA* protein sequences generated by EMBOSS Backtranseq with the *Methanothermobacter thermautotrophicus* strain Delta H codon usage table (EMBL-EBI, Hinxton, UK). Based on the *in silico* analysis, additional primer degeneracies were incorporated into forward primer mlas (Steinberg and Regan 2009) to provide greater coverage (5'-

GGYGGTGTMGGNTTCACHCARTA-3'). Primer specificity was assessed using MFE-primer 2.0 (Qu et al. 2012). Reverse primer mcra-rev was used as reported in the literature (5'-CGTTCATBGCGTAGTTVGGRTAGT-3') (Steinberg and Regan 2008). A detailed discussion of primer design and characterization is provided elsewhere (Clancy et al. 2014). Universal primers targeting the V4 region of the 16S rDNA (Caporaso et al. 2011) were used to quantify 16S rRNA for normalization of *mcrA* transcript quantification. Coverage of 16S rRNA primers was verified using TestPrime 1.0 (Klindworth et al. 2012) (Tables S4-1 and S4-2 in Appendix B).

Reverse transcription to generate single-stranded complementary DNA (cDNA) from RNA extracts was performed using the SuperScript VILO cDNA Synthesis Kit according to manufacturer's instruction (Life Technologies, Grand Island, NY). Two-step RT-qPCR, as opposed to one-step in which cDNA synthesis and qPCR occur sequentially in one reaction, was done to allow for sequencing of synthesized cDNA (described below).

Standards for RT-qPCR were prepared by amplifying *mcrA* and 16S rRNA genes and cDNA synthesized transcripts from a pool of 21 DNA and 21 cDNA samples from the bench-scale AnMBR pooled by equal mass (He and McMahon 2011, Sonthiphand et al. 2013). PCR to prepare RT-qPCR standards was performed in 20 μL reactions using the above described primer sets at 500 nM, 0.5 ng of pooled template, 0.3 mg/mL bovine serum albumin (BSA), 10 μL Phusion High-Fidelity Master Mix (NEB, Ipswich, MA), and ultra-pure nuclease free water. Thermocycling conditions to prepare RT-qPCR standards consisted of an initial 2 min denaturation at 95°C, followed by 30 cycles of denaturing at 95°C for 20 s, annealing at 55°C for 15 s, and extension at 72°C for 30 s, followed by a final extension at 72°C for 5 min. PCR products were run on a 1.5% agarose gel, and bands were excised with a sterile scalpel and purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). The purified amplicon pool was quantified via Quantifluor

dsDNA system and fluorospectrometry. Serial dilutions of the purified amplicon pools were prepared as RT-qPCR standards from 10^8 to 10^3 copies. RT-qPCR was conducted on a Mastercycler realplex ep (Eppendorf, Hamburg, Germany) with a total reaction volume of 20 µL. Samples were quantified in triplicate using serial dilutions of template. All standards and a no template control were run in triplicate. Each reaction contained 2 µL template, 500 nM forward and reverse primers, 10 µL 2X Fast-Plus EvaGreen Master Mix (Biotium, Hayward, CA), and ultra-pure nuclease free water. Thermocycling conditions for RT-qPCR targeting 16S rRNA of samples were the same as described above for standards preparation, except that 50 cycles were performed instead of 30. For *mcrA* quantification in samples, thermocycling conditions consisted of an initial 2 min denaturation at 95°C, followed by 5 cycles of denaturing at 95°C for 20 s, annealing at 55°C for 15 s, a temperature ramp at a rate of 0.1°C/s to 72°C to aid in annealing due to the highly degenerate nature of the primer set (Luton et al. 2002, Morris et al. 2014), and extension at 72°C for 30 s, followed by 45 cycles without the temperature ramp and lastly, a final extension at 72°C for 5 min. In addition, a melt curve analysis was performed to verify qPCR specificity. The R^2 and efficiencies for *mcrA* and 16S rRNA standard curves were 0.991 and 0.997 and 75 and 71%, respectively.

4.3.4 16S rDNA and rRNA sequencing

PCR, multiplexing, and sequencing via Illumina MiSeq (San Diego, CA) of 16S rDNA and rRNA was performed by the Center for Microbial Systems (University of Michigan, Ann Arbor, MI) using the above described universal 16S rDNA primer set targeting the V4 region (Caporaso et al. 2011) barcoded and using sequencing primers described in Kozich et al. (2013). PCR reactions were 20 µL and included primers at 500 nM, 10 µL 2x Accuprime buffer 11 (Invitrogen, Carlsbad, CA), 0.15 µL Accuprime TAQ, 0.5 ng template, and nuclease-free water. Thermocycling

conditions consisted of an initial 2 min denaturation at 95°C, followed by 30 cycles of denaturing at 95°C for 20 s, annealing at 55°C for 15 s, and extension at 72°C for 5 min, followed by a final extension at 72°C for 5 min. Amplicons were pooled by equal mass using the SequalPrep Normalization Plate Kit (Life Technologies, Grand Island, NY). Multiplexed amplicons were sequenced via Illumina MiSeq using the MiSeq Reagent Kit V2 (2x250 bp reads) and sequencing primers described in Kozich et al. (2013). 16,587 paired-end reads (2x250 bp) per sample were generated after quality filtering and subsampling. The resulting sequences were processed with mothur (Schloss et al. 2009) following the Schloss MiSeq SOP and classified using the Ribosomal Database Project (Maidak et al., 1997) and Basic Local Alignment Search Tool (BLAST; NCBI, Bethesda, MD).

4.4 Results and Discussion

4.4.1 Slow startup after inoculating the psychrophilic AnMBR with mesophilic sludge

In a previous study, we inoculated a psychrophilic AnMBR with a mixture of two mesophilic sources and one psychrophilic source (Smith et al. 2013). Pyrosequencing of 16S rDNA in each inoculum and AnMBR biomass samples collected after one year of operation suggested that the AnMBR microbial community structures were most similar to the mesophilic inocula. Therefore, we elected to inoculate the AnMBR in the current study with mesophilic sludge only. COD removal during the first 99 days of operation (Phase 1) was limited, averaging 57 ± 12%. Although a downward trend in permeate COD concentrations was apparent, the levels were rarely below 100 mg/L (Figure S4-1, Appendix B). Methane production was consistent with the limited COD removal and approximately half of the methane produced in the system remained dissolved in the permeate (Figure S4-2). The majority of the permeate COD was comprised of acetate and propionate averaging 70 ± 19 and 52 ± 18 mg/L as the compound, respectively (Figure S4-3).

Concentrations of acetate and propionate in the bioreactor and permeate were nearly identical (data not reported) indicating negligible removal across the membranes. Inoculating the system solely with a mesophilic sludge source may have contributed to the slow startup. When using more diverse inoculum sources including biomass from a psychrophilic environment in our previous study, the startup was much faster (Smith et al., 2013). However, membrane fouling was less controlled during our previous study potentially allowing for biofilm treatment.

Soluble COD in the bioreactor (defined as COD passing through a 0.2 µm filter, the same pore size as the membranes in the AnMBR) was consistently greater than permeate COD with an average difference of 110 ± 22 mg/L. Dissolved methane concentrations in the permeate were only slightly greater than equilibrium concentrations predicted by applying Henry's Law and the measured methane partial pressure in the biogas (averaging 1.2 times the predicted equilibrium concentration). Biological removal by the biofilm commensurate with the observed COD difference would have resulted in substantially higher methane oversaturation (discussed further below). Based on this, we hypothesize that the observed COD removal was by physicochemical mechanisms. Nearly complete sulfate reduction consistently occurred in the system with influent and permeate sulfate concentrations averaging 18 ± 2.0 and 0.32 ± 0.57 mg/L, respectively. TSS and VSS concentrations initially declined but then remained stable, suggesting an initial die-off of biomass after inoculating, likely in response to the psychrophilic temperature and low OLR which was unable to support the initial biomass concentration (Figure S4-4). Negligible net biomass growth occurred thereafter. The initial decline in biomass inventory corresponded to a decrease in microbial community diversity based on 16S rDNA sequencing (Figure S4-4) suggesting that the psychrophilic temperature or other extrinsic conditions disproportionately affected specific members of the inocula.

Our previous study reported a significantly higher COD removal of 92 ± 5% and soluble COD removal by the membrane biofilm accounted for 21 ± 8% of total COD removal (Smith et al. 2013). We further reported that increased membrane fouling resulted in greater soluble COD removal across the membrane, possibly due to biological activity in the foulant layer (Smith et al. 2013). However, the improvement in COD removal when promoting membrane fouling was low, 10 ± 4 mg/L (Smith et al. 2013), likely because most of the readily biodegradable substrate was already removed by the suspended biomass. We hypothesized that development of a membrane biofilm would improve effluent quality to a greater extent when the permeate contained residual biodegradable substrates (e.g., acetate or propionate) and used this strategy to attempt to improve AnMBR performance in the current study.

4.4.2 Biofilm promotion improves effluent quality but results in dissolved methane oversaturation

To improve permeate quality, a controlled membrane fouling experiment was conducted to encourage biofilm development on P2 and P3 by independently reducing the biogas sparging flow rates (Phase 2). Three different levels of membrane fouling were targeted, low fouling (LF; P1), medium fouling (MF; P2), and high fouling (HF; P3). During Phase 2, P1, P2, and P3 TMP averaged -0.96 ± 1.5, 27 ± 9.0, and 45 ± 8.9 kPa, respectively, indicating the targeted fouling levels were achieved (Figure 4-1). Hereafter, P1, P2, and P3 are referred to based on their fouling level (LF, MF, and HF, respectively).

Differences in permeate COD concentrations were observed throughout Phase 2 and corresponded to the level of membrane fouling (Figure 4-2a). HF permeate consistently had the lowest COD with a concentration of 22 mg/L at the end of Phase 2. Permeate VFA levels showed a similar trend with differences in acetate and propionate concentrations as high as 37 mg/L and 25 mg/L, respectively, between MF and HF permeate (Figures 4-2b and 4-2c). Acetate and propionate

concentrations in HF permeate at the end of Phase 2 were 3.5 and 3.6 mg/L, respectively. The VFA concentrations in the bioreactor and LF permeate were similar throughout Phase 2 indicating minimal biological activity across the membrane with limited fouling. These observations indicate that controlled membrane fouling can substantially improve effluent quality in AnMBR and further suggest that the activity of syntrophic propionate oxidizing populations and their methanogenic partners can be promoted through membrane biofilm development (see below).

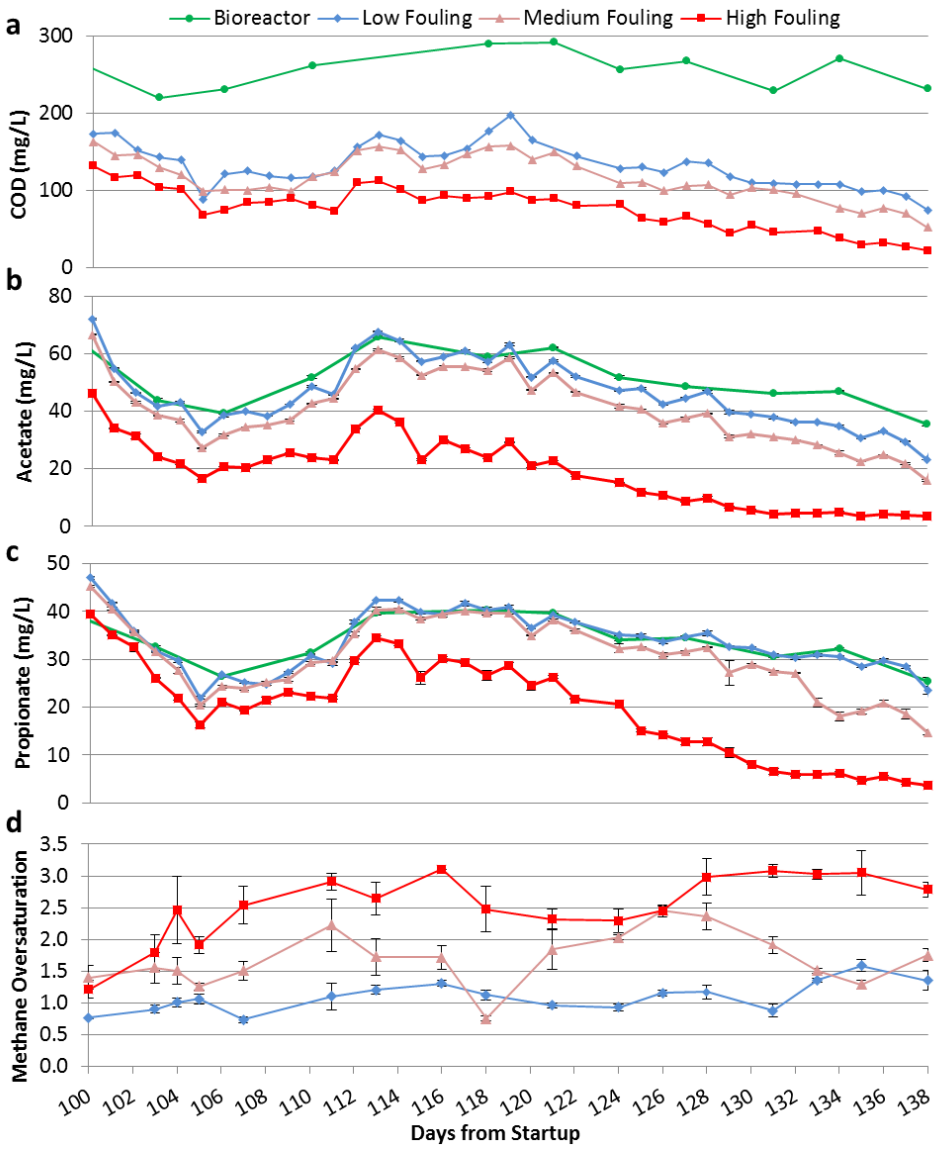

Figure 4-2. Effect of different degrees of biofilm development (Low Fouling, Medium Fouling, and High Fouling) on permeate quality during Phase 2 of AnMBR operation. (a) Bioreactor (soluble) and permeate COD concentrations. (b) Bioreactor and permeate acetate concentrations. Error bars represent the standard deviation of triplicate IC injections. (c) Bioreactor and permeate propionate concentrations. Error bars represent the standard deviation of triplicate IC injections. (d) Dissolved methane oversaturation in permeate assuming a Henry's law constant of 34,300 atm at 15°C (Tchobanoglous et al. 2003) and methane partial pressure in the biogas. The methane content of the biogas was approximately 90% with the balance being carbon dioxide. The high methane content was likely due to high carbon dioxide solubility at the psychrophilic temperature and/or the feed composition (Aiyuk and Verstraete 2004).Error bars represent the standard deviation of duplicate dissolved methane extractions and triplicate GC injections of each dissolved methane extract.

Consistent with this, dissolved methane concentrations in MF and HF permeates indicated significant oversaturation of methane (Figure 4-2d) suggesting that methanogenesis occurred in the biofilm and that methane produced in the biofilm left the system in the dissolved form. From days 107-138, dissolved methane concentrations in LF, MF, and HF permeate averaged 1.1 ± 0.22, 1.7 ± 0.44, and 2.6 ± 0.30 times the concentrations predicted by Henry's Law, respectively. The dissolved methane concentration in the bioreactor (not measured) was likely near saturation due to vigorous biogas sparging. The observation that the dissolved methane concentration in LF permeate was close to saturation during phase 2, as it was during phase 1 for all three permeates, provided further evidence of minimal biological activity during low fouling conditions. Given the high degree of methane oversaturation in MF and HF permeates, a tradeoff was apparent between improved effluent quality and increased effluent dissolved methane. Dissolved methane may be challenging to recover downstream of an AnMBR without substantial energy input (Bandara et al. 2011) and off-gas from a recovery process may be unsuitable for electricity production via cogeneration due to low methane content (Cookney et al. 2012). If released to the atmosphere, this "lost" energy source could be a potent greenhouse gas emission (Smith et al. 2014).

4.4.3 Biofilm promotion leads to a specialized microbial community enriched in active methanogens

The complexity of anaerobic microbial communities and reported differences in suspended and biofilm AnMBR community structure (Gao et al. 2010, Ma et al. 2013, Smith et al. 2013, Yu et al. 2012) suggest that careful monitoring of community structure during development of AnMBR operational strategies, such as the promotion of controlled membrane fouling discussed above, is important. RNA-based approaches targeting either 16S rRNA (e.g., (Eichler et al. 2006, Foesel et al. 2013, Hunt et al. 2013, Männistö et al. 2013)) or transcripts of functional genes (e.g., the methyl coenzyme-M reductase [*mcrA*] gene in methanogens (Freitag and Prosser 2009)) may be more

useful than DNA-based approaches in characterizing microbial community function in AnMBRs given the slow growth rates and low biomass yields of anaerobic microbes, high biomass retention provided by membrane separation, and relatively short operational periods commonly studied in AnMBRs. We therefore applied high throughput sequencing of both 16S rDNA and 16S rRNA and confirmed methanogenic activity data with RT-qPCR targeting the *mcrA* gene while studying controlled membrane fouling during Phase 2.

Substantial differences between microbial community structure (16S rDNA) and activity (16S rRNA) were observed in suspended and biofilm biomass (Figure 4-3). The 16S rDNA sequence data indicated that the suspended and biofilm community comprised <10% methanogens. The hydrogenotrophic methanogens were more abundant than the aceticlastic methanogens in the suspended biomass 26 days after startup and in the biofilm biomass at the end of Phase 2 (Figure S4-5a) suggesting the hydrogen utilization pathway became more important after biomass adaptation to the psychrophilic temperature. A shift towards hydrogenotrophic methanogenesis has also been observed previously in other anaerobic systems when transitioning from mesophilic to psychrophilic conditions using DNA-based molecular analyses and specific methanogenic activity assays (Collins et al. 2006, Connaughton et al. 2006, McHugh et al. 2004) and has been explained by increased hydrogen solubility and thus increased substrate availability for hydrogenotrophic metabolisms (Lettinga et al. 2001). In our system, 16S rRNA sequencing indicated that the relative importance of the aceticlastic and hydrogenotrophic methanogenesis pathways was similar (Figure S4-5b). Further, a substantially greater relative activity of methanogens was observed in the MF and HF biofilms relative to the suspended biomass or LF biofilm on day 138. Specifically, methanogens represented 33 and 34% relative activity in MF and HF biofilms, respectively, in comparison to only 15% in the suspended biomass and the LF biofilm

(Figure 4-3). These observations correlated with the low levels of acetate and propionate and high dissolved methane oversaturation by the end of Phase 2 (Figure 4-2) and suggest that a high level of methanogenesis occurred in the MF and HF biofilms.

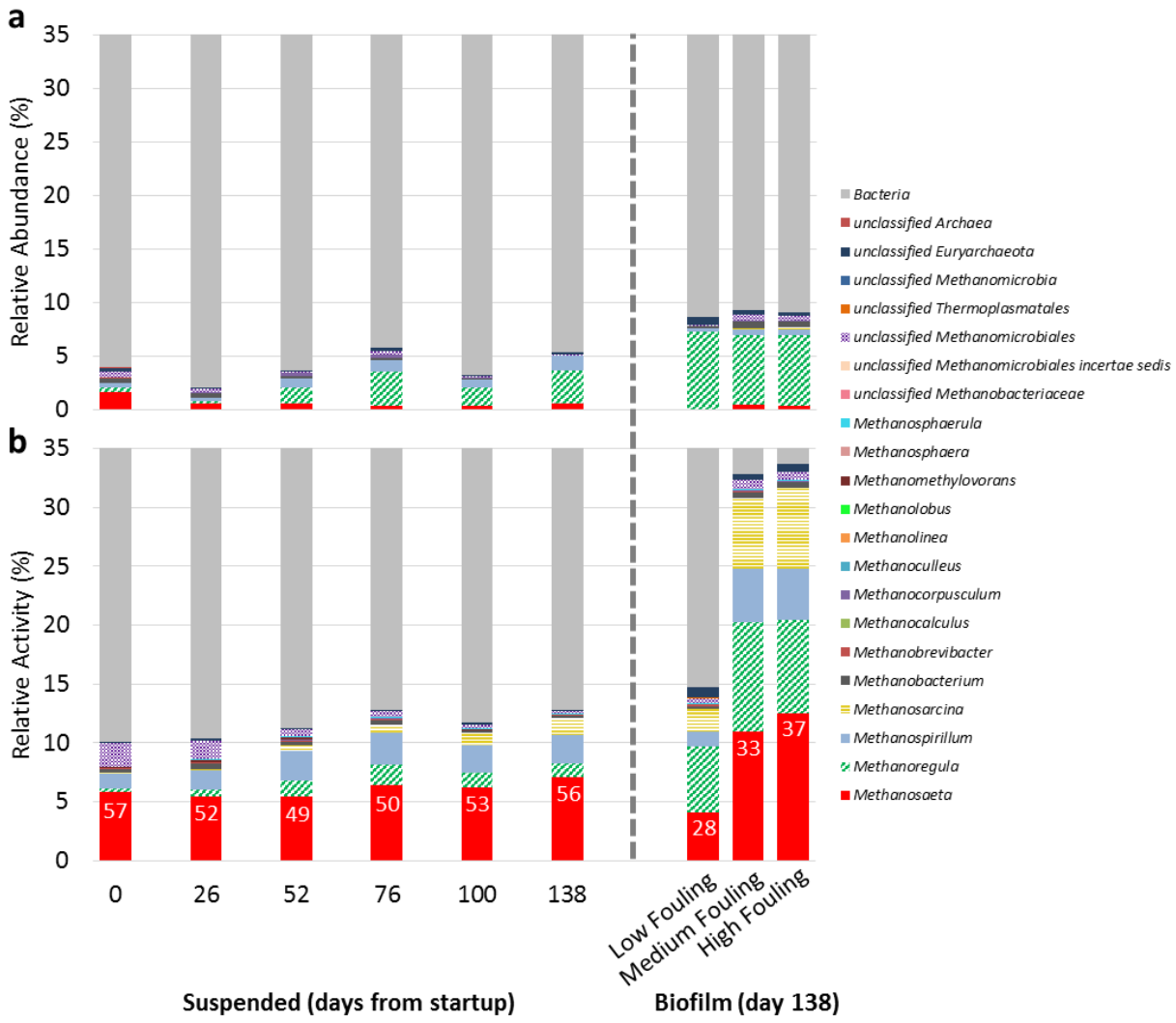

Figure 4-3. (a) Relative abundance of methanogens identified to the genus level based on 16S rDNA sequencing and (b) relative activity of methanogens identified to the genus level based on 16S rRNA sequencing in suspended biomass from startup to the end of Phase 2 and in biofilms at the end of Phase 2. Data are expressed as a percentage and were normalized using the total number of 16S rDNA sequences (a) and 16S rRNA sequences (b) (including both *Bacteria* and *Archaea*). Numbers within bars in (b) represent the relative activity of *Methanosaeta* spp. to all *Archaea*. A truncated y-axis (0 to 35%) is shown to accentuate changes in methanogen abundance and activity.

Methanosaeta is the only methanogen that exclusively produces methane through the aceticlastic pathway (Smith and Ingram-Smith 2007) and does not rely on syntrophy with a bacterial partner. In the suspended biomass, the relative abundance of *Methanosaeta* spp. decreased from 1.6% on day 0 to 0.55% on day 138 (Figure 4.3a). However, their relative activity was fairly stable (Figure 4-3b) resulting in an increase in the activity/abundance ratio over time. The consistently low relative abundance and high relative activity of *Methanosaeta* spp. in the suspended biomass suggests that growth was negligible, possibly due to the psychrophilic temperature. The lack of convergence between 16S rDNA and rRNA sequencing of *Methanosaeta* spp. and stable biomass inventory (Figure S4-4) over 138 days supports this notion.

Methanosarcina produces methane from acetate, hydrogen, and other C1 compounds (Mladenovska and Ahring 1997, Welander and Metcalf 2005) and has thus been categorized as mixotrophic. Relative activity of *Methanosarcina* spp. increased over time in suspended biomass and comprised 18 and 21% of relative methanogenic activity in MF and HF biofilm biomass, respectively. *Methanosarcina* spp. were either not detected or detected at ≤0.23% relative abundance in all biomass using 16S rDNA sequencing. *Methanosarcina* spp. were not detected via 16S rDNA sequencing in another psychrophilic AnMBR study (Bandara et al. 2012) and were detected at <0.50% of the archaeal community in our previous work at 15°C (Smith et al. 2013). *Methanosarcina* has a greater maximum growth rate and half-saturation coefficient than *Methanosaeta* which often leads to the dominance of *Methanosaeta* when acetate concentrations are low such as in continuously fed anaerobic digestion (Conklin et al. 2006) or during low-strength wastewater treatment. It is unclear why the activity of *Methanosarcina* was high in this study, particularly in the biologically active biofilms, as the acetate concentration was near the

threshold level at which *Methanosarcina* spp. are typically inactive (11 – 71 mg/L acetate, (Jetten et al. 1992)). Psychrotolerant *Methanosarcina* spp. have been observed in the environment (Simankova et al. 2001, von Klein et al. 2002) but a specific mechanism for low-temperature adaptation that would give a competitive advantage over *Methanosaeta* or other methanogens in psychrophilic AnMBR is unclear. However, *Methanosarcina* has a unique surface structure containing hundreds of distinct proteins (De Vrieze et al. 2012, Francoleon et al. 2009), as opposed to the typical one or two abundant proteins in a cell's surface layer, that may aid in cell attachment to fixed-surfaces and other cells (De Vrieze et al. 2012). We hypothesize that *Methanosarcina's* metabolic flexibility and unique surface structure potentially gave it a metabolic and physical advantage in the biofilm relative to other methanogens, leading to the observed high activity.

Methanoregula spp. and *Methanospirillum* spp. were the dominantly active hydrogenotrophic methanogens classified in suspended and biofilm biomass comprising 24 and 14% of methanogens in 16S rRNA characterization of HF biofilm biomass, respectively. Based on 16S rDNA sequencing, *Methanospirillum* spp. comprised only 5.5 and 6.4% relative abundance of methanogens in MF and HF biofilm biomass, respectively, whereas *Methanoregula* spp. was detected as the dominant methanogen comprising 70 and 73% relative abundance of methanogens in MF and HF biofilm biomass, respectively. *Methanoregula* spp., a mesophilic hydrogenotrophic methanogen, was only recently cultivated from a full-scale UASB (Yashiro et al. 2011) and an acidic peat bog (Bräuer et al. 2011). Growth for both cultivated species was demonstrated at temperatures as low as 10°C suggesting tolerance to psychrophilic temperature. The activity/abundance ratio of *Methanoregula* spp. was 0.41 and 0.33 in MF and HF biofilm biomass, respectively, whereas the ratio for *Methanospirillum* spp. was 2.5 and 2.0 in MF and HF biofilm biomass, respectively, suggesting that *Methanospirillum* spp. activity per cell was significantly

greater than *Methanoregula* spp. in the biofilm biomass. 16S rRNA operon numbers (described further below) for *Candidatus Methanoregula* and *Methanospirillum hungatei* have been reported as 1 and 4 per genome (Lee et al. 2009), respectively, which would only exacerbate the differences in activity/abundance if taken into account. The low activity/abundance ratio of *Methanoregula* spp. suggests that their colonization of the biofilm was by physical means such as attachment to the membrane surface or to other cells rather than a metabolic strategy.

It is important to note the limitations of our approach to infer microbial abundance and activity. 16S rRNA operon number varies from 1-15 copies per genome (Klappenbach et al. 2000), which can lead to over or under-representation of specific phylogenies if a constant operon number is assumed across all phylogenies (Větrovský and Baldrian 2013). Normalization of sequencing results to operon number is still challenging given databases such as the Ribosomal RNA Database (rrnDB) are still in development (Lee et al. 2009). Further, 16S rRNA abundance varies between phylogenies based on cell size and other factors. Its abundance is also not directly linked to a specific cellular function (e.g., methanogenesis) and does not always correlate well with activity even with pure cultures under steady-state conditions (Blazewicz et al. 2013). Further, inactive or dormant microorganisms can contain high amounts of rRNA (Sukenik et al. 2012). Another limitation of our study is the lack of absolute abundance or gene expression. Doing so requires accurate quantitative nucleic acid extraction which can be challenging from sludge samples. Differences in suspended and biofilm biomass composition such as elevated concentrations of extracellular polymeric substances (EPS) in biofilm biomass (Smith et al. 2013) likely reduce extraction efficiency and quality of nucleic acids extracted which could subsequently reduce PCR efficiency. These matrix effects may influence microbial characterization due to biases in nucleic acids extraction, PCR, reverse transcription, or other steps (Martin-Laurent et al. 2001). These

concerns were motivation for validating our approach by monitoring changes in the expression of the *mcrA* gene. The *mcrA* gene codes for the final step in methanogenesis (Thauer 1998) and has been used as a phylogenetic marker for methanogenic communities (Juottonen et al. 2006, Luton et al. 2002, Rastogi et al. 2008). Very few studies have targeted *mcrA* transcripts to infer activity of methanogens (Freitag and Prosser 2009, Ma et al. 2012, Morris et al. 2014).

RT-qPCR results quantifying *mcrA* transcripts correlated well with performance observations and 16S rRNA sequencing indicating significantly higher methanogenic activity in MF and HF biofilm biomass relative to suspended or LF biofilm biomass (Figure 4-4). Taken together, these results provide strong evidence that a specialized microbial community was promoted in the biofilm enriched in highly active aceticlastic and hydrogenotrophic methanogens.

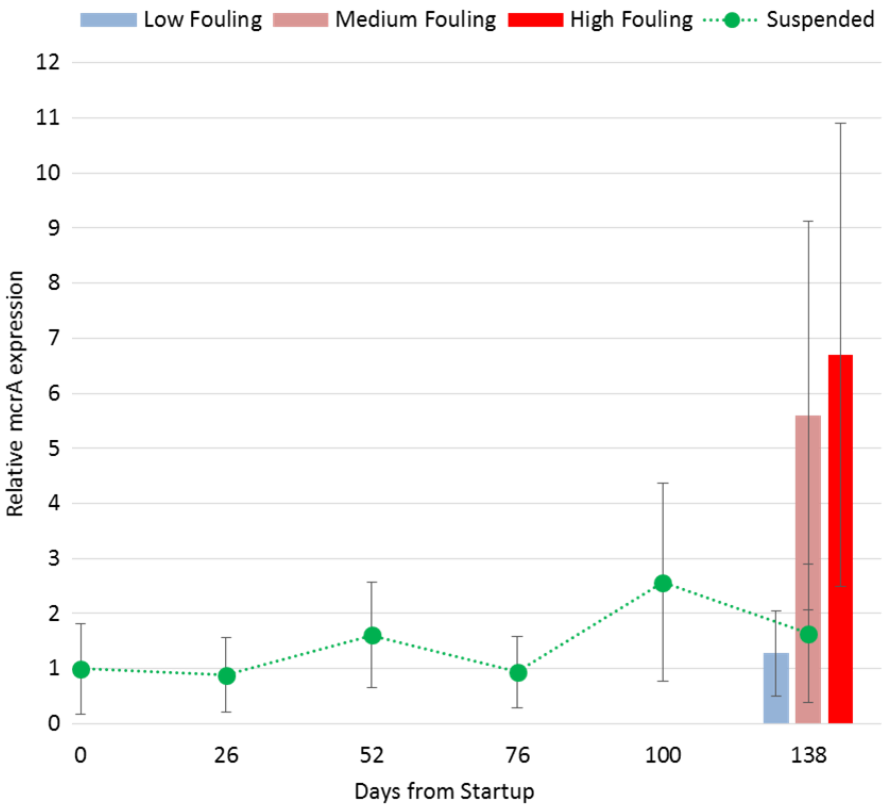

Figure 4-4. Relative expression of *mcrA* transcript copies to 16S rRNA copies in suspended and biofilm biomass. Expression was normalized to the suspended biomass on day 0. Error bars represent the standard deviation of the ratio of triplicate qPCR reactions at serial dilutions of cDNA template concentration.

4.4.4 Phylogenetically distinct syntrophic bacterial OTU was highly active in the biofilm

Relative activity of fatty acid-oxidizing obligately syntrophic bacteria exhibited a similar correlation with performance observations (Figure 4-5). On day 0, a high level of activity of syntrophs was observed in suspended biomass which quickly fell to 0.33% activity of total bacteria by day 26, likely due to the mesophilic inoculum responding to the psychrophilic operational temperature. In addition, high shear induced by biogas sparging may have disrupted cell aggregates in syntrophic association. We calculated an average velocity gradient (g) in our system of 410 s^{-1} which is much higher than recommended values for effective anaerobic digestion, 50-80 s^{-1}

(Tchobanoglous et al. 2003). Research has suggested that high shear can be detrimental to anaerobic digester performance under high loading rates due to increased hydrolysis and fermentation resulting in acidification (Stroot et al. 2001, Vavilin and Angelidaki 2005). In AnMBR under very low loading rates, high shear may be inhibitory instead through disruption of syntrophic interactions. High shear at a similar intensity to our system has been found to favor *Methanosarcina* spp. over *Methanosaeta concilii* (Hoffmann et al. 2008) and likely has implications on syntrophic partners. Activity of syntrophic bacteria was regained in suspended biomass by day 76. Relative to the suspended biomass on day 138, syntrophs were less active in LF biofilm biomass, similarly active in MF biofilm biomass, and more active in HF biofilm biomass. Relative abundance of syntrophic bacteria was substantially lower than relative activity. Relative abundance of syntrophic bacteria in suspended biomass quickly fell and was not regained throughout the experimental period. Relative abundance of syntrophic bacteria to total bacteria in suspended, LF, MF, and HF biofilm biomass on day 138 was 0.57, 0.23, 0.53, and 0.40%, respectively. The stark differences observed between 16S rDNA and rRNA analyses reflects the importance of using RNA-based approaches for molecular characterization of microbial communities in systems where growth is limited and biomass retention is high such as low-temperature AnMBR.

An OTU unclassified at the genus level according to RDP belonging to family *Syntrophomonadaceae* comprised a significant proportion of the relative activity of syntrophs in MF and HF biofilm biomass (i.e., 72 and 64%, respectively) but comprised only 7.7% relative activity of syntrophs in the suspended biomass on day 138. A representative sequence from the OTU had 95% identity with *Syntrophomonas zehnderi,* an obligately syntrophic microorganism (Sousa et al. 2007), according to BLAST. Interestingly, genera in *Syntrophomonadaceae* have only

been observed to syntrophically oxidize C4 compounds (e.g., butyrate) and higher order organics (Stams et al. 2012). In our system, butyrate was non-detectable and thus it is surprising that a butyrate oxidizing syntroph would have such high activity in the biofilm particularly relative to propionate oxidizing syntrophs (e.g., *Smithella*) given the significant propionate removal in the biofilm. The unclassified OTU may be a yet to be described species of *Syntrophomonadaceae* capable of C3 oxidation. Alternatively, Gan et al. (2012) using DNA-based stable isotope probing (SIP) proposed a novel pathway in which *Smithella* spp. first dismutates propionate to acetate and butyrate followed by butyrate oxidation by *Syntrophomonas* spp. via a trophic interaction. Based on the significant removal of propionate in the biofilm, non-detectable levels of butyrate in the bioreactor, and activity of both *Smithella* spp. and the unclassified OTU belonging to *Syntrophomonadaceae*, it is feasible that this novel pathway occurred here. In this hypothetical scenario, butyrate may remain non-detectable acting as a transient metabolite. This scenario would require cooperation between two syntrophic bacteria and a hydrogenotrophic methanogen and thus may benefit from the hypothesized increase in spatial organization afforded to the biofilm community relative to suspended biomass. The unclassified OTU may also be more active in the biofilm than suspended biomass due to differential preferences in growth mode (i.e., suspended versus attached).

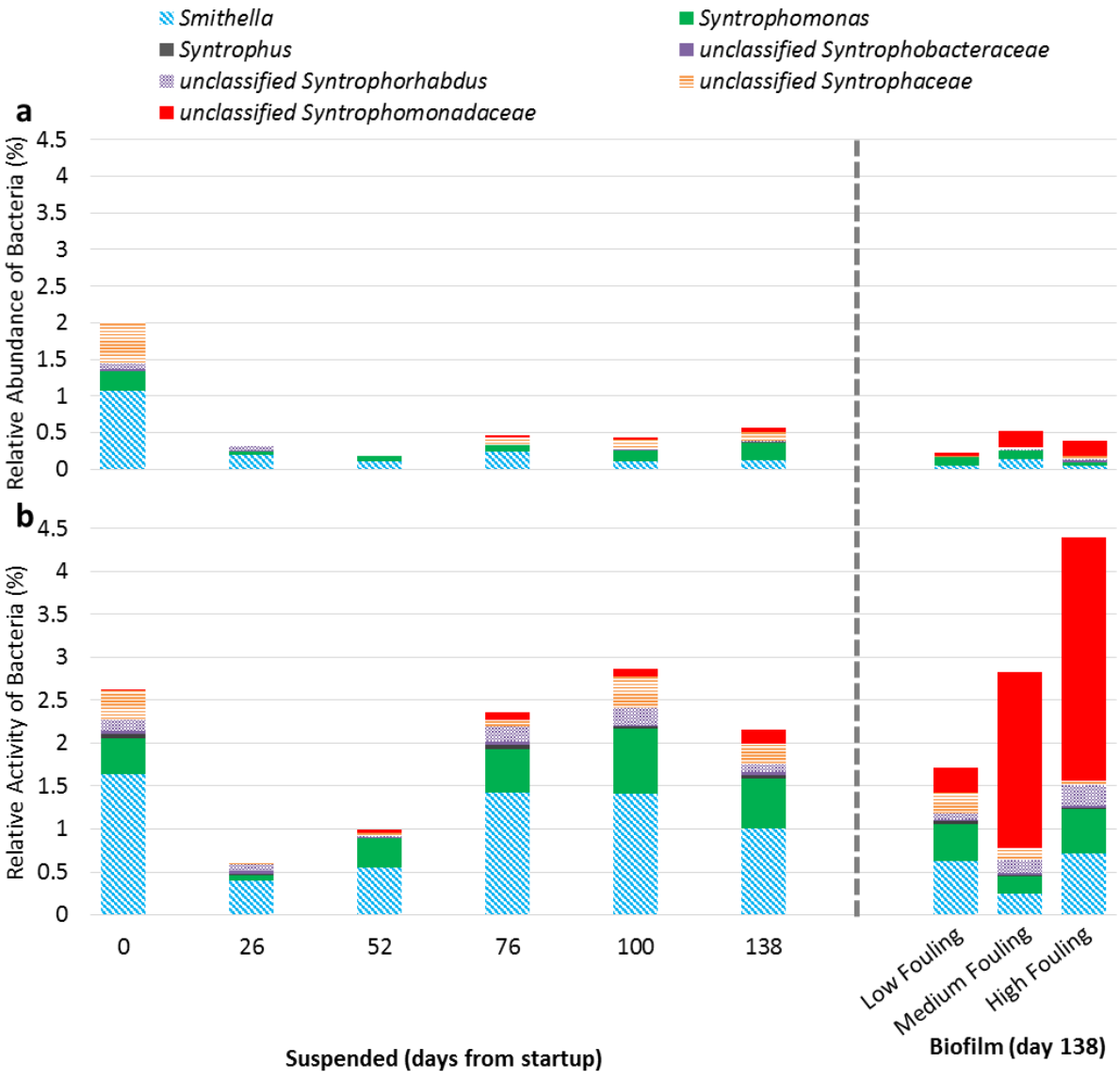

Figure 4-5. (a) Relative abundance of syntrophic bacteria at the genus level to bacteria based on 16S rDNA sequencing and (b) relative activity of syntrophic bacteria at the genus level to bacteria based on 16S rRNA sequencing in suspended and biofilm biomass. A truncated y-axis (0 to 4.5%) is shown to accentuate changes in abundance and activity.

Known syntrophic acetate oxidizing bacteria (Hattori 2008) were not detected in suspended or biofilm biomass. This is consistent with previous observations of their presence primarily under thermophilic conditions (Hao et al. 2010, Mayumi et al. 2011, Rui et al. 2011) due to the

thermodynamic efficiencies gained at higher temperatures in their metabolism. Acetate removal in the biofilm of the AnMBR likely occurred exclusively through the aceticlastic methanogenic pathway via *Methanosaeta* spp. and *Methanosarcina* spp. However, our knowledge on syntrophic acetate oxidizers is expanding (e.g., a novel acetate-oxidizing member of *Synergistes* group 4 was recently identified (Ito et al. 2011)) and this metabolism cannot be excluded from AnMBR without further experimental evidence (e.g., DNA/RNA-SIP with 13C-labeled acetate).

The AnMBR biofilm may support other syntrophic interactions. The sulfate reducer *Desulfovibrio vulgaris* has been identified as capable of growth syntrophically with a hydrogenotrophic methanogen on lactate in the absence of sulfate (Scholten et al. 2007). The majority of sulfate reduction in our system occurred in suspended biomass with sulfate concentrations <1 mg/L in the bioreactor at the end of Phase 2 (prior to biofilm biomass sampling). Relative activity of certain known sulfate reducers correlated well with performance data. For example, *Desulfobulbus* spp. had a relative activity of 2.2% in the suspended biomass but only 0.25% in HF biofilm biomass (Figure S4-6). However, *Desulfovibrio* spp. relative activity was greater in the biofilm biomass, 7.2 versus 4.3% in HF biofilm and suspended biomass, respectively, despite limited measured sulfate reduction occurring by the biofilm. It is possible that in the absence of sulfate, members of *Desulfovibrio* transition to syntrophic metabolisms that are potentially supported in the biofilm due to lower intercellular distances or more generally, spatial organization of the community.

The biofilm may provide an environment conducive to direct interspecies electron transfer (DIET) via nanowires or direct cell-to-cell electron transfer (Summers et al. 2010) as opposed to transfer of other intermediates such as hydrogen or formate (Rotaru et al. 2012). In fact, DIET has been proposed as a potential pathway in methanogenic aggregates (Morita et al. 2011). However, *Geobacter* spp., a genus with members likely to participate in DIET through syntrophic ethanol

degradation (Summers et al. 2010), had lower relative activity in the biofilm than suspended biomass. The highly active unclassified *Syntrophomadaceae* OTU observed in LF and HF biofilm biomass could potentially be involved in DIET although genome sequencing of *Syntrophomonas wolfei* did not identify the outer membrane cytochromes believed to be necessary for DIET (Sieber et al. 2010). DIET may occur between other unidentified syntrophic partners in the biofilm or hydrogen/formate electron transfer may be more prevalent in this system. We hypothesize that reduced mass-transfer limitations, increased substrate availability, and spatial organization of the biofilm community all play a role in the high microbial activity observed. Future research should evaluate the relative contribution of these potential explanations.

4.4.5 Biofilm treatment is maintained in the absence of TMP

During Phases 3 and 4, biogas sparging on HF was increased to evaluate if biological treatment in the biofilm could be maintained without high TMP (i.e., high fouling operation was switched to low fouling operation; HF-LF). HF-LF permeate COD during Phases 3 and 4 averaged 24 ± 7.1 mg/L (Figure S4-7), a similar effluent quality to that obtained at the end of Phase 2. Further, HF-LF permeate propionate concentration was below detection by the start of Phase 4 (Figure S4-8) implying that the activity of syntrophic propionate oxidizers improved during this time period, despite low TMP. Dissolved methane oversaturation remained high, averaging 2.2 ± 0.49, and was thus primarily driven by biological activity in the biofilm rather than high TMP or a combination of the two. One concern with our comparative evaluation is that pump slippage from high TMP resulted in reduced flux for HF which increased substrate contact time in the biofilm which could have affected our observations. However, HF flux was restored after returning TMP to near zero suggesting that the potentially higher substrate contact time did not impact our comparison. These results demonstrate that biofilm treatment can be maintained in the absence of TMP and suggest

that the active microbial community in the biofilm is tightly adhered to the membrane surface. The active community is either distinct from the layer of foulants contributing to high TMP or has sufficient biological activity to maintain treatment under low fouling conditions (i.e., less biomass).

After restoring MF TMP to near zero (Phase 3), fouling was increased in attempt to replicate the performance obtained previously with HF (Figure 4-1). Medium fouling to high fouling (MF-HF) permeate COD during Phase 4 was 37 ± 7.0 mg/L, approaching a similar effluent quality to HF permeate in Phase 2. Dissolved methane oversaturation in MF-HF permeate increased, averaging 2.6 ± 0.68. These observations provide evidence that biofilm promotion via reduced biogas sparging to enhance treatment performance is replicable.

Because we were able to maintain biological activity after returning to near zero TMP, biofilm promotion strategies may only require an inoculation period in which the membrane is colonized from the suspended biomass and can then be operated at low TMP. Long-term operation with membrane fouling is undesirable from an operations standpoint and thus our demonstration of biofilm treatment at low TMP is encouraging. However, the industry's current reliance on aggressive chemical cleaning in membrane installations is a concern. Chemical cleaning might disrupt the active biofilm community and treatment benefits would not be maintained although this has yet to be determined experimentally. It is important to note that we were able to return to a low TMP after extended periods of fouling by solely adjusting biogas sparging flow rate without chemical cleaning. In a full-scale system, fouling control could be accomplished without chemical cleaning using this approach or an alternative strategy that sustains biological activity in the biofilm. The energy demands of a higher biogas sparging flow rate to do so need to be weighed against the benefits of operation without chemical cleaning.

4.4.6 Biofilm treatment is an attractive operational strategy for low-temperature AnMBR

We have shown that by rethinking common perceptions of membrane fouling, we can improve effluent quality in AnMBR domestic wastewater treatment. However, improved effluent quality was at the cost of elevated dissolved methane concentrations in the permeate. Dissolved methane recovery from the permeate would be necessary to recover this potential energy source and prevent greenhouse gas emissions. Multiple lines of evidence (i.e., 16S rRNA sequencing, RT-qPCR targeting the *mcrA* gene, and performance observations) show that controlled membrane fouling leads to development of a biologically active membrane biofilm enriched in highly active aceticlastic and hydrogenotrophic methanogens and syntrophic bacteria. DNA-based molecular analyses were insufficient to describe microbial community activity and functional significance in this study. Conversely, RNA-based analyses were consistent with performance observations and we thus recommend a combination of RNA and DNA approaches to evaluate microbial community dynamics in systems with low growth and high biomass retention such as low-temperature AnMBR. Future research should evaluate the underlying mechanisms behind the biofilm community's high biological activity (e.g., reduced mass transfer limitations, lower intercellular distances, or other factors), the impact of biofilm promotion strategies on long-term membrane fouling, and the biofilm's response to chemical membrane cleaning. Operating without chemical membrane cleaning may be preferable if treatment performance is reliant on the biofilm. A biologically active biofilm may also have additional unexplored benefits in AnMBR such as removal of antibiotic resistance genes (e.g., as demonstrated in aerobic MBR (Riquelme Breazeal et al. 2012)) or other micropollutants. Finally, future research is necessary to develop low-energy dissolved methane recovery technologies capable of a high degree of dissolved methane recovery at a usable methane content. This is crucial under conditions of high dissolved methane

oversaturation that may prevail in AnMBR particularly considering on-going efforts to reduce fouling control energy demands.

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Chapter 5. Anaerobic Membrane Bioreactor Treatment of Domestic Wastewater at Psychrophilic Temperatures Ranging from 12 to 3°C

5.1 Abstract

Anaerobic membrane bioreactor (AnMBR) treatment of a simulated domestic wastewater was evaluated at psychrophilic temperatures of 12, 9, 6, and 3°C. Chemical oxygen demand (COD) removal > 95% was maintained at temperatures as low as 6°C, but fell to 86 ± 4.0% at 3°C. The membrane biofilm's contribution to biological treatment increased as temperature decreased in response to a decrease in suspended biomass treatment. High dissolved methane oversaturation occurred due to an increase in methanogenesis in the biofilm at lower temperatures. High-throughput sequencing of 16S rRNA to infer microbial activity in the biofilm and performance observations suggested that a diversification of metabolisms in the biofilm (i.e., methanogenesis and syntrophic VFA oxidation as well as fermentation of more complex organics) occurred as temperature decreased. Hydrogenotrophic methanogenesis as opposed to aceticlastic methanogenesis was the preferred pathway in the biofilm but not in suspended biomass, possibly due to better spatial microbial organization in the biofilm supporting syntrophy. Membrane fouling became more severe as temperature decreased indicating a potential barrier to AnMBR implementation at such low temperatures. This research demonstrated that AnMBR treatment of domestic wastewater at very low temperatures is feasible. Future research in fouling control, dissolved methane recovery, and improving suspended biomass treatment to reduce reliance on

Smith, A.L., Skerlos, S. and Raskin, L., 2014a. Anaerobic membrane bioreactor treatment of domestic wastewater at psychrophilic temperatures ranging from 12-3°C. in preparation for Environmental Science & Technology.

biofilm treatment and limit methane oversaturation are necessary to justify AnMBR treatment at such low temperatures.

5.2 Introduction

With few exceptions, anaerobic biological waste treatment processes to date are operated at mesophilic (30-40°C; (Lettinga et al. 2001)) or thermophilic (50-60°C; (Kim et al. 2002)) temperatures. However, given the low energy content of domestic wastewater, the energy demand to maintain a mesophilic reactor temperature for anaerobic treatment of domestic wastewater far outweighs the energy recovery possible in most climates (Martin et al. 2011). Thus, for anaerobic treatment of domestic wastewater to become a reality, operation at ambient temperatures is necessary. In the U.S., the annual mean temperature of untreated domestic wastewater varies from approximately 3 to 27°C, with a nationwide average of about 16°C (Tchobanoglous et al. 2003). Globally, a large portion of the population lives in temperate climates and low domestic wastewater temperatures are common in winter months. The seasonal temperature fluctuations in these climates require treatment technologies with a high degree of resilience to ensure effluent discharge criteria are consistently met. Anaerobic membrane bioreactor (AnMBR) systems have recently come to the forefront as promising options for mainstream anaerobic treatment of domestic wastewater at various temperatures (Lin et al. 2013, Ozgun et al. 2013, Smith et al. 2012). Understanding the lower temperature limits for AnMBR treatment is imperative to determine climate-based barriers to implementation.

Despite the importance of assessing AnMBR operation at psychrophilic temperatures (< 20°), only a few studies have evaluated performance in this temperature range (Chu et al. 2005, Ho and Sung 2010, Martinez-Sosa et al. 2012, Shin et al. 2014, Smith et al. 2014a, Smith et al. 2013, Wen et al. 1999) and no studies to date have explored temperatures less than 8°C. Chemical oxygen demand

(COD) removal greater than 85% has been reported at temperatures as low as 15°C (Chu et al. 2005, Ho and Sung 2010, Smith et al. 2014a, Smith et al. 2013, Wen et al. 1999). However, Chu et al. (2005) observed COD removals of only 76-81% at 11°C. A few studies reported an increase in the amount of COD removal across the membrane when the operational temperature decreased (Chu et al. 2005, Ho and Sung 2010, Wen et al. 1999) suggesting the membrane biofilm as opposed to the suspended biomass is more important for COD removal at psychrophilic conditions. Chue at al. (2005) also evaluated an increase in the hydraulic retention time (HRT) to improve treatment performance at low temperatures.

In addition to understanding the impact of lower temperature on AnMBR treatment performance, recognizing the impact of lower temperature operation on the distribution of methane in the gas versus the liquid phase is important to achieve AnMBR implementation for domestic wastewater treatment. Failing to recover dissolved methane from AnMBR permeate impairs the energy balance while also allowing the release of a potent greenhouse gas to the atmosphere, increasing the global warming potential of treatment (Smith et al. 2014b). Multiple AnMBR studies have reported dissolved methane oversaturation (Kim et al. 2011, Smith et al. 2013, Yeo and Lee 2013) and we established a positive correlation between dissolved methane oversaturation and methanogenic activity in the membrane biofilm (Smith et al. 2014a). Lower operational temperatures result in increased methane solubility resulting in greater dissolved methane losses. The potential for greater reliance on the membrane biofilm as opposed to the suspended biomass for treatment at psychrophilic conditions as indicated above could exacerbate this concern by increasing dissolved methane oversaturation. Research on dissolved methane recovery is still developing, but to date dissolved methane recovery is energy intensive and may not produce an off-gas of sufficient methane content for cogeneration (Bandara et al. 2011, Cookney et al. 2012).

Regardless, understanding methane fate in AnMBR, especially for low temperature operation, is necessary to gauge the practicality of AnMBR implementation.

Understanding the impact of psychrophilic conditions on AnMBR treatment performance and on methane fate cannot be accomplished without appreciating the structure and activity of the diverse microbial populations in the suspended biomass and the membrane biofilm of the AnMBR. In low temperature anaerobic treatment, propionate oxidation and methanogenesis are typically considered rate-limiting metabolisms (Bialek et al. 2013, Rebac et al. 1995). Further, aceticlastic methanogens have been reported as more strongly affected by low temperature than their hydrogenotrophic counterparts (Lettinga et al. 1999, Nozhevnikova et al. 1997). A shift towards hydrogenotrophic methanogenesis in anaerobic treatment at psychrophilic temperatures has been reported using DNA-based molecular analyses and specific methanogenic activity assays (Collins et al. 2006, Connaughton et al. 2006, McHugh et al. 2004). This shift may occur due to increased hydrogen solubility which increases substrate availability and thermodynamically reduces energy requirements for hydrogenotrophic methanogenesis (Lettinga et al. 2001). Given the complexity of anaerobic microbial communities involved in degrading complex and undefined mixtures of organics present in domestic wastewater in general, challenges specific to AnMBRs due to the development of distinct microbial communities in the suspended biomass and membrane biofilm (Smith et al. 2014a), and the temperature dependent impact of the availability of hydrogen and the distribution of methane, evaluating the metabolic pathways and response of specific microbial populations to temperature changes is critical to understand operational strategies to improve AnMBR performance.

The objective of this study was to evaluate AnMBR operation at decreasing temperatures to assess the potential for AnMBR treatment of domestic wastewater in temperate climates. A bench-scale

AnMBR with a history of operation with controlled fouling to allow for membrane biofilm treatment at 15ºC, was operated for five to six weeks each at 12, 9, 6, and 3°C. Illumina sequencing of 16S rRNA genes (rDNA) and 16S rRNAs was applied to evaluate microbial community structure and activity dynamics in suspended and biofilm biomass in response to the decrease in operational temperature.

5.3 Materials and Methods

5.3.1 AnMBR Operation and Chemical Assays

A bench-scale AnMBR (Smith et al. 2014a) was operated to evaluate system performance at varying psychrophilic temperatures while treating a simulated domestic wastewater (Aiyuk and Verstraete 2004, Smith et al. 2013). The bench-scale AnMBR was previously operated at 15°C for 172 days (Smith et al. 2014a). Reactor temperature was controlled using a water jacket connected to a Polystat 6-L recirculating water bath (Cole-Parmer, Vernon Hills, IL). Water bath temperature was adjusted based on temperature measurement of a submerged probe located in close proximity to the membrane surface. The bench-scale AnMBR contained three individual membrane housings, designated P1, P2, and P3, and generated three permeate streams.

The AnMBR temperature was reduced from 15 to 12ºC on day 173, then to 9ºC on day 216, 6ºC on day 252, and 3ºC on day 286 (Table 5-1) to represent a range of potential temperatures experienced during winter in a domestic wastewater treatment plant in a temperate climate. The bench-scale AnMBR was initially operated at an HRT of 17 ± 1.0 h, that corresponded to an organic loading rate (OLR) of 630 mg COD/L•d. However, membrane fouling became more severe throughout the operational period resulting in flux reduction due to pump slippage and an increase in HRT (Table 5-1). Biomass was only removed from the AnMBR for sampling purposes, which resulted in a solids retention time (SRT) of approximately 300 days. The biogas sparging flow rate

for P3 was 3.0 L/min (5.8 $m^3/h•m^2$). The biogas sparging flow rate for P1 and P2 was decreased to 1.5 – 2.0 L/min from days 173 to 200 to permit biofilm development (described further below) and 3.0 L/min from days 201 to 313. Backflushing was performed for 30 s every 10 min of bioreactor operation initially but was modified on day 253 in attempt to improve flux and lower HRT by increasing the duration to 1 min and decreasing the interval time to 5 min.

Influent, permeate, biogas, and bioreactor content sampling, sample preservation, and storage were performed as described previously (Smith et al. 2014a). COD, total suspended solids (TSS), and volatile suspended solids (VSS) were determined using procedures outlined in Standard Methods (APHA 2005). Soluble COD was determined by filtering samples through a 0.2 µm filter to replicate the physical removal of the membrane (same pore size). Concentrations of volatile fatty acids (VFAs) (acetate, propionate, formate, butyrate, and valerate) and sulfate were determined by ion chromatography (ICS-1600, Dionex, Sunnyvale, CA) (Smith et al. 2014a).

Biogas methane content and dissolved methane concentration were measured with a gas chromatograph (Gow-Mac, Bethlehem, PA) (Smith et al. 2014a). Biogas production was measured by collecting gas in a 1-L Tedlar bag and quantifying the production daily using a wet-type gas meter (Actaris Metering Systems, Dordrecht, The Netherlands).

Table 5-1. AnMBR operational temperature, HRT (average ± standard deviation), OLR, and flux (average ± standard deviation).

				Flux (LMH)		
Days from Startup	**Temperature (°C)**	**HRT (h)**	**OLR (mg COD/L•d)**	**P1**	**P2**	**P3**
173-215	12	17 ± 1.0	630	2.5 ± 0.23	2.2 ± 0.21	2.5 ± 0.14
216-251	9	19 ± 1.3	560	2.2 ± 0.16	2.1 ± 0.15	2.1 ± 0.14
252-285	6	26 ± 3.5	410	1.9 ± 0.22	1.8 ± 0.21	1.2 ± 0.17
286-313	3	29 ± 2.2	370	1.6 ± 0.16	1.5 ± 0.15	1.2 ± 0.10

5.3.1 Nucleic acids extraction and cDNA synthesis

Suspended and biofilm biomass samples from the AnMBR were collected (Smith et al. 2014a) on days 215, 251, 285, and 313 at the end of each temperature phase, pelletized by centrifugation at 5,000 x *g* for 5 min at 4°C, decanted, and immediately stored at -80°C. Biomass samples for RNA extraction were prepared similarly except for the addition of RNAlater (Qiagen, Valencia, California) prior to storage. DNA and RNA were extracted from pelletized biomass and DNA and RNA quality and quantity were assessed as described previously (Smith et al. 2014a). Reverse transcription to generate single-stranded complementary DNA (cDNA) from RNA extracts was performed using the SuperScript VILO cDNA Synthesis Kit (Life Technologies, Grand Island, NY).

5.3.2 16S rDNA and rRNA sequencing

Universal primers targeting the V4 region (Caporaso et al. 2011) were used to amplify 16S rDNA and rRNA as described previously (Smith et al. 2014a). PCR of 16S rDNA and rRNA from DNA extracts and synthesized cDNA, respectively, taken during reactor operation at 12, 9, and 6°C was performed by the Center for Microbial Systems (University of Michigan, Ann Arbor, MI). PCR conditions included 20 µL reactions with the aforementioned primers at 500 nM, 10 µL 2x Accuprime buffer 11, 0.15 µL Accuprime TAQ (Invitrogen, Carlsbad, CA), 0.5 ng template, and nuclease-free water. Thermocycling conditions consisted of an initial 2 min denaturation at 95°C, followed by 30 cycles of denaturing at 95°C for 20 s, annealing at 55°C for 15 s, and extension at 72°C for 5 min, followed by a final extension at 72°C for 5 min. DNA and cDNA from 3°C biomass was amplified using the above described primer sets at 500nM, 0.3 mg/mL bovine serum albumin (BSA), 10 µL 2x Phusion High-Fidelity Master Mix (NEB, Ipswich, MA), 0.5 ng template, and nuclease-free water. Thermocycling conditions consisted of an initial 2 min

denaturation at 95°C, followed by 30 cycles of denaturing at 95°C for 20 s, annealing at 55°C for 15 s, and extension at 72°C for 30 s, followed by a final extension at 72°C for 5 min. Amplicons were pooled by equal mass using the SequalPrep Normalization Plate Kit (Life Technologies, Grand Island, NY). Multiplexed amplicons were sequenced by the Center for Microbial Systems via Illumina MiSeq using the MiSeq Reagent Kit V2 (samples from 12, 9, and 6°C; 2x250 bp reads) and V3 (samples from 3°C; 2x300 bp reads). 13,922 paired-end reads per sample were generated after quality filtering and subsampling. The resulting sequences were processed with mothur (Schloss et al. 2009) following the Schloss MiSeq SOP and classified using the Ribosomal Database Project (Maidak et al., 1997) and Basic Local Alignment Search Tool (BLAST; NCBI, Bethesda, MD).

5.4 Results and Discussion

5.4.1 COD removal remained excellent when reducing the AnMBR temperature from 12°C to 6°C, but was impacted at 3°C

The bench-scale AnMBR was previously operated for 172 days at 15°C (Smith et al. 2014a). During the last phase of that operational period, COD removal was > 95%, a substantial fraction of which was accomplished by the membrane biofilm. P1, P2, and P3 were operated under varying levels of membrane fouling by adjusting biogas sparging flow rates during days 100-138 to evaluate the effect of differential fouling levels on biofilm treatment (Smith et al. 2014a). We observed a decrease in permeate COD greater than 50 mg/L, primarily due to acetate and propionate removal, when membranes were operated under high fouling conditions based on transmembrane pressure (TMP). We also observed a limited impact on biofilm treatment after reducing TMP to near zero once a biologically active biofilm had been developed. This implies that membranes can be inoculated by operating at a high TMP temporarily and thereafter, membrane fouling can be reduced without loss of biofilm treatment. At the end this study, P3 had

a mature biofilm and P2 was in the process of developing a biologically active biofilm. During the current study, we elected to also operate P1 under conditions supporting biofilm development to maximize overall treatment performance. Because of the membranes' different histories, permeate COD was initially different but converged over time as a mature biofilm was formed on each membrane. The overall COD removal was largely unchanged as temperature was reduced to 12, 9, and even 6°C, averaging 95 ± 1.5%. However, a decrease in microbial activity in the suspended biomass was observed suggested by a steady increase in soluble COD in the bioreactor (Figure 5-1). Biofilm activity was critical to maintain the high total COD removal as temperature decreased. At 3°C, the suspended biomass was primarily hydrolyzing particulate COD as indicated by similar influent total and bioreactor soluble COD concentrations at this temperature (Figure 5-1). These data suggest that fermentation, syntrophic VFA oxidation, and methanogenesis primarily took place within the biofilm biomass. Permeate COD averaged 70 ± 21 mg/L during operation at 3°C corresponding to a COD removal of 86 ± 4.0%.

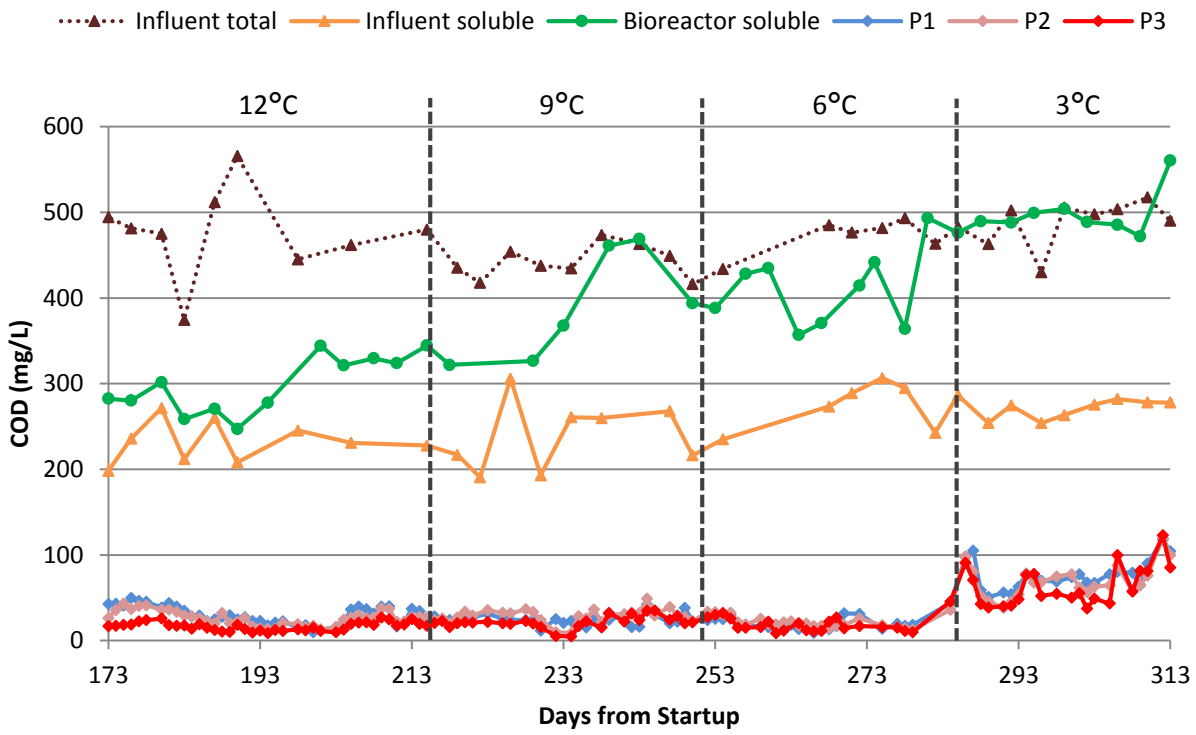

Figure 5-1. Influent (total and soluble), bioreactor (soluble), and permeate (P1, P2, and P3) COD concentration from days 173-313.

VFA removal was similarly unaffected by temperature decrease from 12 to 6°C. The average permeate acetate and propionate concentrations for each of the first three temperature periods was < 5 mg/L (Figure 5-2). Other VFAs (formate, butyrate, and valerate) were below their detection limits (Figure S5-1, Appendix C). Concentrations of acetate and propionate in P3 permeate were initially lower than P1 and P2 permeate due to the different membrane histories described above. As with COD, acetate and propionate concentrations in P1, P2, and P3, converged over time after P1 and P2 developed mature biofilms. At 3°C, an increase in acetate, propionate, and formate concentrations in the permeate was observed. Immediately after the temperature decrease from 6 to 3°C, permeate acetate and propionate concentrations exceeded those in the bioreactor likely because the biofilm was fermenting organic compounds and methanogens and syntrophic bacteria in the biofilm had yet to adapt to the temperature decrease. Acetate and propionate concentrations

in the permeate were similar during operation at 3°C suggesting temperature based inhibition occurred for aceticlastic methanogens as well as syntrophic propionate oxidizing bacteria in the biofilm. However, the differences in acetate and propionate concentrations in the bioreactor and permeates indicated that biological removal of acetate and propionate by the biofilm still occurred at 3°C (Figure 5-2) and therefore, these specific populations were not completely inhibited.

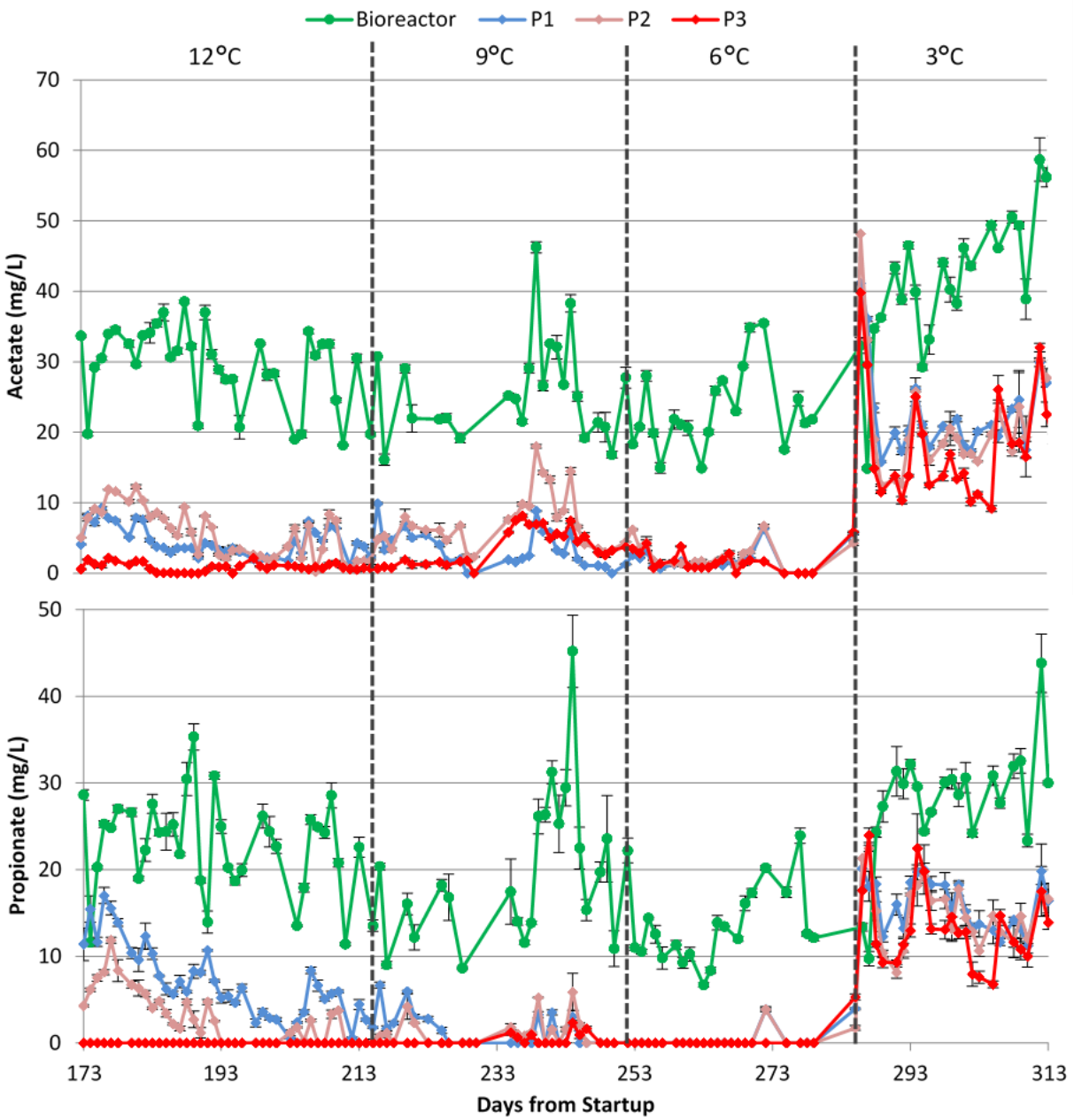

Figure 5-2. Bioreactor (soluble) and permeate (P1, P2, and P3) concentrations of (a) acetate and (b) propionate. Error bars represent the standard deviation of triplicate IC injections.

COD removal was potentially impacted by the increase in HRT as temperature decreased, particularly at 6 and 3°C (Table 5-1). A relationship between COD removal and HRT in AnMBR has previously been reported at low temperatures (Chu et al. 2005). The increase in HRT in this study was due to membrane fouling and the correspondingly high TMP, which reduced the flux

due to pump slippage. For each 3°C temperature decrease, the TMP increased ~20 kPa over the course of several hours. Membrane fouling concerns such as extracellular polymer substances (EPS) have been shown to increase as temperatures decrease in aerobic MBRs (Wang et al. 2009). However, the inverse has been shown in AnMBRs in a comparison of mesophilic and psychrophilic temperatures (Robles et al. 2013). The rapid onset of fouling observed here suggests that the fouling may have been non-biological in nature. Future work is necessary to evaluate membrane fouling at such low temperatures and at higher, more realistic fluxes.

5.4.2 The reliance on the biofilm for treatment lead to significant dissolved methane oversaturation

Dissolved methane concentrations in the permeate were indicative of significant methanogenic activity in the biofilm. An increase in methane oversaturation (calculated by measuring the dissolved methane concentrations in the permeate and by calculating the equilibrium concentrations predicted by applying Henry's law using constants of 32,400, 30,600, 28,800, and 27,100 atm (Tchobanoglous et al. 2003) for 12, 9, 6, and 3°C, respectively, and the measured methane partial pressure in the headspace) was observed as temperature decreased (Figure 5-3) with an average methane oversaturation of 2.0 ± 0.41, 2.9 ± 0.48, 3.6 ± 0.87, and 4.1 ± 1.2 at 12, 9, 6, and 3°C, respectively. We previously reported methane oversaturation as high as 3.1 during operation at 15°C. We hypothesize that the higher oversaturation reported here resulted from an increased dependence on the biofilm for treatment and therefore, relatively greater methane production in the biofilm. A high degree of variability in methane oversaturation was apparent as temperature decreased (e.g, between approximately 2 and 7 times oversaturation during operation at 3°C; Figure 5-3). Oversaturation positively correlated with the biofilm's contribution to COD removal (Figure 5-4) as a function of temperature indicating a strong link between dependence on the biofilm for COD removal and additional dissolved methane in the permeate.

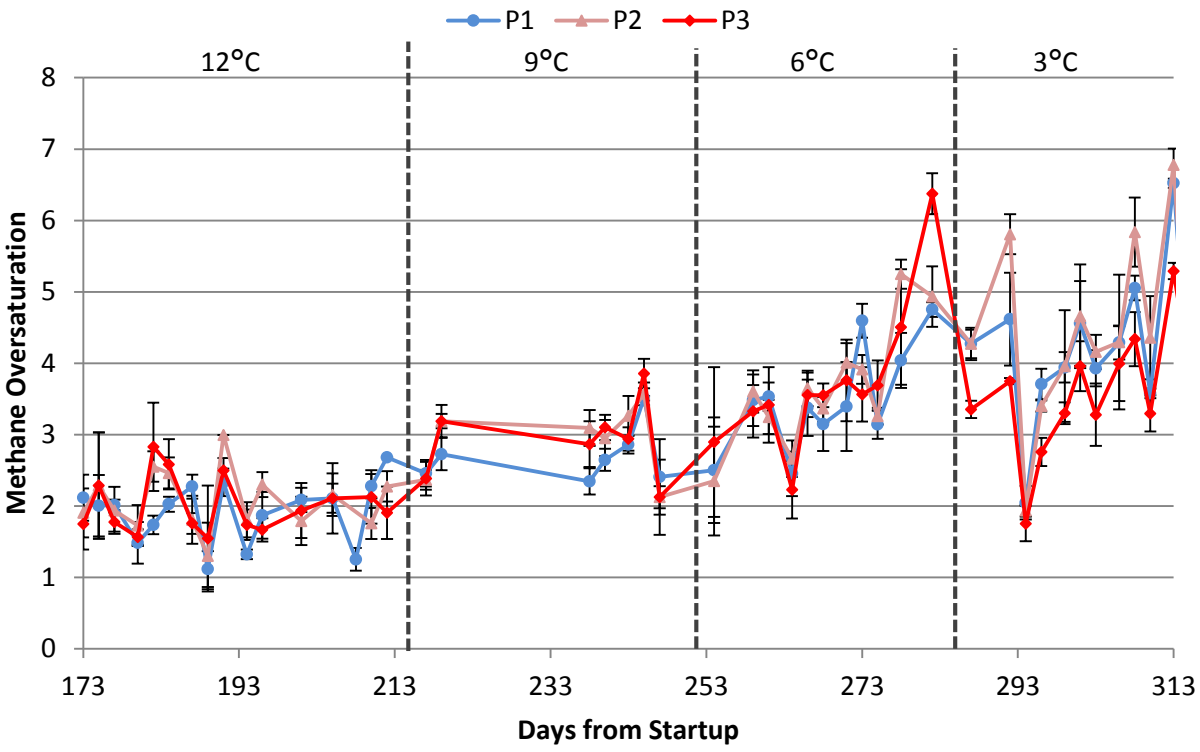

Figure 5-3. Permeate (P1, P2, and P3) dissolved methane oversaturation. Error bars represent the standard deviations of duplicate dissolved methane extractions and triplicate GC injections of each extract. Saturation was calculated according to Henry's law using constants of 32,400, 30,600, 28,800, and 27,100 atm (Tchobanoglous et al. 2003) for 12, 9, 6, and 3°C, respectively, and the measured methane partial pressure in the headspace. A value of 1 indicates saturation of methane whereas a value of 2 indicates two times the concentration at saturation.

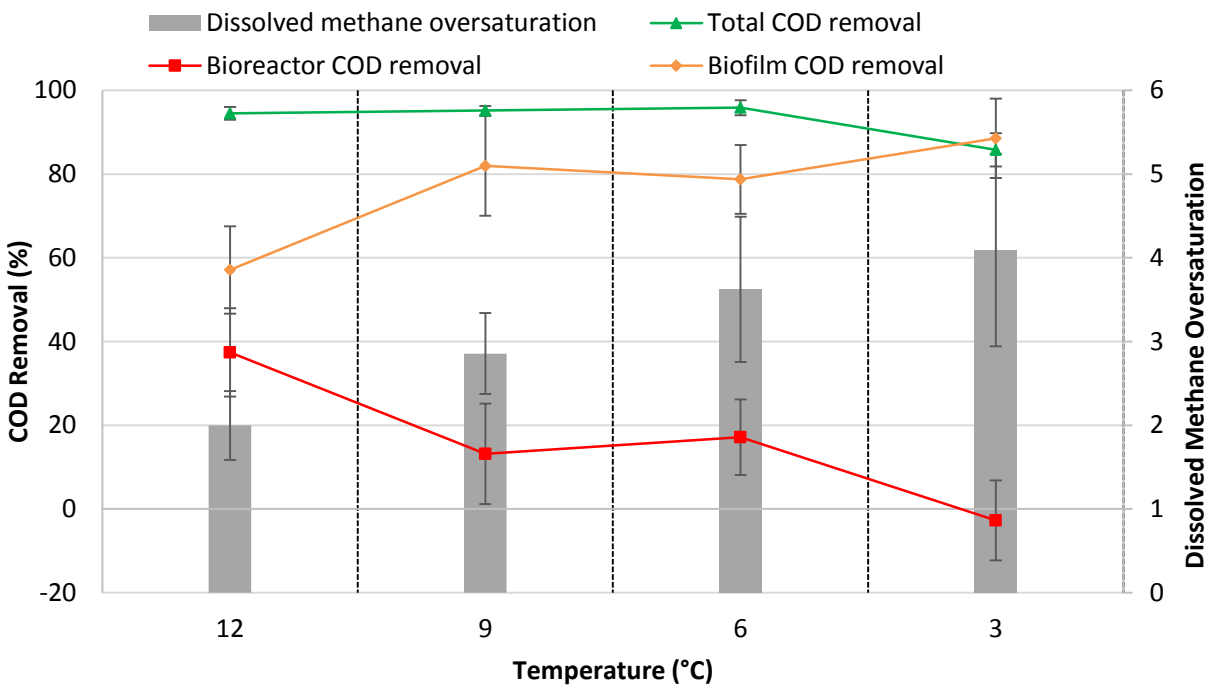

Figure 5-4. Average COD removal (total, bioreactor, and biofilm) on primary y-axis and dissolved methane oversaturation in permeate on secondary y-axis as a function of operational temperature. Total COD removal is the summation of bioreactor and biofilm COD removal. Error bars represent the standard deviation of all measurements at each temperature.

Dissolved methane was the major constituent of COD_{out} in the COD mass balance at all operational temperatures comprising on average 59 ± 13%, 61 ± 6.4%, 75 ± 7.7%, and 62 ± 9.5% at 12, 9, 6, and 3°C, respectively (Figure S5-2). The high contribution of dissolved methane to COD_{out} was partly due to the high methane content in the headspace, > 90% at 12, 9, and 6°C. The high methane content may have been due to high carbon dioxide solubility at low temperatures and/or influenced by the synthetic wastewater composition (Aiyuk and Verstraete 2004). The decrease in contribution of dissolved methane to COD_{out} at 3°C was primarily a result of elevated permeate COD relative to other operational temperatures. Biogas production at 3°C was erratic and largely negligible in the overall COD mass balance. The lack of biogas production at 3°C resulted in declining headspace methane content over time. Suspended solids concentrations in the bioreactor remained stable throughout operation (Figure S5-3) suggesting negligible net biomass growth.

This further suggests that influent particulates did not accumulate in the system and were hydrolyzed.

Biofilm treatment may be a requirement at low operational temperatures for AnMBR to achieve effluent discharge criteria. Therefore, significant methane oversaturation may be unavoidable. Alternatively, extended operation at low temperatures beyond what was done in this study may provide sufficient time for adaptation by the suspended biomass. However, prolonged periods for psychrophilic biomass adaptation are unrealistic at the full-scale. Another strategy may be to inoculate AnMBRs with a psychrophilic or psychrotolerant biomass as opposed to only a mesophilic biomass. Currently, psychrophilic or psychrotolerant anaerobic biomass is rare in engineered systems as most are operated in the mesophilic temperature range. This has prompted researchers to investigate seeding anaerobic systems with psychrophilic biomass from the environment (Petropoulos et al. 2013). Future work should evaluate these approaches and others to improve suspended biomass treatment and limit dissolved methane oversaturation caused by biofilm treatment. Downstream treatment technologies for dissolved methane recovery should also be explored as an alternative.

5.4.3 RNA-based 16S rRNA sequencing revealed significant changes in the functional microbial community

RNA-based methods to assess microbial activity, as opposed to DNA-based methods evaluating microbial community structure, may be particularly helpful in environments with low microbial growth and high biomass retention, such as in psychrophilic AnMBRs. DNA-based approaches would likely only be representative of the functional microbial community after considerable operational time which is a limitation in bench or pilot-scale studies. Further, DNA-based approaches may detect extracellular DNA and DNA from inactive community members. We previously explored AnMBR at 15°C using this microbial characterization approach and observed

significant differences between 16S rDNA and rRNA sequences, especially for methanogens and syntrophic bacteria. Lower temperatures further reduce microbial growth and could further reduce the sensitivity of DNA-based molecular methods.

Although 16S rRNA sequencing to infer microbial activity has a number of limitations (e.g., poor correlation between growth rate and rRNA concentration in some instances, rRNA presence in dormant cells, and limited information on non-growth activities and rRNA concentration; (Blazewicz et al. 2013)), we believe that the comparative evaluation taken here between suspended and biofilm biomass and over time is still useful and more informative than 16S rDNA sequencing alone. Quantifying functional gene expression may be a more accurate method to determine activity of specific populations. However, using this approach is challenging to assess the whole community given the high diversity in AnMBR biomass. 16S rRNA sequencing is thus a valuable tool to broadly characterize active members of a microbial community, despite the methodological limitations. We also previously verified that methanogenic activity based on 16S rRNA sequencing and expression of the methyl coenzyme-M reductase (*mcrA*) gene, a functional gene in methanogens, correlated well (Smith et al. 2014a) suggesting that 16S rRNA sequencing is a valid tool to study psychrophilic AnMBR communities.

Sequencing results at 12, 9, 6, and 3°C supported the notion that 16S rRNA sequencing is more suitable than 16S rDNA sequencing at describing the functional microbial community in low-temperature AnMBR. Relative methanogenic activity based on 16S rRNA in all biomass was > 2x the relative abundance based on 16S rDNA (Figure 5-5). However, 16S rDNA sequencing did reveal changes in the methanogenic community as temperature decreased. For example, *Methanoregula* spp., a mesophilic hydrogenotrophic methanogen (Bräuer et al. 2011), was the dominant methanogen in terms of abundance at 12°C comprising 46% and 60 ± 14% of *Archaea*

in suspended biomass and biofilm biomass, respectively. However, at 9°C *Methanoregula* spp. comprised only 9% of *Archaea* in suspended biomass and less than 8.0% in biofilm biomass suggesting abrupt inhibition between these temperatures. 16S rRNA sequencing supported this conclusion with a decrease in relative activity of *Methanoregula* spp. in the biofilm from 27 ± 8.2% to 3.6 ± 1.4% as temperature decreased from 12 to 9°C. *Methanoregula* spp. activity remained low thereafter. Two studies that cultivated *Methanoregula* observed growth at temperatures as low as 10°C but not at 4°C (Bräuer et al. 2011, Yashiro et al. 2011). 16S rDNA and rRNA sequencing here confirms temperature based inhibition between 12 and 9°C. Shifts in relative activity of other methanogens were less severe as temperature decreased and therefore, temperature based inhibition may have been less prevalent than in *Methanoregula*. It is important to note that *Methanoregula* was represented by 36 OTUs over all biomass samples whereas other dominant methanogens such as *Methanospirillum, Methanosaeta, and Methanosarcina* were represented by 45, 61, and 39 OTUs, respectively, suggesting greater intragenus diversity and potentially more temperature based resilience. For example, the diversity of *Methanospirillum* according to the inverse of Simpson's Diversity Index (Hunter and Gaston 1988) increased in suspended biomass as temperature decreased implying that OTUs within the genus had differing response to the temperature decrease.

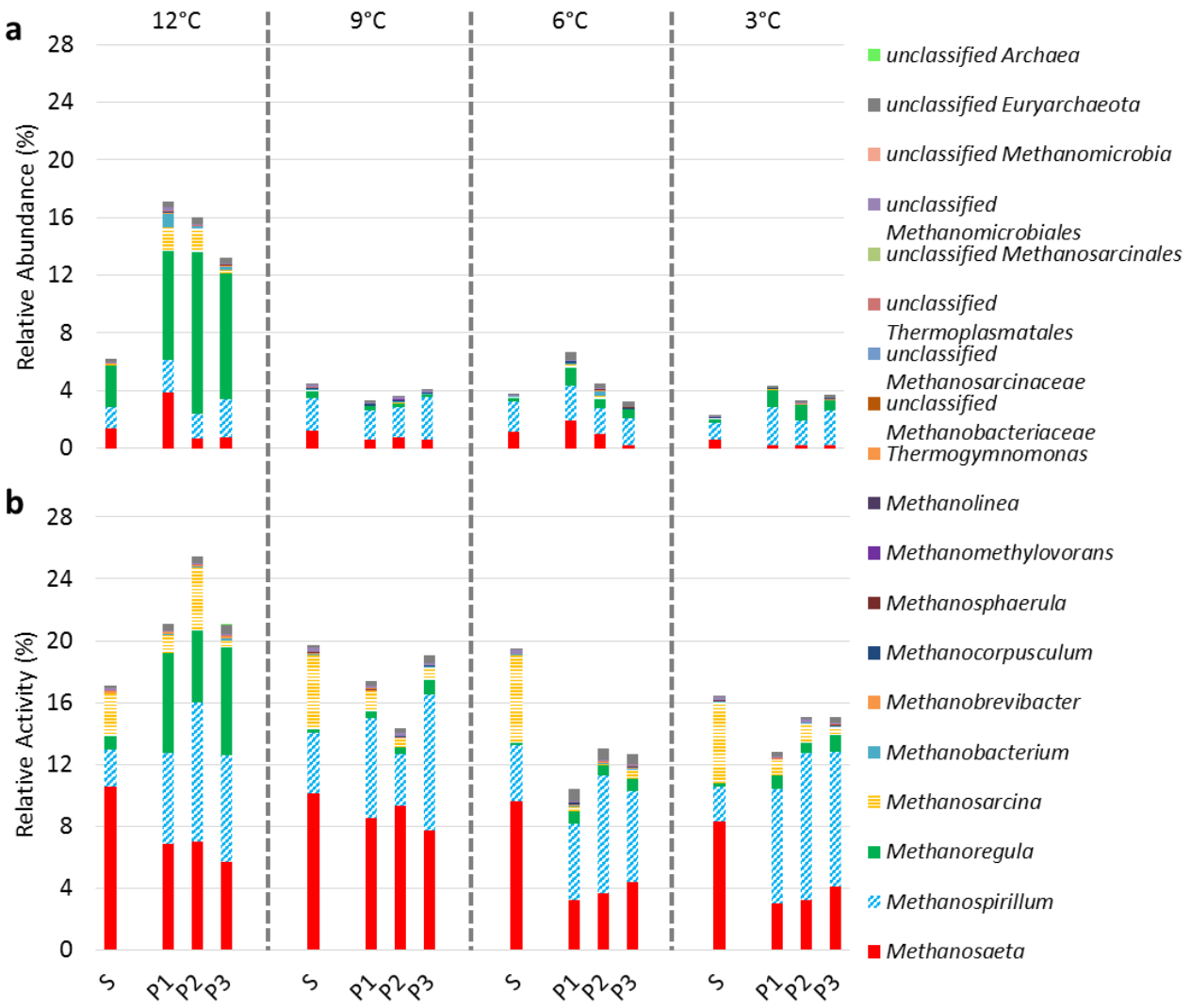

Figure 5-5. (a) Relative abundance based on 16S rDNA sequencing and (b) relative activity based on 16S rRNA sequencing of methanogens to total community in suspended (S) and biofilm (P1, P2, and P3) biomass at operational temperatures of 12, 9, 6, and 3°C.

The declining relative activity of methanogens in biofilm biomass as temperature decreased suggests a diversification of microbial metabolisms in the biofilm. The increasing contribution of dissolved methane to COD_{out} (Figure S5-2) indicates that methanogenic activity in the biofilm increased as temperatures decreased to 6°C whereas the increase in soluble bioreactor COD indicates a decrease in suspended biomass activity. This suggests that the absolute activity of biofilm biomass and temperature were negatively correlated. Therefore, the observed decline in

relative activity of methanogens signifies an increase in its range of microbial metabolisms (e.g., fermentations in addition to methanogenesis and syntrophic VFA oxidation) rather than a reduction in methanogenic activity in the biofilm. We hypothesize that biofilm biomass is more active and resilient to temperature than suspended biomass due to increased spatial organization of the microbial community enhancing syntrophy and/or reduced mass-transport limitations and increased substrate availability in the biofilm.

A limitation of this study is the lack of absolute abundance or activity in our molecular characterization. Quantitative nucleic acid extraction from sludge is challenging particularly when matrix differences such as those between suspended biomass and biofilm biomass are unavoidable. Constituents such as EPS, which we previously reported as higher in biofilm biomass from fouled membranes (Smith et al. 2013), can decrease extraction efficiency and extract quality which makes quantitative characterization difficult. Constituents such as EPS and other microbial products may also vary as a function of temperature (Wang et al. 2009) creating additional complications. Therefore, here we rely on relative molecular characterization and process performance data to make inferences regarding absolute activity.

A ThetaYC-based (Yue and Clayton 2005) principal coordinate analysis (PCoA) of 16S rRNA revealed significant changes in the biofilm community as temperature decreased. High variability (i.e., poor clustering) between biofilm biomass was observed at 12 and 9°C but not at 6 and 3°C which is consistent with the three biofilms converging performance-wise over time based on effluent quality as each biofilm matured. The suspended biomass community was distinct from the biofilm community but remained relatively constant at the varying temperatures suggesting limited changes in the community's membership or individual relative activity of each member as a

function of temperature (Figure 5-6). This suggests that the temperature decrease non-specifically reduced activity of the suspended biomass community.

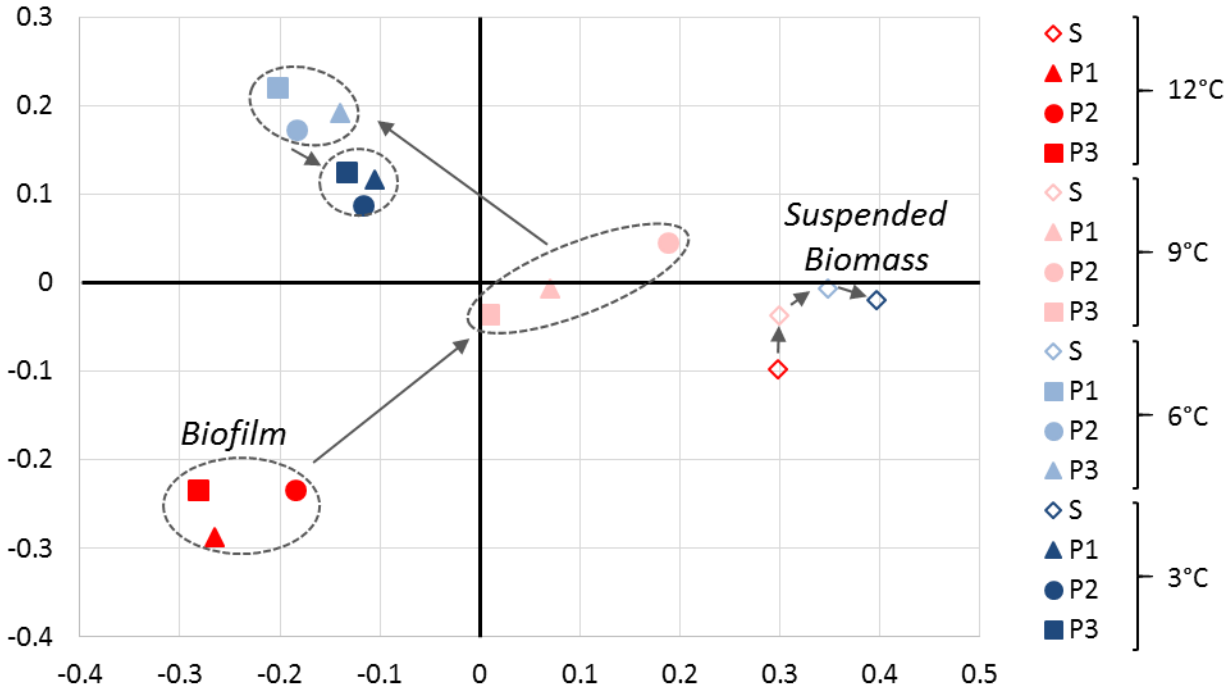

Figure 5-6. ThetaYC-based PCoA of microbial community based on 16S rRNA sequencing of suspended (S) and biofilm (P1, P2, and P3) biomass at operational temperatures of 12, 9, 6, and 3°C. The x and y-axes represent 45 and 20% of the variation, respectively. The top 20 classified phylotypes are shown in Figure S5-5.

5.4.4 The dominant type of methanogenic pathway is specific for suspended and biofilm biomass at all temperatures

Hydrogenotrophic and aceticlastic methanogenic pathways were favored in biofilm and suspended biomass, respectively (Figure S5-4). Higher activity of hydrogenotrophic methanogens relative to their aceticlastic counterparts in biofilm biomass suggests a metabolic advantage, possibly due to spatial organization of the community supporting syntrophic interactions. However, syntrophic bacterial activity was almost always higher in the suspended biomass which contradicts this hypothesis (Figure 5-7). It is important to again mention that our molecular approach to infer

activity is relative. If overall absolute microbial activity is significantly higher in biofilm biomass compared to suspended biomass, absolute syntrophic bacterial activity could indeed be higher in the biofilm than the suspended biomass. At all operational temperatures except 9°C, an unclassified OTU belonging to family *Syntrophomonadaceae* was significantly more active in the biofilm than the suspended biomass. *Syntrophomonadaceae* typically only oxidize C4 and higher order organic compounds (Stams et al. 2012) and therefore, its high activity in the biofilm given that butyrate and valerate were non-detectable in the bioreactor is remarkable. We previously hypothesized that this unclassified OTU may instead play a role in a novel pathway in which *Smithella* spp., a propionate oxidizing syntroph, first dismutates propionate to acetate and butyrate followed by butyrate oxidation by *Syntrophomonas* spp. via a trophic interaction (Gan et al. 2012) or alternatively that this species possesses a unique capability of C3 oxidation that is unknown in other members of its family.

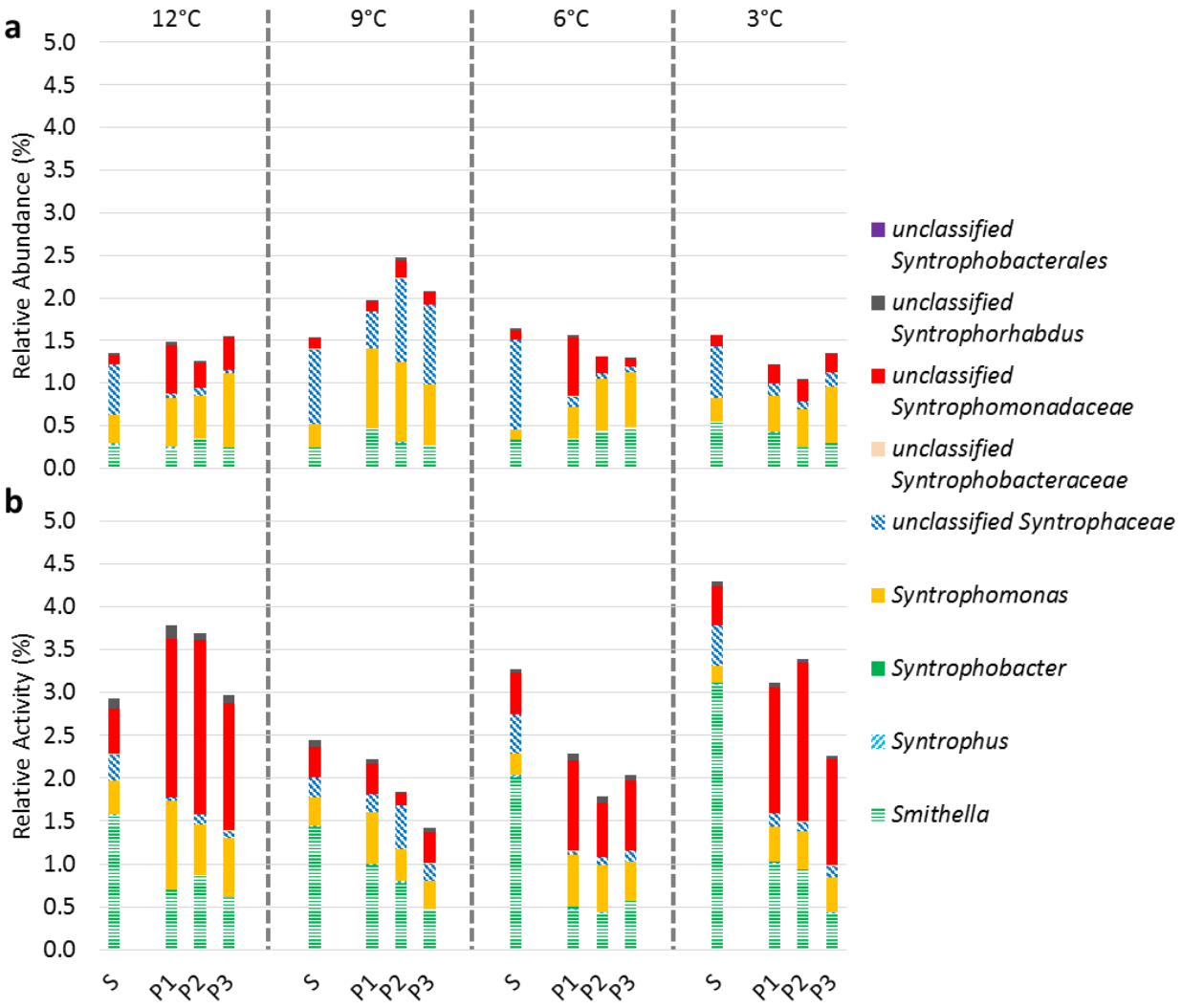

Figure 5-7. (a) Relative abundance based on 16S rDNA sequencing and (b) relative activity based on 16S rRNA sequencing of syntrophic VFA oxidizing bacteria to total community in suspended (S) and biofilm (P1, P2, and P3) biomass at operational temperatures of 12, 9, 6, and 3°C.

It is important to note that *Methansarcina,* a mixotrophic methanogen capable of metabolizing acetate, hydrogen, and other C1 compounds (Mladenovska and Ahring 1997), increased activity in suspended biomass as temperature decreased. The molecular methods employed here are not resolute enough to determine substrate utilization of *Methanosarcina*. We hypothesize that

Methanosarcina may have an advantage over other methanogens in low temperatures due to its metabolic flexibility. For example, *Methanosarcina* might have the capability to transition from aceticlastic to hydrogenotrophic methanogenesis as temperature decreases and the thermodynamics of hydrogenotrophic methanogenesis become more favorable (Lettinga et al. 2001). Typically, *Methanosarcina* is thought to outcompete *Methanosaeta* when acetate concentrations are high due to its higher growth rate but lower substrate affinity (Conklin et al. 2006). The high activity of *Methanosarcina* in suspended biomass in our system is unusual given acetate concentrations were always low which should theoretically favor *Methanosaeta*. Competition between *Methanosarcina* and *Methanosaeta* at such low temperatures has yet to be reported and therefore, it is difficult to conclude which selective pressures (e.g., temperature or substrate availability) led to the functional methanogenic community observed here.

5.4.5 Biofilm treatment may be a prerequisite for AnMBR domestic wastewater treatment at low psychrophilic temperatures

This study assessed the lower temperature limits for AnMBR treatment of domestic wastewater. COD removal > 95% was maintained at temperatures as low as 6°C. However, this high treatment performance would not have been possible without biological treatment in the biofilm. We hypothesize that biofilm biomass is more resilient than suspended biomass to decreases in operational temperature, possibly because of spatial organization of the microbial community enhancing syntrophy and resulting in a metabolic advantage. This hypothesis was supported by 16S rRNA sequencing in which hydrogenotrophic methanogenesis was the dominant methanogenic pathway in the biofilm biomass, but not suspended biomass. Future work could explore this possibility using fluorescence in situ hybridization (FISH) targeting syntrophic bacteria and hydrogenotrophic methanogens in the biofilm and suspended biomass to visually investigate spatial juxtapositioning. Alternatively, the biofilm could have a metabolic advantage

relative to suspended biomass due to reduced mass-transport limitations and increased substrate availability. Biofilm treatment, however, introduces concerns regarding long-term and potentially irreversible membrane fouling, a need for chemical cleaning in membrane installations, and high methane oversaturation. Low operational temperature is detrimental to the AnMBR energy balance without the development of low-energy dissolved methane recovery technologies. Future work should evaluate strategies to improve suspended biomass treatment at low temperature to decrease the reliance on the biofilm thereby decreasing dissolved methane oversaturation and the aforementioned concerns. We have demonstrated that AnMBR treatment at temperatures down to 6°C is feasible but future research is necessary to establish the practicality of doing so.

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Chapter 6. Navigating Wastewater Energy Recovery Strategies: A Life Cycle Comparison of Anaerobic Membrane Bioreactor and Conventional Treatment Systems with Anaerobic Digestion

6.1 Abstract

The objective of this study was to evaluate emerging anaerobic membrane bioreactor (AnMBR) technology in comparison with conventional wastewater energy recovery technologies. Wastewater treatment process modeling and systems analyses were combined to evaluate the conditions under which AnMBR may produce more net energy and have lower life cycle environmental emissions than high rate activated sludge with anaerobic digestion (HRAS+AD), conventional activated sludge with anaerobic digestion (CAS+AD), and aerobic membrane bioreactor with anaerobic digestion (AeMBR+AD). For medium strength domestic wastewater treatment under baseline assumptions at 15°C, AnMBR recovered 49% more energy as biogas than HRAS+AD, the most energy positive conventional technology considered, but had significantly higher energy demands and environmental emissions. Global warming impacts associated with AnMBR were largely due to emissions of effluent dissolved methane. For high strength domestic wastewater treatment, AnMBR recovered 15% more net energy than HRAS+AD and the environmental emissions gap between the two systems was reduced. Future developments of AnMBR technology in low energy fouling control, increased flux, and management of effluent methane emissions would make AnMBR competitive with HRAS+AD.

Smith, A.L., Stadler, L.B., Cao, L., Love, N.G., Raskin, L. and Skerlos, S.J., 2014c. Navigating Wastewater Energy Recovery Strategies: A Life Cycle Comparison of Anaerobic Membrane Bioreactor and Conventional Treatment Systems with Anaerobic Digestion. Environmental Science & Technology 48(10), 5972-5981.

Rapid advancements in AnMBR technology must continue to achieve its full economic and environmental potential as an energy recovery strategy for domestic wastewater.

6.2 Introduction

Anaerobic processes that recover energy in the form of biogas are gaining attention in the wastewater treatment industry. Anaerobic membrane bioreactor (AnMBR) systems are emerging as a promising technology for mainstream (as opposed to sidestream) treatment of domestic wastewater because they can generate a high-quality effluent during operation at reasonable hydraulic retention times (HRT < 8 hours) and low temperatures (~15°C), while producing a fraction of the sludge compared to aerobic treatment (Chu et al. 2005, Ho and Sung 2010, Smith et al. 2013, Wen et al. 1999). AnMBRs generate methane-rich biogas directly through anaerobic conversion of organics in domestic wastewater. This energy recovery must be balanced against system energy consumption to reduce net energy use. Although AnMBRs eliminate aeration and associated energy demands, substantial energy is currently needed to prevent membrane fouling. A previous energy balance (Martin et al. 2011) and cost analysis (Lin et al. 2011) on AnMBRs concluded that fouling control contributes significantly to overall energy demand and operational costs. Dissolved methane in AnMBR effluent represents another concern as both a lost energy source and a greenhouse gas emission if not recovered. Recently, research on AnMBRs has expanded substantially (Lin et al. 2013, Ozgun et al. 2013, Smith et al. 2012), including several pilot-scale demonstrations (Dagnew et al. 2011, El-Mashad and Zhang 2010, Gimenez et al. 2011, Martinez-Sosa et al. 2011b). To date however, AnMBR has not been evaluated from a systems perspective against more established technologies. A comprehensive comparison of AnMBR and established wastewater energy recovery technologies is needed to prioritize future research on AnMBRs.

A high rate activated sludge system followed by anaerobic digestion of produced sludge (HRAS+AD) is an established treatment strategy capable of energy recovery. HRAS research began in the 1940s (Wuhrmann 1954) and today many full-scale HRAS systems exist. Similarly, AD of sewage sludge has been in practice for over 100 years (Speece 2008). Interest in HRAS+AD has recently increased due to its compatibility with emerging low-energy nitrogen removal processes (Miller et al. 2012) (e.g., mainstream ammonia oxidation [nitritation] and anaerobic ammonia oxidation (Kartal et al. 2010)). In contrast to AnMBR, biogas in HRAS+AD is generated during AD of sludge rather than directly from wastewater. HRAS+AD is operated at a short HRT of 1.5 – 3 hours and solids retention time (SRT) of <2 days (Tchobanoglous et al. 2003), thereby maximizing sludge production while minimizing oxygen demand. Therefore, the total energy recovery possible through anaerobic digestion is maximized while aeration energy demands are minimized. However, limitations exist to HRAS treatment such as poor settleability of mixed liquor and low BOD_5 removal, particularly at SRTs less than 1.5 days, which can limit the use of HRAS for direct discharge (Bisogni and Lawrence 1971, Shao et al. 1992). These limitations become less of a concern when implemented in conjunction with downstream low-energy nutrient removal processes (e.g., A-stage of A/B processes).

This paper compares AnMBR and HRAS+AD technologies through the application of wastewater treatment process modeling, life cycle costing (LCC), net energy balance (NEB), and life cycle assessment (LCA) methods. Despite their different approaches to energy recovery, AnMBR and HRAS+AD generate effluents with similar five-day biochemical oxygen demand (BOD_5) and ammonia concentrations (no nitrification), thus providing reasonably equivalent functional units as needed for life cycle comparisons. The comparisons may be impacted by factors such as wastewater temperature, strength, and sludge disposal practice. For instance, lower wastewater

temperature affects the energy balance by increasing oxygen transfer efficiency (positive impact on NEB) and methane solubility (negative impact on NEB). Higher strength wastewater increases oxygen demand during aerobic treatment and energy recovery. The comparisons are also affected by sludge management practices since HRAS+AD and AnMBR vary in quantity of sludge produced.

In this work we address these factors via an extensive sensitivity analysis on uncertain parameters. Scenarios varying wastewater temperature, strength, and sludge disposal practice are discussed. Monte Carlo simulation was employed to address uncertainty and sensitivity to model parameters (Table S8 of the Supplementary Information (SI) in Appendix D) that would affect the comparison of HRAS+AD and AnMBR from cost, energy, and environmental impact perspectives. To project the development of AnMBR technology as it evolves to full-scale, a set of uncertainty parameters was created considering best-case efficiency gains that could be achieved in the next decade. We start with a comparison of AnMBR performing as reported in the literature (pilot and lab-scale systems) and then compare AnMBR as it may exist after future development (full-scale implementation) through the uncertainty analysis.

6.3 Methods

6.3.1 System Boundary and Functional Unit

The ISO 14040 framework (Finkbeiner et al. 2006) was used to compare the environmental attributes of AnMBR and HRAS+AD. As additional points of reference, aerobic membrane bioreactor with anaerobic digestion (AeMBR+AD) and conventional activated sludge with anaerobic digestion (CAS+AD) were included in the study. CAS+AD and AeMBR+AD employ similar solids separation processes to HRAS+AD and AnMBR, respectively, but are not specifically designed to enhance energy recovery. Thus, these additional comparisons illustrate the

effect of converting to systems designed for enhanced energy recovery. The comparison of AnMBR and AeMBR+AD is also relevant for water reuse schemes in which a solids-free effluent is desirable. Process flow diagrams are provided in Figures S6-2 - S6-5 of SI. In total, 16 scenarios for each treatment system were evaluated: two domestic wastewater strengths (430 mg/L and 800 mg/L chemical oxygen demand (COD)) (Tchobanoglous et al. 2003), two wastewater temperatures (15 and 25°C), and four sludge disposal practices (landfilling, land application, incineration, and an aggregate that represents average U.S. sludge disposal practices). The baseline scenario was defined as the treatment of medium strength domestic wastewater at 15°C using the aggregate sludge disposal practice (13% landfilling, 62% land application, 25% incineration (U.S. Environmental Protection Agency 1999)), and the operational parameter values specified in Table 6-1. It was verified that construction phase environmental impacts are negligible relative to life cycle impacts as reported by Renou et al. (2008), and thus only the use-phase impacts were included. The unit processes included in the system boundary are summarized in Figure 6-1, with details provided in Section 3 of the SI.

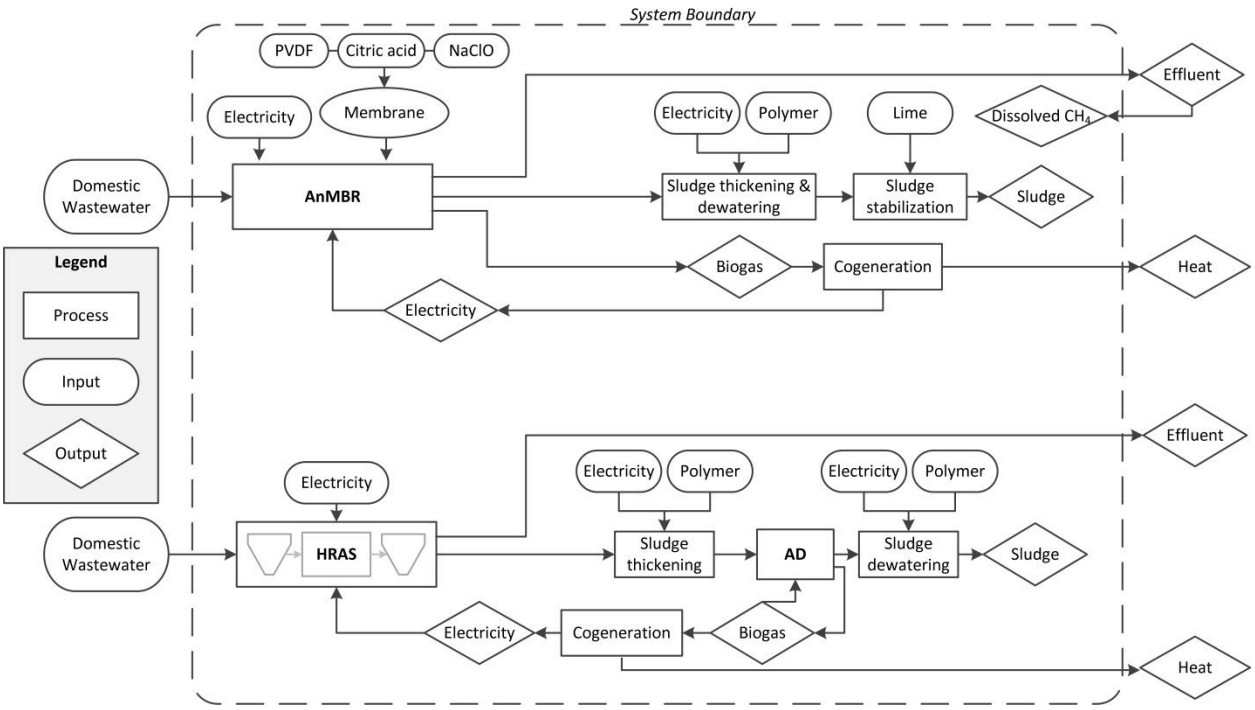

Figure 6-1. System Boundary of AnMBR and HRAS+AD. CAS+AD and AeMBR+AD system boundaries are presented in Figure S1.

The functional unit was defined as the treatment of 5 million gallons per day (MGD) (18,950 m^3d^{-1}) of domestic wastewater to achieve at a minimum U.S. EPA secondary treatment effluent standards (<30 mg L^{-1} BOD_5 and 30 mg L^{-1} total suspended solids (TSS) (U.S. Environmental Protection Agency 1988)). Energy recovery was assumed to occur via on-site biogas combustion using combined heat and power (CHP), or cogeneration. Treatment capacity was selected based on the U.S. EPA's CHP Partnership 2007 report, which suggested that influent flow rates greater than 5 MGD are required to produce biogas via anaerobic digestion in quantities sufficient for economically feasible CHP systems (Naik-Dhungel 2010).

6.3.2 System Design and Modeling for Life Cycle Inventory

HRAS+AD, CAS+AD, and AeMBR+AD were modeled using GPS-X (Hydromantis, Inc.) as discussed in the SI. AnMBR was modeled based on the performance of several existing pilot and lab-scale systems (Baek et al. 2010, Chu et al. 2005, Ho and Sung 2009, Hu and Stuckey 2006,

Huang et al. 2011, Martin et al. 2011) and using steady-state equations described in SI Equations 1-3. This approach was used because no verified AnMBR performance model is yet available and existing anaerobic digestion models (e.g., ADM1 (Batstone et al. 2002)) do not accurately simulate low temperature, low strength AnMBR treatment. The baseline scenario for this analysis uses the model process parameters provided in Table 6-1. Although each system achieves the effluent standards defined by the functional unit, differences do exist, especially in effluent suspended solids concentration and nitrogen speciation (Table S6-6).

Table 6-1. Model Process Parameters for Baseline Scenario (15°C medium strength wastewater).

System	SRT	HRT	Recycle Ratio	Flux	SGD
	(d)	(h)		(L m^{-2} h^{-1}; LMH)	(m^3 m^{-2} h^{-1})
CAS	10	8	--	--	--
HRAS	1.5	2	--	--	--
AeMBR	10	8	2Q	20	0.082
AnMBR	200	8	2Q	10	0.23

SRT=solids retention time; HRT=hydraulic retention time; Q=influent flow rate; SGD=specific gas demand.

Table 6-2. Uncertainty parameter values for current versus future AnMBR.

	Baseline	Current		Future	
Parameter*	Value	Worst Case	Best Case	Worst Case	Best Case
Flux (LMH)	10	7	17	10	30
Sparging (SGD; m^3 m^{-2} h^{-1}/frequency; % on)	0.23/100	0.50/100	0.10/100	0.23/100	0.082/25
Dissolved methane recovered (%)	0	0	0	0	100

*Only parameters differing between the current and future AnMBR uncertainty are shown. All uncertainty parameters are shown in Table S6-8.

The membrane properties used for AnMBR and AeMBR were based on hollow fiber membranes. Manufacturing information was used to determine materials and weights of the membrane components and chemical cleaning requirements (specifications provided in Table S6-3). Permeate pumping energy was calculated assuming a transmembrane pressure of 10 kPa. Membrane lifetimes were assumed to be 10 years. The specific gas demand (SGD), which is the gas flow required for membrane sparging to prevent fouling, was assumed to be 0.23 $m^3 m^{-2} h^{-1}$ for AnMBR based on pilot-scale evaluation of membrane fouling at different SGDs (Robles et al. 2012). Reported pilot-scale AnMBR SGDs vary widely (0.10 – 1.2 $m^3 m^{-2} h^{-1}$) and have not yet been optimized for energy. An SGD of 0.23 $m^3 m^{-2} h^{-1}$ was selected from the only long-term study that compared multiple SGDs at reasonably high fluxes (10 LMH) (Robles et al. 2012). For AeMBR an SGD of 0.082 $m^3 m^{-2} h^{-1}$ was selected based on manufacturer full-scale recommendations for hollow fiber membranes (Hong 2012).

Wastewater strength is known to impact mixed liquor characteristics and concentration, however its influence on membrane fouling is poorly understood (Lousada-Ferreira et al. 2010), particularly for AnMBR. Therefore it was assumed that membrane fouling control requirements were independent of wastewater strength. Membrane fluxes of 10 and 20 LMH were assumed for AnMBR and AeMBR, respectively, based on pilot-scale AnMBR (Gimenez et al. 2011, Martinez-Sosa et al. 2011a, Robles et al. 2012) and full-scale AeMBR data (Judd 2010). Much effort is being directed to understanding MBR fouling and optimizing fouling control strategies (e.g. reducing SGD, use of intermittent sparging and large bubble scour). These efforts were incorporated into this work by performing uncertainty and sensitivity analyses to evaluate the impact of SGD, intermittent sparging, and flux on energy and environmental impacts using the values and ranges

reported in Table 6-2. For the current AnMBR uncertainty parameter ranges, SGD and flux values were assumed based on reported operation of pilot-scale AnMBRs (Dagnew et al. 2011, Gimenez et al. 2011, Martinez-Sosa et al. 2011b, Pretel et al. 2013, Robles et al. 2012). Future AnMBR uncertainty parameter ranges assumed SGD, sparging intervals, and flux will approach that of current AeMBR operation in the best case, while worst case values were the baseline current AnMBR values (Table 6-2).

AnMBR methane production was calculated assuming 350 L of methane was produced per kg COD removed at standard temperature and pressure and was adjusted for the different wastewater temperatures (Grady et al. 2011). Sulfate reduction, which reduces the COD available for conversion to methane (Gimenez et al. 2011, Smith et al. 2013), was taken into account when calculating methane generation. Several studies using anaerobic bioreactors for domestic wastewater treatment with and without membrane separation have reported methane oversaturation in the effluent (Hartley and Lant 2006, Kim et al. 2011, Singh et al. 1996, Smith et al. 2013). The dissolved methane concentration in AnMBR effluent was calculated using Henry's Law and assumed to be 1.5 times oversaturation, which represents the average oversaturation reported to date (Bandara et al. 2012, Kim et al. 2011, Smith et al. 2013). It was further assumed that dissolved methane in AnMBR effluent would eventually be released to the atmosphere. We considered the future possibility that complete dissolved methane recovery and its subsequent use for energy generation becomes technically and economically feasible (Table 6-2).

Waste activated sludge was assumed to be thickened using a gravity belt-filter press with polymer addition for all systems. Primary and waste activated sludge were blended prior to anaerobic digestion for HRAS and CAS. Blended sludge (HRAS and CAS) and waste activated sludge (AeMBR) were anaerobically digested at a 20 day SRT and 35°C to achieve Class B biosolids

(Tchobanoglous et al. 2003, U.S. Environmental Protection Agency 1993). The dissolved methane concentration in AD effluent was calculated assuming five times oversaturation (Pauss et al. 1990) relative to Henry's Law and considered a direct emission. Sludge was dewatered by centrifugation with polymer addition for all systems. Since AnMBR sludge would not benefit from stabilization using anaerobic digestion, dewatered AnMBR sludge was lime stabilized to achieve Class B biosolids (U.S. Environmental Protection Agency 1993) for landfilling and land application, whereas for incineration AnMBR sludge was only dewatered.

NEB was calculated as the sum of all electrical energy demands for treatment minus electricity generated via CHP. Electricity requirements for pumps, blowers, and mixing were estimated using SI Equations 4 and 5. The electricity requirements for gravity belt thickening and centrifuge dewatering were estimated using equations adapted from CAPDETWorks (Guest 2012). Heat generated by CHP was used to heat the digesters to 35°C and any excess heat was considered waste. The electricity generated was used to offset average U.S. grid electricity. Non-electrical energy demands such as those incurred outside of the treatment plant for sludge transportation and disposal (e.g., diesel) were included as environmental impacts but not included in NEB. Emissions data for U.S. grid electricity and other unit processes within the system boundary were collected from U.S. LCI (Norris 2004), Ecoinvent (Frischknecht et al. 2005), and ELCD (European Commission Joint Research Centre 2010) databases.

6.3.3 Impact Assessment

Environmental impacts were characterized using Tools for the Reduction and Assessment of Chemical and Other Environmental Impacts (TRACI) developed by the U.S. EPA (Bare et al. 2002). Except for gaseous emissions of dissolved methane, all impacts to the environment caused by the effluent were excluded from the analysis. Direct emissions of methane from AnMBR and

AD effluents were evaluated assuming a global warming potential 25 times that of an equivalent mass of carbon dioxide (IPCC 2007). Biogenic carbon dioxide emissions during treatment were not included in the impact assessment as they are not considered to contribute to net greenhouse gas effects (Monteith et al. 2005).

6.3.4 Uncertainty and Sensitivity Analysis

The aggregate impacts of data uncertainty were evaluated by Monte Carlo analysis (50,000 simulations). Table S6-8 lists the 14 uncertainty parameters, which represent variations in efficiency, technology, membrane performance, and sludge transport distance. The majority of the uncertainty parameters were associated with AnMBR due to the emerging status of the technology relative to HRAS+AD. Two sets of uncertainty parameter ranges, representing current and future development, were evaluated for AnMBR as shown in Table 6-2. Uncertainty parameter values for HRAS+AD, CAS+AD, AeMBR+AD, and current AnMBR were assigned using either literature values or conservatively estimated values when literature data were not available. The set of parameters for future AnMBR development were assigned assuming it could achieve operating conditions comparable to full-scale AeMBRs today. Uncertainty parameter distributions were assumed as triangular when data were available to suggest a likely midpoint value. Uniform distributions were used in the absence of midpoint estimates. A sensitivity analysis was performed to determine the sensitivity of net energy demand and emissions categories to each uncertainty parameter. A category was defined as "sensitive" to an uncertainty parameter if the resulting correlation coefficient had an absolute value greater than 0.6.

6.3.5 Life Cycle Costing

LCC was performed for AnMBR, HRAS+AD, CAS+AD, and AeMBR+AD assuming a treatment plant life of 40 years. CAPDETWorks (Hydromantis, Inc.) was used to estimate the capital and

operational costs of each system (Tables S6-11 – S6-16). Supplemental costs added to CAPDETWorks estimations included membrane-related costs (materials and chemicals), AnMBR cover and gas handling equipment, biogas CHP units, and quicklime for AnMBR sludge stabilization. LCC was applied and net present value determined at three discount rates: 5%, 8%, and 10%.

6.4 Results and Discussion

6.4.1 Life Cycle Costs of Energy Recovery Systems are Lower than Conventional Systems

It was first observed that the sludge disposal practice was a key determinant as to whether AnMBR or HRAS+AD had a lower life cycle cost (in terms of net present value). Although AnMBR capital costs were greater than HRAS+AD, HRAS+AD produced more sludge and the higher sludge disposal cost of HRAS+AD off-set the higher capital cost of AnMBR when sludge was landfilled (Figure 6-2). HRAS+AD had the lowest life cycle cost when sludge was land applied or incinerated.

Both HRAS+AD and AnMBR had lower life cycle costs than their reference systems (CAS+AD and AeMBR+AD, respectively) for all sludge disposal practices. Despite CAS+AD having comparable capital costs to HRAS+AD, CAS+AD had higher life cycle costs due to electricity costs associated with aeration. AeMBR+AD had the highest life cycle cost for all sludge disposal practices primarily because of higher capital costs due to the combination of the membrane system and anaerobic digester, as well as higher electricity costs for aeration. In situations where membrane separation and energy recovery are both required, AnMBR is more economical than AeMBR+AD for all sludge disposal practices.

As AnMBR technology improves, its capital and operational costs are likely to decrease. For instance, increasing flux from 10 to 20 LMH reduces membrane capital costs by 46%. If one assumes that chemical and energy use per unit membrane area remain constant, then operational costs for membrane cleaning and fouling control also decrease with increasing flux. In this case, doubling the flux reduces life cycle AnMBR costs by 12-13%, resulting in a lower life cycle cost as compared with HRAS+AD when solids are landfilled or incinerated and comparable when solids are land applied.

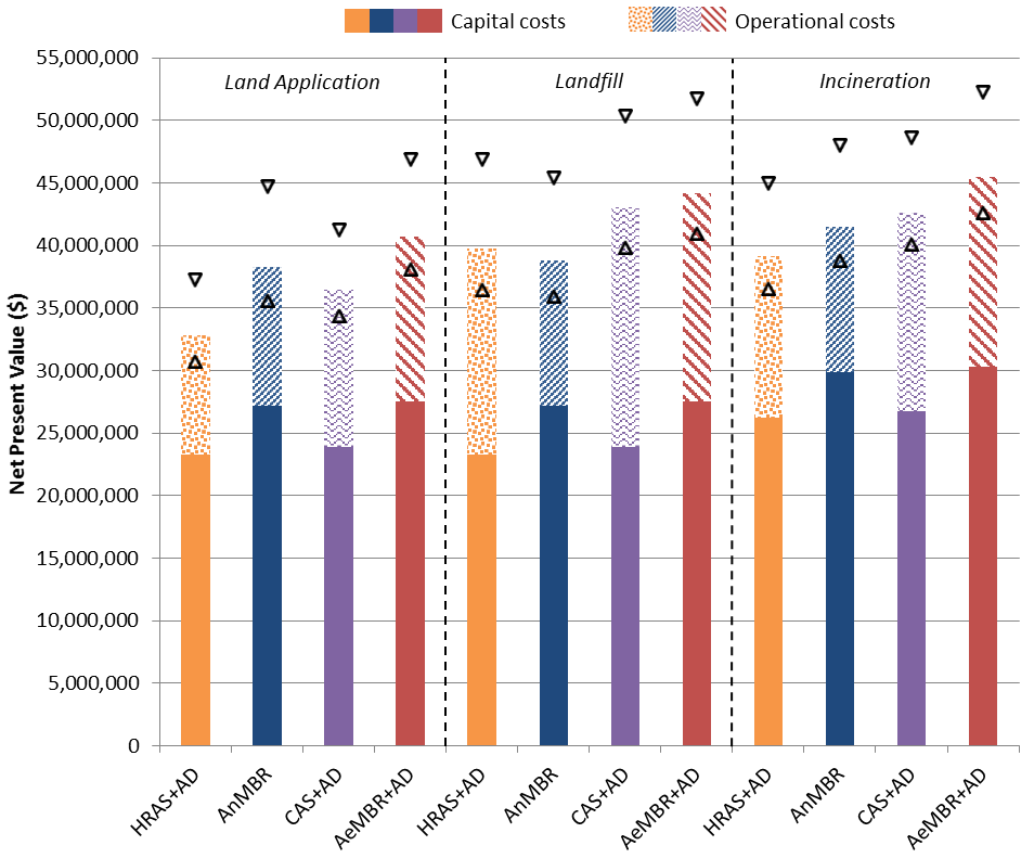

Figure 6-2. Net present value of HRAS+AD, AnMBR, CAS+AD, and AeMBR+AD at 15°C for each sludge disposal practice. Bars represent a discount rate of 8%. Triangles (▽,△) represent a discount rate of 5% and 10%.

6.4.2 Future Developments are needed for AnMBR to be Energy Competitive with HRAS+AD

HRAS+AD was the only system to achieve a positive NEB, meaning it created more energy than it consumed, in the baseline scenario. Despite AnMBR recovering more energy than HRAS+AD, AnMBR consumed almost 4 times as much energy (Figure 6-3). AnMBR energy demand was primarily attributable to gas recirculation (i.e., sparging) energy for fouling control which represented 86% of the energy demand. This energy consumption was 5 times greater than the blower energy demand for HRAS+AD aeration. Energy consumption by AnMBR was highly sensitive to SGD and flux, as described later. Energy demands for sludge thickening and dewatering were three times greater for HRAS+AD, accounting for 22% of HRAS+AD energy demand, whereas they accounted for <2% of AnMBR energy demand.

A higher wastewater temperature of 25°C decreased the NEB of HRAS+AD, CAS+AD, and AeMBR+AD due to increased aeration energy demands arising from lower oxygen transfer efficiencies. However, the higher temperature lowered AD heating requirements. This is because waste heat from CHP was sufficient for heating the digesters at 25°C, whereas biogas was required for heating at a wastewater temperature of 15°C that would otherwise have been used to generate electricity. Taken together, the positive NEB of HRAS+AD decreased by 13% at 25°C. The negative NEB of AnMBR reduced by 21%, primarily due to lower methane solubility leading to increased collection of biogas (Figure S6-6).

Systems designed for enhanced energy recovery achieved better NEBs than their respective reference systems (HRAS+AD > CAS+AD and AnMBR > AeMBR+AD). Unlike HRAS+AD, CAS+AD did not achieve a positive NEB, mostly because it required more energy for aeration given its SRT of 10 days versus 1.5 days for HRAS. CAS+AD also recovered less energy because

a significant fraction of the energy contained in the soluble and particulate fractions of wastewater, as well as the energy contained in the biomass formed during treatment, were oxidized at the longer SRT rather than converted to biogas in AD. AeMBR+AD NEB was lower than AnMBR because AeMBR+AD recovered significantly less energy and required energy for aeration in addition to sparging energy. Among all treatment systems, AeMBR+AD had the worst NEB.

The uncertainty analysis for AnMBR resulted in a broad 95% confidence interval for the NEB (Figure 6-3) due to the potential for future AnMBR development as it progresses to full-scale (Table 6-2). For the treatment of medium strength wastewater, there is significant potential for AnMBR to have a more positive NEB than HRAS+AD if the energy required for sparging is reduced (i.e., lower SGD and/or biogas sparging frequency) or flux is increased. For example, reducing SGD from 0.23 m^3 m^{-2} hr^{-1} to that of AeMBR (0.082 N m^3 m^{-2} hr^{-1}) would result in comparable NEBs for AnMBR and HRAS +AD. Alternatively, flux could be increased from 10 to 28 LMH to attain comparable NEBs. Achieving modest improvements in SGD, sparging frequency, and flux simultaneously is a more realistic approach than focusing on any one parameter alone. For example, if SGD were to remain the same but sparging frequency was reduced from 100% to 50% "on" and flux increased from 10 LMH to 15 LMH, AnMBR would yield a more positive NEB than HRAS+AD.

Improvements in AnMBR operational parameters are likely as it evolves to full-scale operation. Pilot AnMBRs have already been successfully operated at fluxes of 15 LMH and greater (Dagnew et al. 2011, Robles et al. 2012), although fluxes in the range of 8-10 LMH are more common (Smith et al. 2012). There are also full-scale AeMBRs that operate using cyclic sparging (on for 10 seconds and off for 30 seconds). Improvements in AeMBR sparging, such as intermittent and large-bubble sparging, are still evolving and have yet to be explored in AnMBR. Granular

activated carbon (GAC) in a fluidized bed has been proposed as an alternative strategy to membrane fouling control that may significantly reduce energy demand by eliminating the need for membrane sparging entirely (Kim et al. 2011). However, this strategy must also be evaluated for its impact on membrane lifetime and additional energy demands for GAC fluidization. Other novel approaches to membrane fouling control, such as rotating ceramic disc membranes (Jaffrin 2008), or emerging membrane materials such as electrospun nanofibers (Bjorge et al. 2009), have yet to be evaluated in AnMBR. Recovery of effluent dissolved methane could also improve the AnMBR NEB significantly as discussed below. As AnMBR technology matures, research will shift from focusing on performance feasibility to minimizing energy demands and maximizing energy recovery, making it more competitive relative to HRAS+AD for energy recovery from medium strength wastewater.

For high strength domestic wastewater, AnMBR achieved a more positive NEB than HRAS+AD. HRAS+AD energy demand was dependent on wastewater strength, increasing 80% relative to medium strength wastewater due to increased aeration requirements. AnMBR energy consumption increased less than 1% for high strength versus medium strength wastewater because membrane sparging, which made up the majority of AnMBR energy demand, was assumed to be independent of wastewater strength. In addition, energy recovery increased by 130% for AnMBR but only by 89% for HRAS+AD. AnMBR energy recovery was more sensitive to wastewater strength because AnMBR converts a greater fraction of organics in wastewater to methane. Further, dissolved methane in AnMBR effluent was not assumed to increase at higher wastewater strength because AnMBR mixed liquor was methane-saturated and changes in strength likely do not change methane solubility. Therefore, methane losses in the effluent were not assumed to increase and all methane generated from the additional organics in higher strength wastewater was recoverable

biogas. Even without future development, AnMBR has a more positive NEB than HRAS+AD for high strength domestic wastewater and the margins will only increase with further AnMBR developments to reduce energy consumption.

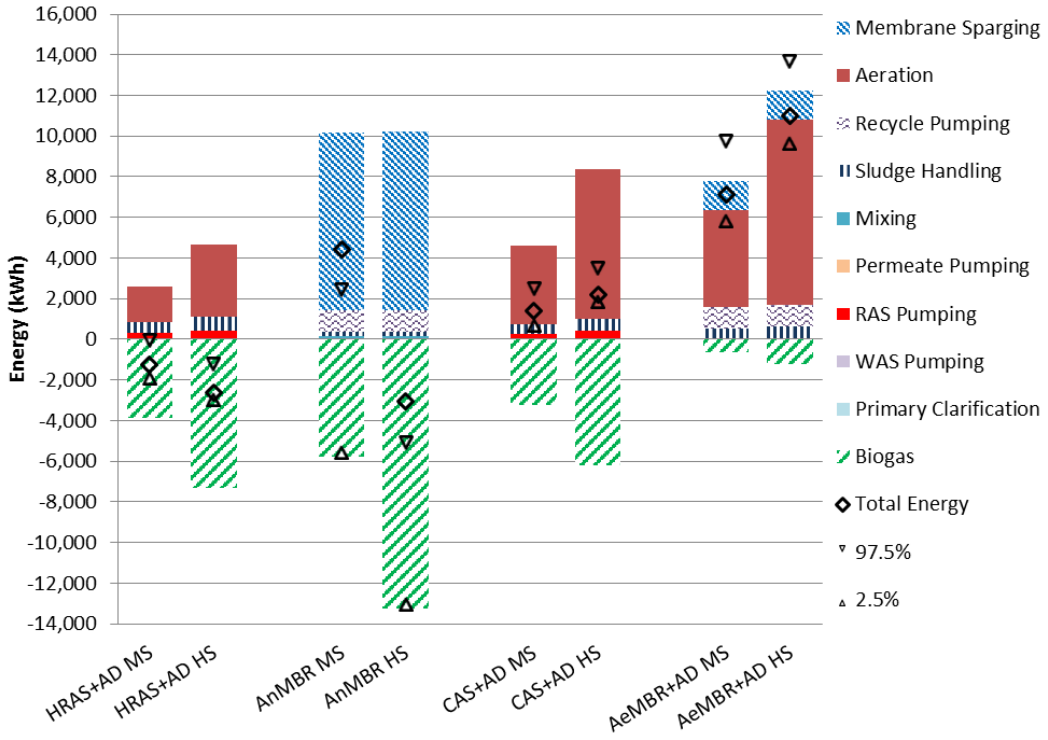

Figure 6-3. Net energy balance (NEB) for HRAS+AD, AnMBR, CAS+AD, and AeMBR+AD for medium strength (MS) and high strength (HS) wastewater treatment at 15°C. Triangles (∇,Δ) represent the 95% confidence interval of net energy demand from the uncertainty analysis. For AnMBR, triangles represent uncertainty based on the future parameter values.

6.4.3 Environmental Impacts of AnMBR Require Attention

AnMBR featured the highest global warming (GW) impact compared with all other systems for medium strength wastewater (Figure 6-4). Seventy-five percent of this impact was from effluent dissolved methane. The magnitude of these emissions is primarily due to mainstream anaerobic treatment (i.e., the volume of effluent generated containing dissolved methane) rather than a function of operational temperature. GW impact decreased only 16% when the temperature was increased from 15 to 25°C (data not shown). Most of the remaining AnMBR GW impact was from

electricity use (19%) which will decrease significantly if AnMBR becomes operationally closer to AeMBR. For HRAS+AD, CAS+AD, and AeMBR+AD, nearly all GW impact was caused by electricity use which resulted in a linear relationship between GW and NEB (Figure 6-4). Effluent AnMBR methane emissions must be avoided for AnMBR to approach the limiting linear relationship between GW and net energy demand seen in Figure 6-4.

Effluent dissolved methane handling has emerged as a key concern for mainstream anaerobic treatment processes. One approach is to recover the dissolved fraction for additional energy generation. However, no energetically and economically feasible approach for methane recovery has been demonstrated to date. Membrane degasification of dissolved methane from anaerobic effluents has been demonstrated (Bandara et al. 2011, Cookney et al. 2012). In one study the recovered gas had a low methane content (approximately 20%), which was not suitable for cogeneration and the process was also energy intensive, requiring two orders of magnitude more energy than could be theoretically recovered (Bandara et al. 2011) Another study estimated a substantially lower energy requirement but did not experimentally verify their assumptions (Cookney et al. 2012). A simpler approach may involve stripping dissolved methane during re-aeration, which would be required prior to discharge for an anaerobic effluent, using a covered process to capture the off-gas. Relatively minor aeration energy would be required as the effluent contains minimal biological activity. With any non-selective dissolved gas recovery system, carbon dioxide, which is significantly more soluble than methane, may also be stripped and dilute recovered methane. The stripped biogas could theoretically be blended with the bioreactor biogas to generate a biogas suitable for cogeneration. This gap in current understanding suggests that research efforts should focus on demonstrating low-energy methane recovery strategies and quantifying their impact on the AnMBR NEB.

Until efficient recovery technologies are developed, other options may be more energetically favorable for mitigating GW impact. In principle, a downstream process could biologically oxidize dissolved methane while achieving nitrification and/or nitrogen removal. For example, nitritation to convert ammonia to nitrite, coupled with anaerobic oxidation of methane using nitrite as the electron acceptor (Waki et al. 2009) could be feasible for downstream AnMBR treatment. Dissolved methane could reduce or eliminate the need for an exogenous electron donor and carbon source otherwise required in denitrification. Nitritation/anammox could also be applied downstream of AnMBR or HRAS+AD, as both produce effluents with low carbon-to-nitrogen (C:N) ratio. Nitritation followed by anammox consumes less energy than conventional nitrification-denitrification and does not require an additional electron donor and carbon source. However, the majority of dissolved methane would likely be oxidized by aerobic methanotrophs rather than stripped during nitritation (Daelman et al. 2012) and could exert a significant oxygen demand.

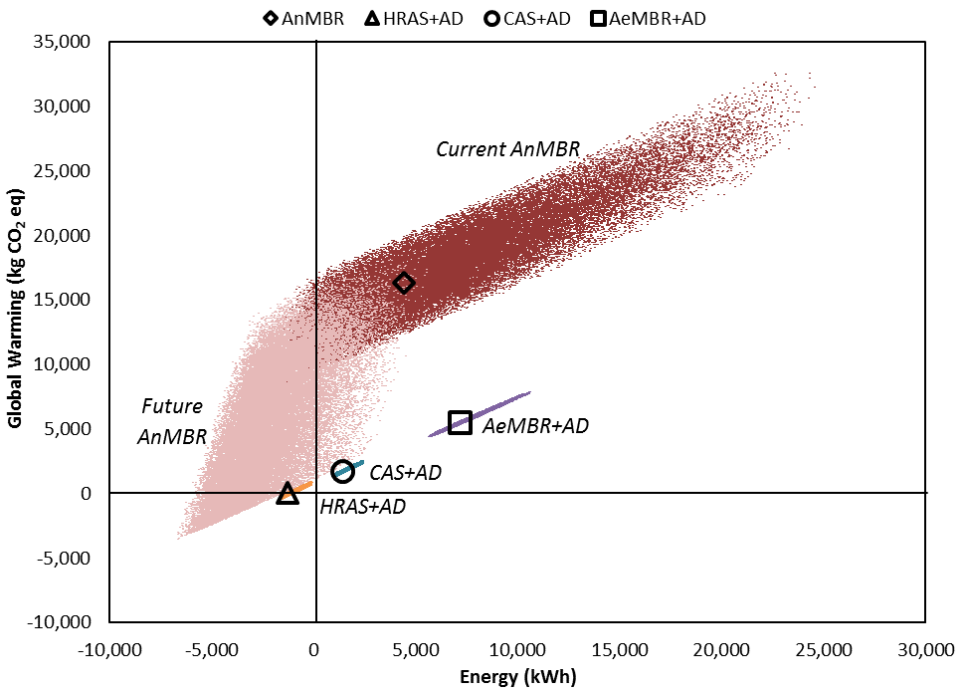

Figure 6-4. Global warming versus net energy balance (NEB) for HRAS+AD, AnMBR, CAS+AD, and AeMBR+AD for medium strength wastewater at 15°C. Open markers represent the baseline conditions. Colored dots indicate the values outputted by Monte Carlo simulations for HRAS+AD, AnMBR (current and future), CAS+AD, and AeMBR+AD.

In addition to GW impact, AnMBR had greater environmental impacts in all other categories relative to HRAS+AD (Figure 6-5). This was primarily because of greater electricity use associated with high sparging requirements (Tables S6-8 – S6-11). As with energy, environmental impacts were highly sensitive to SGD and flux which are expected to improve as the technology matures. Other significant environmental impacts arose due to membrane cleaning (50% of eutrophication impacts), membrane manufacturing (24% of carcinogenics and 23% of ecotoxicity impacts), and cogeneration. Negative eutrophication impacts for HRAS+AD and CAS+AD were derived from fertilizer offset, which was a function of the quantity of sludge that was land applied. With respect to eutrophication potential, AnMBR was disadvantaged by generating less sludge than HRAS+AD. Assuming future development in-line with AeMBR efficiency gains and effluent

methane management, AnMBR can have comparable environmental impacts in all TRACI categories except eutrophication relative to HRAS+AD for medium strength wastewater.

When treating high strength domestic wastewater, AnMBR achieved lower environmental impacts than CAS+AD in five of the nine TRACI impact categories (acidification, non-carcinogens, respiratory effects, ecotoxicity, and smog) and exhibited comparable impacts to HRAS+AD (Figure 6-5). If effluent dissolved methane can be managed along with more energy-efficient fouling control, AnMBR becomes an even more attractive option for higher strength domestic wastewater.

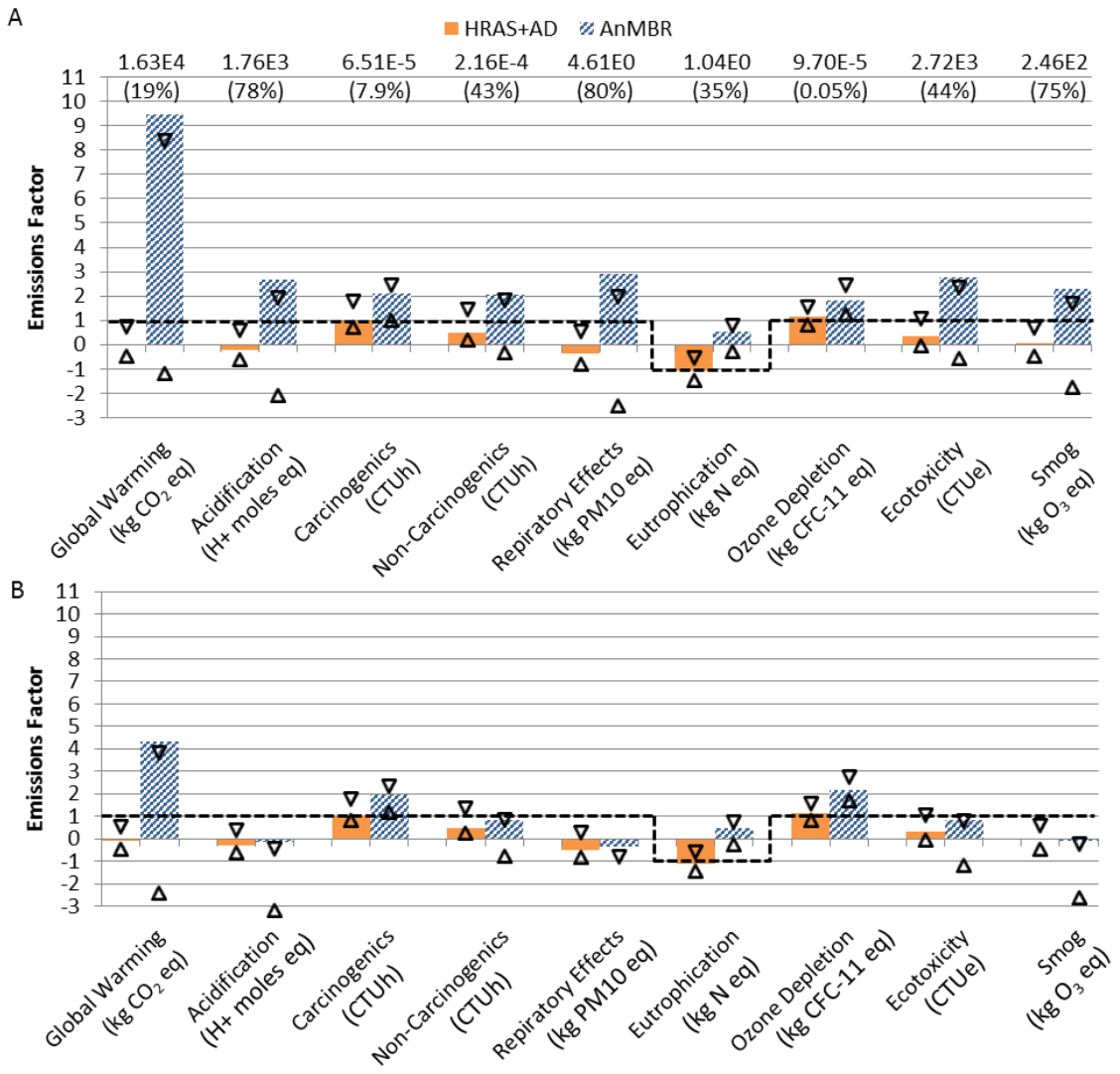

Figure 6-5. Environmental impacts compared by TRACI impact category for HRAS+AD and AnMBR at 15 °C and aggregate sludge disposal practice for (A) medium strength domestic wastewater and (B) high strength domestic wastewater. HRAS+AD and AnMBR impacts are normalized to CAS+AD impacts (emissions factor) as it represents a conventional technology for comparison. The dashed line represents CAS+AD emissions. The emissions factor for CAS+AD is 1 in all categories except eutrophication, which is -1, based on negative impacts from artificial fertilizer offset. Triangles (▽,△) represent the 95% confidence interval from the uncertainty analysis. Numbers above AnMBR bars in (A) indicate absolute value of that impact. Percentages in parentheses above AnMBR bars in (A) indicate percentage of that impact from grid electricity.

6.4.4 AnMBR could have Benefits beyond Energy Recovery

Membrane solids separation as opposed to gravity separation (as used in HRAS) has distinct benefits in certain applications. For example, AnMBR might be advantageous in potable reuse applications as it provides an effluent low in suspended solids and colloidal material compatible with downstream reverse osmosis treatment (Tam et al. 2007). AnMBR effluent could also be used in non-potable reuse applications such as agricultural irrigation given its effluent is rich in nutrients. Further, membrane separation may have additional benefits beyond producing a low-solids effluent, such as mitigating the release of antibiotic resistant bacteria and antibiotic resistance genes in receiving waters (Riquelme Breazeal et al. 2012). On the other hand, if downstream treatment with a suspended growth process is needed for nutrient and methane removal these potential advantages could be lost.

Decentralization of wastewater treatment is one strategy for addressing urban development without massive infrastructure overhaul. AnMBR is worth considering in this context given the higher strength of domestic wastewater in decentralized systems (Libralato 2013). These advantages must be weighed against implementation of small-scale or microturbine CHP systems which may be less cost effective and less efficient relative to larger CHP systems implemented in centralized wastewater treatment plants.

6.4.5 AnMBR must be Developed Further to Achieve Cost and Environmental Benefits

AnMBR will be competitive with HRAS+AD for medium strength domestic wastewater and the clear choice for energy recovery from higher strength domestic wastewater as reductions in AnMBR energy demands are realized along with strategies to address GW concerns. This will require improvements in SGD, use of intermittent sparging, increased flux, and dissolved methane management. Future AnMBR research should prioritize these areas while broadening the system

boundary to consider downstream treatment and alternative wastewater reuse applications ranging from irrigation to drinking water production.

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Chapter 7. Conclusions and Engineering Significance

7.1 Overview

The primary objective of this dissertation was to expand and advance available tools to recover resources from wastewater. The dissertation specifically focused on using anaerobic biotechnology for direct energy recovery from domestic wastewater, a waste stream that has long been considered incompatible with the goal of direct energy recovery. This work began with a preliminary investigation of anaerobic membrane bioreactor (AnMBR) treatment of simulated domestic wastewater and actual domestic wastewater at 15°C (Chapter 3; (Smith et al. 2013)). This work suggested that the membrane foulant layer or biofilm may play a role in treatment and motivated an in-depth investigation of the biofilm's ability to improve effluent quality (Chapter 4; (Smith et al. 2014b)). We then applied this approach to gauge the lower temperature limits of AnMBR domestic wastewater treatment (Chapter 5; (Smith et al. 2014a)). Finally, an assessment framework was developed to assess the life cycle environmental and economic impacts of AnMBR systems compared to conventional wastewater treatment systems with a focus on highlighting operational and design targets AnMBR needs to achieve for the technology to move to full-scale (Chapter 6; (Smith et al. 2014c)).

7.2 The membrane biofilm can significantly improve AnMBR treatment

The primary objective of AnMBR is to treat wastewater to protect the aquatic environment while recovering energy. This requires that AnMBR consistently produces a high quality effluent under stresses such as variable wastewater strength and composition, and seasonal and daily temperature fluctuations. One approach to improve treatment and increase operational resilience is to provide

an additional barrier of treatment such as a membrane biofilm. We originally observed an indication that fouled membranes improve effluent quality (Chapter 3; (Smith et al. 2013)). However, improvements in effluent quality were minor, likely due to the limited biodegradable substrates (e.g., acetate and propionate) available during this operational period. We hypothesized that the biofilm could significantly improve treatment under conditions in which biodegradable substrates are more available such as when suspended biomass treatment is inadequate due to low temperature, a sharp increase in organic loading rate (OLR), or other factors.

Improving AnMBR treatment through biofilm development was explored by operating a bench-scale AnMBR with independent membrane housings under different levels of fouling as determined by transmembrane pressure (TMP) (Chapter 4; (Smith et al. 2014b)). During this operational period, the AnMBR was running under conditions that led to relatively high availability of acetate and propionate to the biofilm due to insufficient treatment by suspended biomass. Membranes with the highest level of fouling almost completely removed acetate and propionate and significantly decreased permeate chemical oxygen demand (COD) relative to membranes operated under low fouling conditions. However, COD removal in the biofilm corresponded to substantial dissolved methane oversaturation in the permeate, suggesting a downside to this approach to improve effluent quality.

16S rRNA sequencing indicated that controlled membrane fouling led to development of a biologically active membrane biofilm enriched in highly active aceticlastic and hydrogenotrophic methanogens and syntrophic bacteria. The increase in methanogenic activity was confirmed using reverse transcription quantitative PCR (RT-qPCR) targeting the methyl coenzyme-M reductase (*mcrA*) gene. DNA-based molecular analyses (16S rDNA sequencing) were insufficient to describe microbial community activity and functional significance in this study. This work enabled

us to recommend a combinations of RNA and DNA-based molecular analyses to study AnMBR and other systems in which microbial growth is limited due to low temperatures, low OLR, or other factors.

Membranes operated under the highest level of membrane fouling were returned to a near zero TMP to evaluate if biological activity in the biofilm could be maintained in the absence of TMP. Low effluent COD was maintained in further operation indicating a negligible impact on biofilm treatment. This suggests that the active biofilm is tightly adhered to the membrane surface and potentially distinct from the layer of foulants contributing to high TMP. Dissolved methane oversaturation persisted suggesting that oversaturation is primarily driven by methanogenesis in the biofilm and not by high TMP.

7.3 AnMBR can produce a high quality effluent at temperatures as low as 6°C

Increasing the potential adoption of AnMBR technology requires demonstration of treatment at low temperatures, which occur during winter in most temperate climates. We explored the lower temperature limits of AnMBR treatment of domestic wastewater by operating a bench-scale AnMBR at temperatures of 12, 9, 6, and 3°C (Chapter 5; (Smith et al. 2014a)). Membranes were operated under conditions that supported biofilm development based on previous observations (Chapter 4; (Smith et al. 2014b)) to maximize overall treatment performance.

COD removal > 95% was maintained at temperatures as low as 6°C. COD removal was not affected until temperature was reduced to 3°C, after which it fell to 86 ± 4.0%. An increase in the biofilm's role in treatment was observed as temperature decreased suggesting that suspended biomass was more sensitive than the biofilm to temperature decreases. This greater reliance on the biofilm for treatment led to an increase in dissolved methane oversaturation due to greater

methanogenesis in the biofilm. Membrane fouling became more severe as temperature decreased indicating a potential concern for AnMBR implementation at such low temperatures.

High-throughput sequencing of 16S rRNA indicated a diversification of metabolisms as temperature decreased (i.e., reduced relative activity of methanogens and syntrophic bacteria and increased relative activity of fermenters). A concurrent increase in permeate dissolved methane oversaturation as temperature decreased suggests that methanogenesis in the biofilm increased, despite lower relative activity of methanogens, and therefore, that the overall biological activity in the biofilm also increased. Hydrogenotrophic methanogenesis as opposed to aceticlastic methanogenesis was the preferred pathway in the biofilm but not in suspended biomass, possibly due to better spatial microbial organization in the biofilm supporting syntrophy.

7.4 Full-scale implementation requires dissolved methane recovery and reduction in membrane fouling control energy demands

Dissolved methane in AnMBR permeate represents a significant fraction of the energy produced during treatment and would result in greenhouse gas emissions if released to the atmosphere. Failing to recover dissolved methane from AnMBR permeate thus decreases the favorability of the energy balance while also increasing concerns regarding the environmental impacts of treatment. During a preliminary investigation of AnMBR domestic wastewater treatment at 15°C, dissolved methane oversaturation of approximately 1.5 times that predicted by Henry's Law was observed (Chapter 3; (Smith et al. 2013)). Due to this level of oversaturation, dissolved methane represented 40-50% of methane produced during treatment. We further linked dissolved methane oversaturation directly to methanogenesis in the biofilm by operating a bench-scale AnMBR under different levels of fouling (i.e., biofilm treatment). Dissolved methane oversaturation as high as 3 times that predicted by Henry's Law was observed under the highest level of biofilm treatment

(Chapter 4; (Smith et al. 2014b)). We further observed a strong dependence of dissolved methane oversaturation on operational temperature when relying on biofilm treatment (Chapter 5; (Smith et al. 2014a)). Dissolved methane concentration in the effluent increased both because of the decrease in temperature, which increased methane solubility, and the increase in oversaturation. Dissolved methane oversaturation approached 7 times that predicted by Henry's Law during operation at 3°C. Essentially all of the methane produced at this temperature remained in the dissolved form. Therefore, both biofilm treatment and low operational temperature are detrimental to energy recovery and global warming potential of AnMBR if adequate dissolved methane recovery technologies are not in place.

An environmental and economic evaluation framework was established to compare AnMBR with conventional aerobic wastewater treatment systems considering the impacts related to dissolved methane release to the atmosphere (Chapter 6; (Smith et al. 2014c)). AnMBR had significantly greater global warming impact than aerobic treatment systems with 75% of this impact from effluent dissolved methane. This analysis considered a dissolved methane oversaturation of 1.5 times. Therefore, AnMBR could potentially have even greater global warming impacts based on the work described above.

The environmental and economic evaluation framework also highlighted the significance of fouling control energy demands in AnMBR. During treatment of medium strength domestic wastewater, AnMBR was unable to recover net energy because energy recovery was far outweighed by energy demands associated with biogas sparging. AnMBR is currently better suited to higher strength domestic wastewater treatment. For medium strength domestic wastewater, biogas sparging flow rates need to be reduced to those currently used in full-scale aerobic

membrane bioreactors to achieve net energy recovery. Alternatively, employing intermittent biogas sparging or increasing membrane flux could achieve net energy recovery.

7.5 Future research directions

This dissertation research demonstrated that AnMBR can produce a high quality effluent at temperatures as low as 6°C through membrane biofilm development. The practicality of doing so, considering the majority of methane remains dissolved in the effluent, is questionable if efficient dissolved methane recovery is not in place. One option to reduce dissolved methane concentration is to limit the reliance on biofilm treatment. To do so, novel approaches to improve suspended biomass activity need to be developed such that a high quality effluent can be produced at low temperatures without biofilm treatment.

One approach could include supplying biofilm carriers within the reactor (e.g., granular activated carbon (GAC) as previously demonstrated (Yoo et al. 2012) or a plastic media such as those used in moving bed biofilm bioreactors). However, the underlying mechanisms behind the high biological activity observed in the biofilm, particularly at such low temperatures, is poorly understood. We hypothesize that spatial organization of microbes within the biofilm enhances syntrophic interactions by reducing intercellular distances between syntrophic bacteria and hydrogenotrophic methanogens. High shear within suspended biomass due to biogas sparging may concurrently disrupt these syntrophic relationships in suspension. This hypothesis should be explored using fluorescence in situ hybridization (FISH) targeting these specific populations and comparing their spatial juxtapositioning in suspended and biofilm biomass. However, mass transfer limitations and substrate availability may also play a role or may even be the primary driver in the high biofilm activity observed. If so, adding biofilm carriers to an AnMBR may not appreciably improve suspended biomass activity. Alternative approaches to improving activity by

enhancing these syntrophic interactions beyond biofilm development may be more valuable. For example, it may be possible that direct interspecies electron transfer (DIET) (Morita et al. 2011) plays a role in AnMBR and that incorporating electrically conductive materials such as GAC (Liu et al. 2012), biochar (Chen et al. 2014), or other materials in AnMBR could enhance syntrophy and enable sufficient treatment without the membrane biofilm to limit dissolved methane oversaturation.

A different approach to improve suspended biomass activity may involve rethinking inoculum and startup strategies for AnMBRs. We observed a rapid startup when inoculating a bench-scale AnMBR with a mixture of psychrotolerant and mesophilic biomass. After prolonged operation, the suspended and biofilm communities most closely resembled the mesophilic inocula suggesting that the psychrotolerant inoculum was unnecessary. However, we did not evaluate the suspended and biofilm communities at other time points during startup. There may have been an early reliance on the psychrotolerant inoculum for treatment performance. Startup was significantly slower in future work when we inoculated with only mesophilic biomass. Future research should investigate AnMBR inoculation strategies in more detail. One approach may be to test startup using different mixtures of psychrophilic/psychrotolerant and mesophilic biomass and evaluate performance at a range of operational temperatures. Another approach may be to inoculate an AnMBR solely with biofilm biomass cultured in another system. Modifying operational strategies during startup could also be beneficial in improving syntrophic bacteria and methanogen activity in suspended biomass. For example, an AnMBR could be supplemented with high concentrations of acetate and propionate during startup to rapidly increase the activity of syntrophic bacteria and methanogens in suspended biomass.

Although limiting dissolved methane oversaturation reduces global warming potential, it is insufficient for AnMBR to compete with the global warming potential of existing aerobic treatment processes. AnMBR has a significantly greater global warming potential even under conditions in which dissolved methane is near saturation (Chapter 6; (Smith et al. 2014c)). Although wastewater treatment plants are not currently regulated on greenhouse gas emissions, implementing an AnMBR would be environmentally irresponsible without adequate dissolved methane recovery technologies in place. Current approaches to dissolved methane recovery are energy intensive and produce an off-gas of relatively low methane content which is not suitable for energy recovery via cogeneration (Bandara et al. 2011, Cookney et al. 2012). Therefore, future research is required to advance our ability to recover dissolved methane from AnMBR effluent by developing low-energy dissolved methane recovery technologies that effectively recover a usable off-gas. Future research could also investigate the use of a biological downstream system for dissolved methane removal. For example, methanotrophs in a downstream system could biologically oxidize effluent dissolved methane to carbon dioxide (Hatamoto et al. 2011). Alternatively, anaerobic oxidation of methane coupled to denitrification could be used in a downstream system to remove dissolved methane and use it as an electron donor in nitrogen removal (Luesken et al. 2011). Based on the life cycle evaluation of AnMBR (Chapter 6; (Smith et al. 2014c)), a dissolved methane management solution needs to be low cost and energy to reduce global warming potential without significantly increasing life cycle costs or worsening the net energy balance.

Finally, the net energy balance of AnMBR treatment needs to be improved significantly to warrant full-scale implementation. Energy demands for fouling control, namely high biogas sparging rates currently demonstrated in pilot-scale AnMBR studies, prevent net energy recovery from being

achieved. Future research should evaluate approaches to limit these energy demands such as reducing biogas sparging flow rates, intermittently sparging, and/or increasing membrane flux. Alternatively, research should focus on novel methods of fouling control such as GAC (Shin et al. 2014), rotating ceramic discs (Jaffrin 2008), and alternative membrane materials such as electrospun nanofibers (Bjorge et al. 2009). Future research could also consider improving the net energy balance by supplementing domestic wastewater with another high-strength waste stream such as food waste.

Implementation of AnMBR requires assessment of local and regional water quality concerns and water demands. For example, utilities in the Chesapeake Bay area are currently facing extremely stringent nitrogen regulations (Howarth and Marino 2006). Implementing AnMBR here would require extensive downstream nitrogen removal not yet well researched. This combined system may not be economically viable compared to an activated sludge process with advanced biological nitrogen removal. AnMBR effluent could be used for irrigation purposes rather than direct discharge in which the presence of nutrients may be beneficial. However, water supplies in much of the U.S. are sufficient such that water reuse is unnecessary. The logistics and economic investments required to transport effluent from a wastewater treatment plant to agricultural land are major barriers to implementing water reuse in regions where water is plentiful and undervalued. Reuse of AnMBR effluent for irrigation may be more feasible in draught-prone regions of the U.S. such as southern California, where water reuse is becoming increasingly attractive. The Monterey County Water Recycling Project (MCWRP) (Haddad 2002) in California was established in 1998 and has practiced irrigational reuse of highly treated wastewater to minimize groundwater draw and limit saltwater intrusion into aquifers. AnMBR may have the greatest potential in these scenarios in which a highly treated effluent rich in nutrients can be valued for reuse.

Success of AnMBR in domestic wastewater treatment clearly hinges on not only improvements in AnMBR but also in developing a greater system of integrated technologies to meet location-specific needs. Development of AnMBR compatible technologies for dissolved methane management, nutrient removal/recovery, and water reuse (e.g., reverse osmosis or advanced biofiltration) is needed to expand the opportunities for AnMBR implementation. AnMBR research activity has been high over the past decade and has substantially increased in the past few years. While this research interest is warranted, it should expand to consider a greater system of treatment technologies to better suit a variety of location-specific needs. AnMBR is competitive but not yet an improvement over activated sludge processes based on life cycle economic and environmental impacts. However, activated sludge processes have reached maturity and are limiting our ability to recover resources and adapt to changing water needs. AnMBR is a key opportunity to achieve net energy positive wastewater treatment, provide water reuse opportunities, and even offset carbon with further technological development.

7.6 Literature Cited

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Appendix A. Supplementary Information for Chapter 3

Table S3-1. Recipe for concentrated feed solution used to prepare the synthetic domestic wastewater (DWW) (diluted feed). This recipe was modified from the SYNTHES recipe originally presented by Aiyuk and Verstraete (2004).

Component	Concentrated feed (mg/L)	Diluted feed (mg/L)
Chemical Compounds		
Urea	550	63.8
NH_4Cl	150	17.4
Na-acetate· $3H_2O$	350	40.6
Peptone	150	17.4
*$MgHPO_4$· $3H_2O$	---	29.2
*K_2HPO_4· $3H_2O$	---	13.3
$FeSO_4$· $7H_2O$	400	46.4
$CaCl_2$	600	69.6
*$NaHCO_3$	---	265
Food Ingredients		
Starch	1500	174
Milk powder	1500	174
Dried yeast	600	69.6
Soy oil	250	29.0
Trace Metals		
$Cr(NO_3)_3$ ·$9H_2O$	8	0.93
$CuCl_2$· $2H_2O$	5	0.58
$MnSO_4$· H_2O	10	1.16
$NiSO_4$· $6H_2O$	3	0.35
$PbCl_2$	1	0.12
$ZnCl_2$	3	0.35

*In dilution water only

Table S3-2. Number of sequences obtained per sample after quality screening. The sequences were quality filtered to allow a maximum of 4 bp mismatch with the reverse primer, 0 mismatches with the barcode, 0 ambiguous bases, and an average quality score of 25 over a sliding window of 50 bp over the read length. All reads quality trimmed below 200 bp length were removed. Chimeras were detected and removed from the remaining sequences using the Chimera Slayer algorithm in Mothur.

Sample	*Archaea*	*Bacteria*
AnMBR Biofilm	1080	1227
AnMBR Suspended Biomass	1370	836
UASB	6503	405
Anaerobic Digester	86	537
Anaerobic Lagoon	97	227

Appendix B. Supplementary Information for Chapter 4

Table S4-1. Primer coverage of *Archaea* for 16S rRNA primers F515 (GTGCCAGCMGCCGCGGTAA) and R806 (GGACTACHVGGGTWTCTAAT) targeting the V4 region (Caporaso et al. 2011) according to TestPrime 1.0. TestPrime 1.0 evaluates the coverage of primer pairs by running an *in silico* PCR using the SILVA databases. Zero primer mismatches were allowed.

Domain	Phylum	Class	Order	Family	Genus	Coverage (%)
Archaea						51
Archaea	Crenarchaeota					0
Archaea	Euryarchaeota					82
Archaea	Euryarchaeota	Methanobacteria				93
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales			93
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae		93
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobacterium	89
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	95
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera	82
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanothermobacter	89
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanothermaceae	Methanothermus	100
Archaea	Euryarchaeota	Methanococci				83
Archaea	Euryarchaeota	Methanococci	Methanococcales			83
Archaea	Euryarchaeota	Methanococci	Methanococcales	Methanocaldococcaceae		74
Archaea	Euryarchaeota	Methanococci	Methanococcales	Methanocaldococcaceae	Methanocaldococcus	92
Archaea	Euryarchaeota	Methanococci	Methanococcales	Methanocaldococcaceae	Methanotorris	39
Archaea	Euryarchaeota	Methanococci	Methanococcales	Methanococcaceae	Methanococcus	96
Archaea	Euryarchaeota	Methanococci	Methanococcales	Methanococcaceae	Methanothermococcus	90
Archaea	Euryarchaeota	Methanomicrobia				88
Archaea	Euryarchaeota	Methanomicrobia	Methanocellales			88
Archaea	Euryarchaeota	Methanomicrobia	Methanocellales	Methanocellaceae		89
Archaea	Euryarchaeota	Methanomicrobia	Methanocellales	Methanocellaceae	Methanocella	94
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales			90
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Family Incertae Sedis	Methanocalculus	97
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanocorpusculaceae		82
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanocorpusculaceae	Methanocorpusculum	82
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae		90
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanoculleus	93
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanofollis	89
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanogenium	97
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanolacinia	100
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanomicrobium	76
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanoplanus	100
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanoregulaceae		93
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanoregulaceae	Methanolinea	94
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanoregulaceae	Methanoregula	92
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanoregulaceae	Methanosphaerula	100
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanospirillaceae		83
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanospirillaceae	Methanospirillum	83
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales			88
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosaetaceae		89
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosaetaceae	Methanosaeta	89
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae		88
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanimicrococcus	93
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanococcoides	86
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanohalobium	100
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanohalophilus	89
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanolobus	91
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanomethylovorans	87
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanosalsum	100
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanosarcina	89
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methermicoccaceae		73
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methermicoccaceae	Methermicoccus	73
Archaea	Euryarchaeota	Methanopyri				0
Archaea	Korarchaeota					47
Archaea	Nanoarchaeota					0
Archaea	Thaumarchaeota					8

Table S4-2. Primer coverage of *Bacteria* for 16S rRNA primers targeting the V4 region according to TestPrime 1.0 (see Table S4-1 legend for additional details). The coverage of taxa in which known fatty acid-oxidizing syntrophic bacteria group is specified down to the genus or family levels.

Domain	Phylum	Class	Order	Family	Genus	Coverage (%)
Bacteria						86
Bacteria	Acidobacteria					91
Bacteria	Actinobacteria					81
Bacteria	Aquificae					87
Bacteria	Armatimonadetes					74
Bacteria	Bacteroidetes					87
Bacteria	Caldiserica					8
Bacteria	Chlamydiae					4
Bacteria	Chlorobi					69
Bacteria	Chloroflexi					51
Bacteria	Chrysiogenetes					100
Bacteria	Cyanobacteria					83
Bacteria	Deferribacteres					78
Bacteria	Deinococcus-Thermus					92
Bacteria	Dictyoglomi					88
Bacteria	Elusimicrobia					89
Bacteria	Fibrobacteres					85
Bacteria	Firmicutes					88
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	89
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Pelotomaculum	92
Bacteria	Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae		90
Bacteria	Firmicutes	Clostridia	Thermoanaerobacterales	Family III Incertae Sedis	Tepidanaerobacter	93
Bacteria	Firmicutes	Clostridia	Thermoanaerobacterales	Thermoanaerobacteraceae	Syntrophaceticus	100
Bacteria	Firmicutes	Clostridia	Thermoanaerobacterales	Thermoanaerobacteraceae	Thermacetogenium	92
Bacteria	Fusobacteria					85
Bacteria	Gemmatimonadetes					87
Bacteria	Lentisphaerae					77
Bacteria	Nitrospirae					89
Bacteria	Planctomycetes					80
Bacteria	Proteobacteria					89
Bacteria	Proteobacteria	Alphaproteobacteria				83
Bacteria	Proteobacteria	Betaproteobacteria				91
Bacteria	Proteobacteria	Deltaproteobacteria				88
Bacteria	Proteobacteria	Deltaproteobacteria	Order Incertae Sedis	Syntrophorhabdaceae		92
Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae		88
Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae		90
Bacteria	Proteobacteria	Epsilonproteobacteria				92
Bacteria	Proteobacteria	Gammaproteobacteria				91
Bacteria	Spirochaetae					73
Bacteria	Synergistetes					90
Bacteria	Tenericutes					85
Bacteria	Thermodesulfobacteria					96
Bacteria	Thermotogae					87
Bacteria	Thermotogae	Thermotogae	Thermotogales	Thermotogaceae	Thermotoga	89
Bacteria	Verrucomicrobia					85

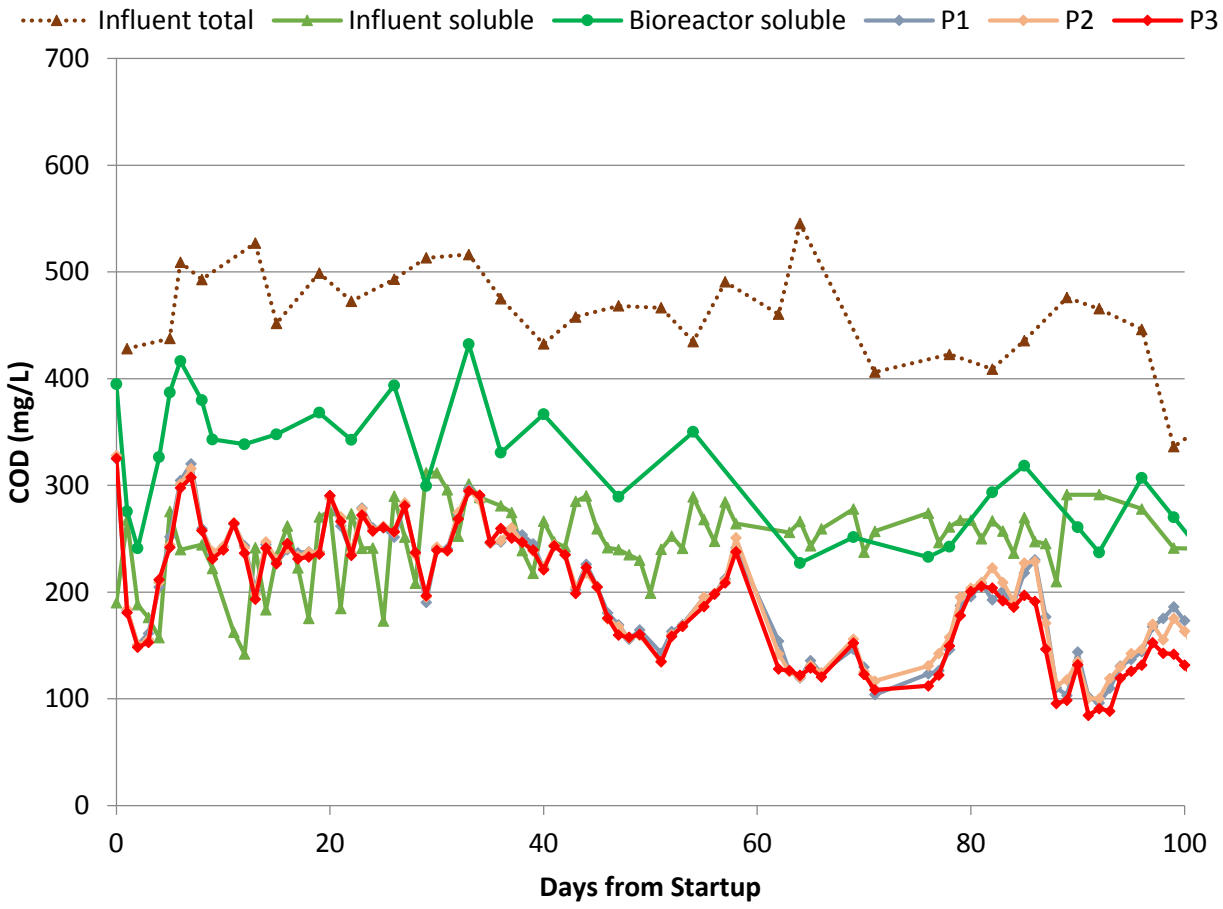

Figure S4-1. Influent (total and soluble), bioreactor (soluble), and permeate COD during days 1-100.

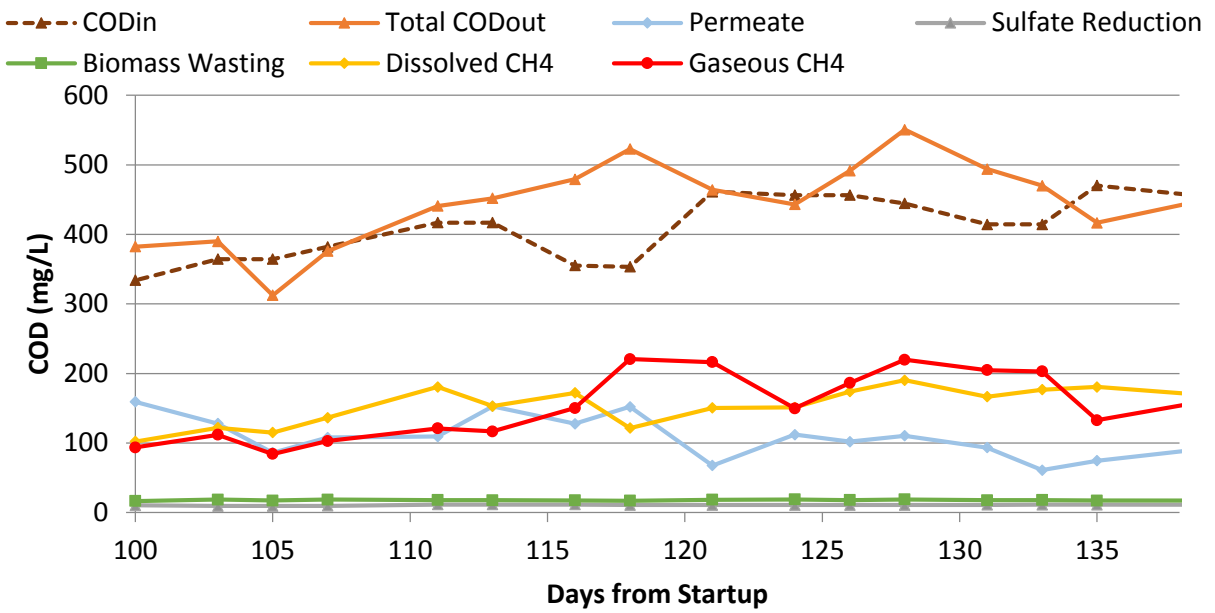

Figure S4-2. COD mass balance for days 100-138. Total COD_{out} is the summation of measured permeate COD, measured dissolved methane, measured gaseous methane, theoretical COD removal from measured sulfate reduction, and theoretical COD from measured biomass wasting.

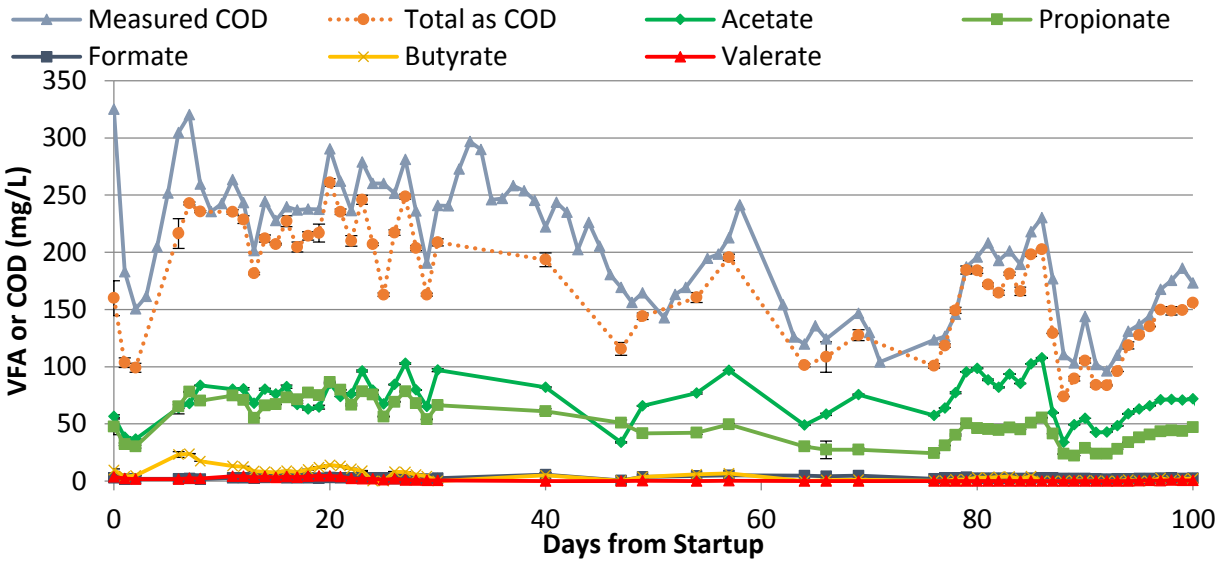

Figure S4-3. P1 VFA concentrations (concentrations are expressed as the actual compound, not as COD), theoretical COD contribution from measured VFAs, and measured COD during days 1-100. Total as COD is the calculated theoretical COD contribution from measured VFAs. Results for P2 and P3 were very similar (data not reported). Error bars represent standard deviations of triplicate IC injections.

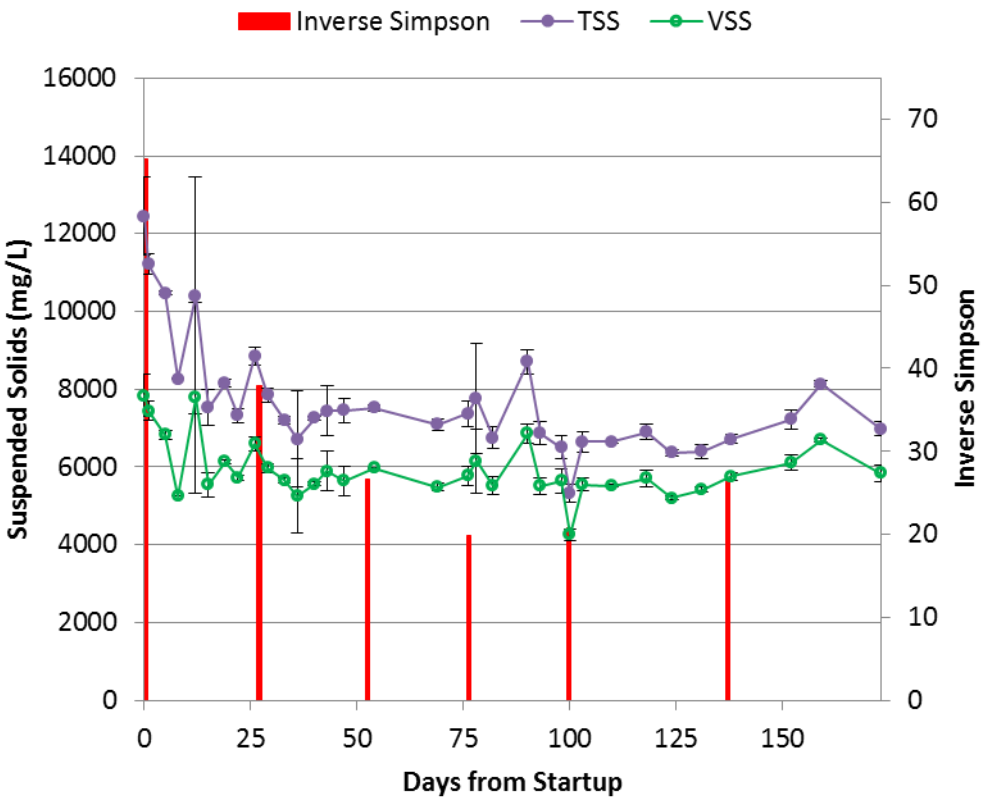

Figure S4-4. Total and volatile suspended solids (TSS; VSS) in the bioreactor during days 1-173 (primary y-axis) and inverse Simpson index in suspended biomass based on 16S rDNA sequencing (secondary y-axis). Error bars represent standard deviation of triplicate sample analysis.

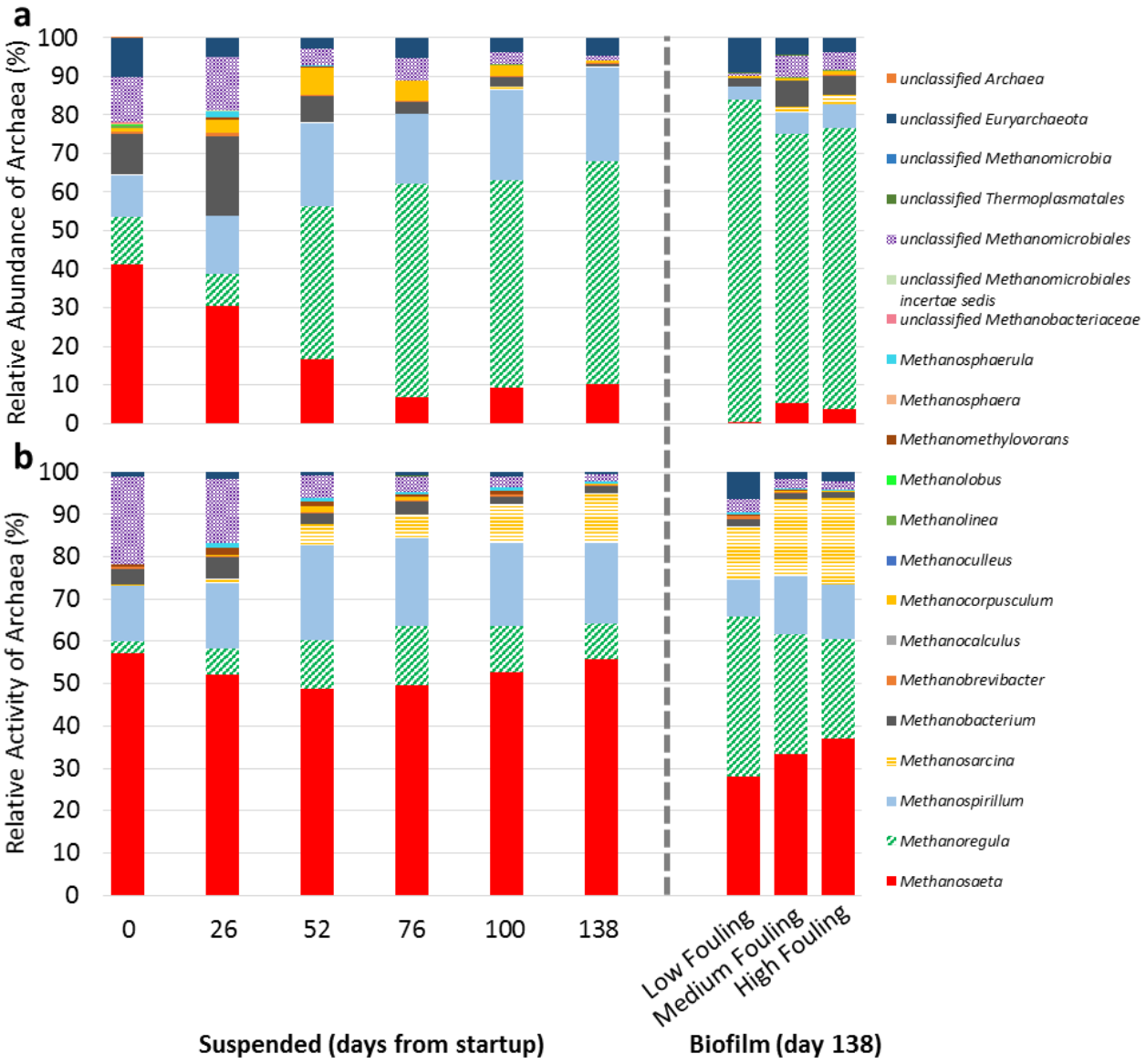

Figure S4-5. (a) Relative abundance of methanogens identified to the genus level based on 16S rDNA sequencing and (b) relative activity of methanogens identified to the genus level based on 16S rRNA sequencing in suspended biomass from startup to the end of Phase 2 and in biofilms at the end of Phase 2. Data are expressed as a percentage and were normalized using the total *Archaeal* 16S rDNA sequences (a) and 16S rRNA sequences (b).

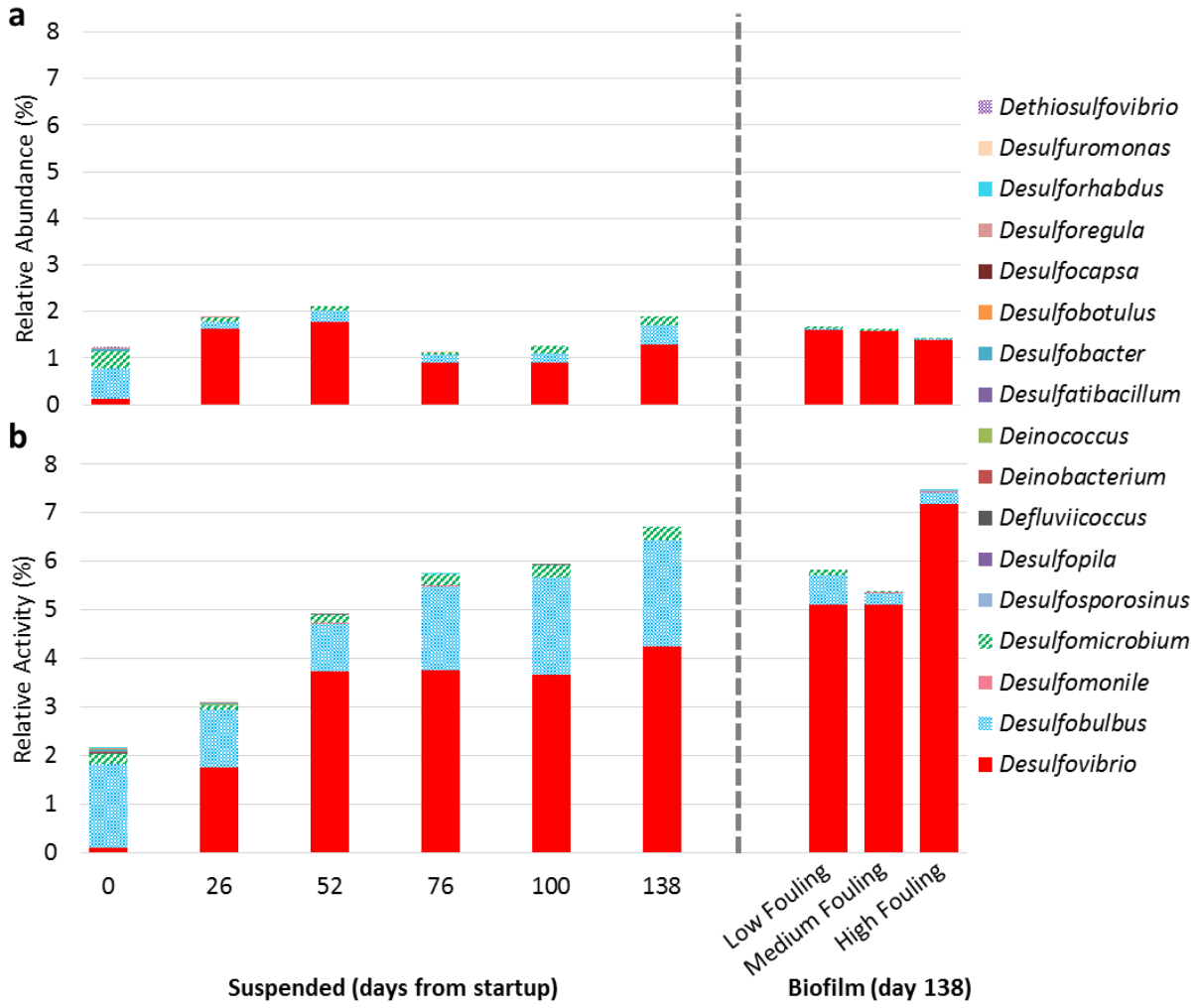

Figure S4-6. (a) Relative abundance of sulfate reducing bacteria identified to the genus level based on 16S rDNA sequencing and (b) relative activity of sulfate reducing bacteria identified to the genus level based on 16S rRNA sequencing in suspended biomass from startup to the end of Phase 2 and in biofilms at the end of Phase 2. Data are expressed as a percentage and were normalized using the total 16S rDNA sequences (a) and 16S rRNA sequences (b) (including *Archaeal* and *Bacterial* sequences). A truncated y-axis (0 to 8%) is shown to accentuate changes in abundance and activity.

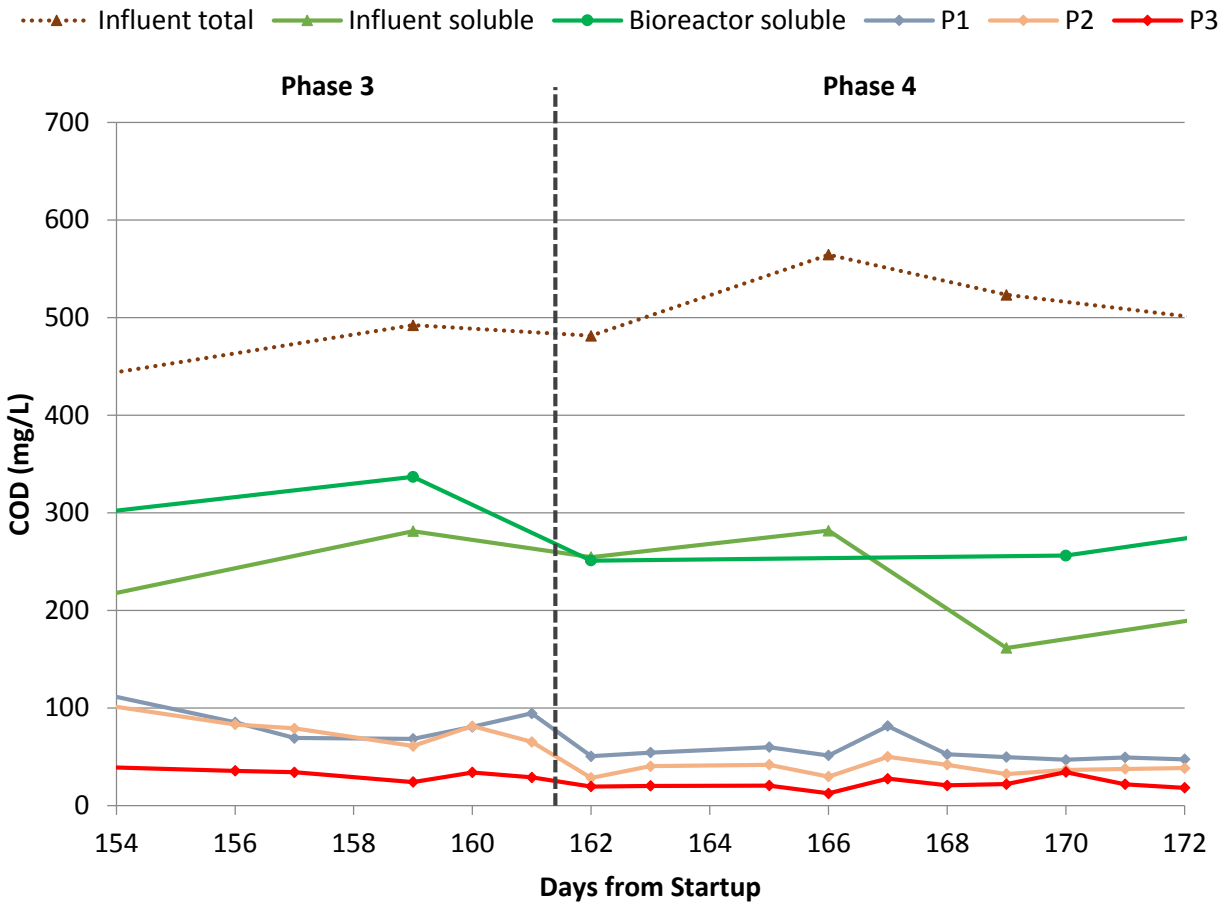

Figure S4-7. Influent (total and soluble), bioreactor (soluble), and permeate COD during Phases 3 and 4.

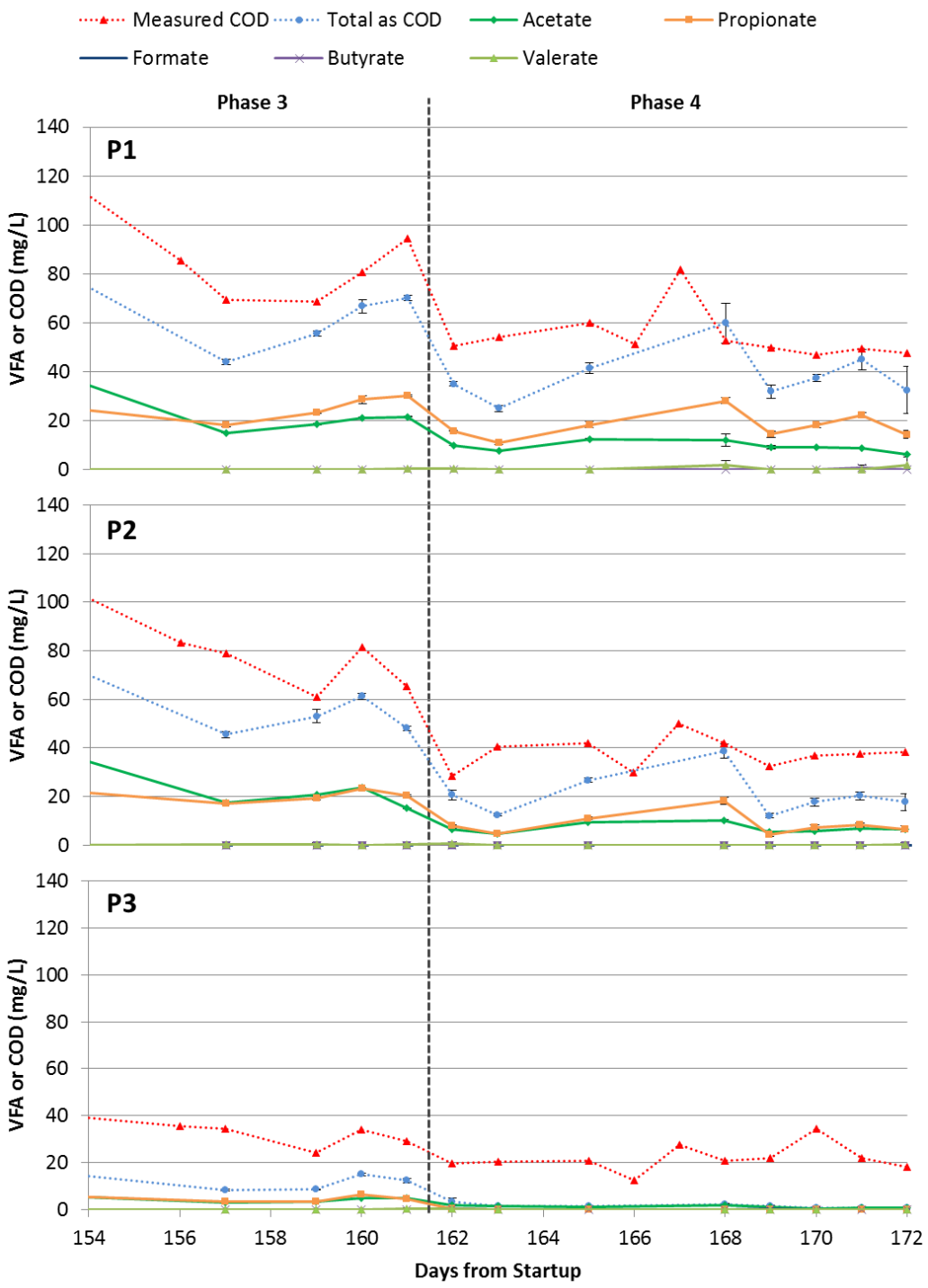

Figure S4-8. P1, P2, and P3 permeate VFA concentrations (concentrations are expressed as the actual compound, not as COD), theoretical COD contribution from measured VFAs, and measured COD during Phases 3 and 4. Total as COD is the calculated theoretical COD contribution from measured VFAs. Error bars represent standard deviations of triplicate IC injections.

References:

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N. and Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences 108(Supplement 1), 4516-4522.

Appendix C. Supplementary Information for Chapter 5

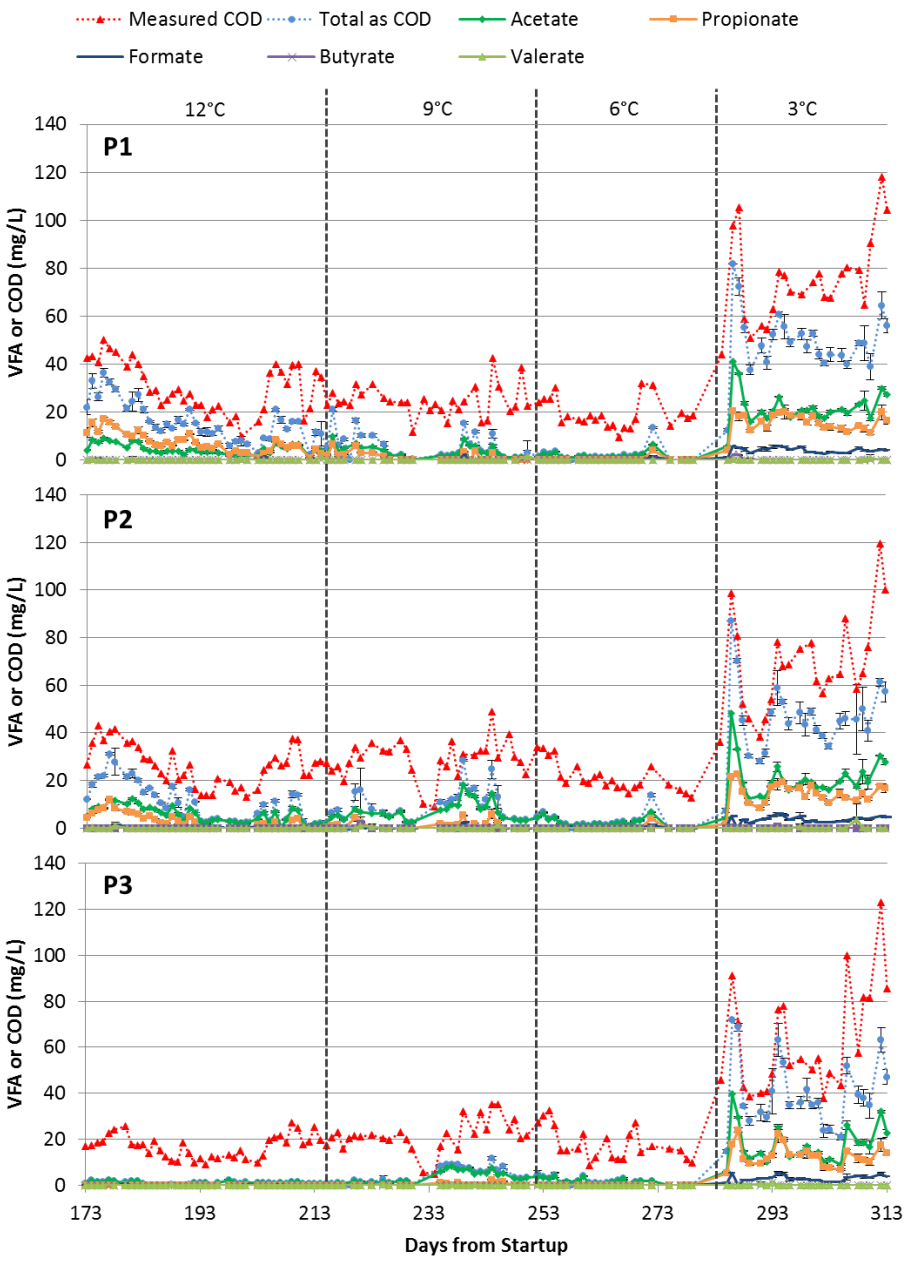

Figure S5-1. P1, P2, and P3 permeate VFA concentrations (concentrations are expressed as the actual compound, not as COD), theoretical COD contribution from measured VFAs, and measured COD during days 173-313. Total as COD is the calculated theoretical COD contribution from measured VFAs. Error bars represent standard deviations of triplicate IC injections.

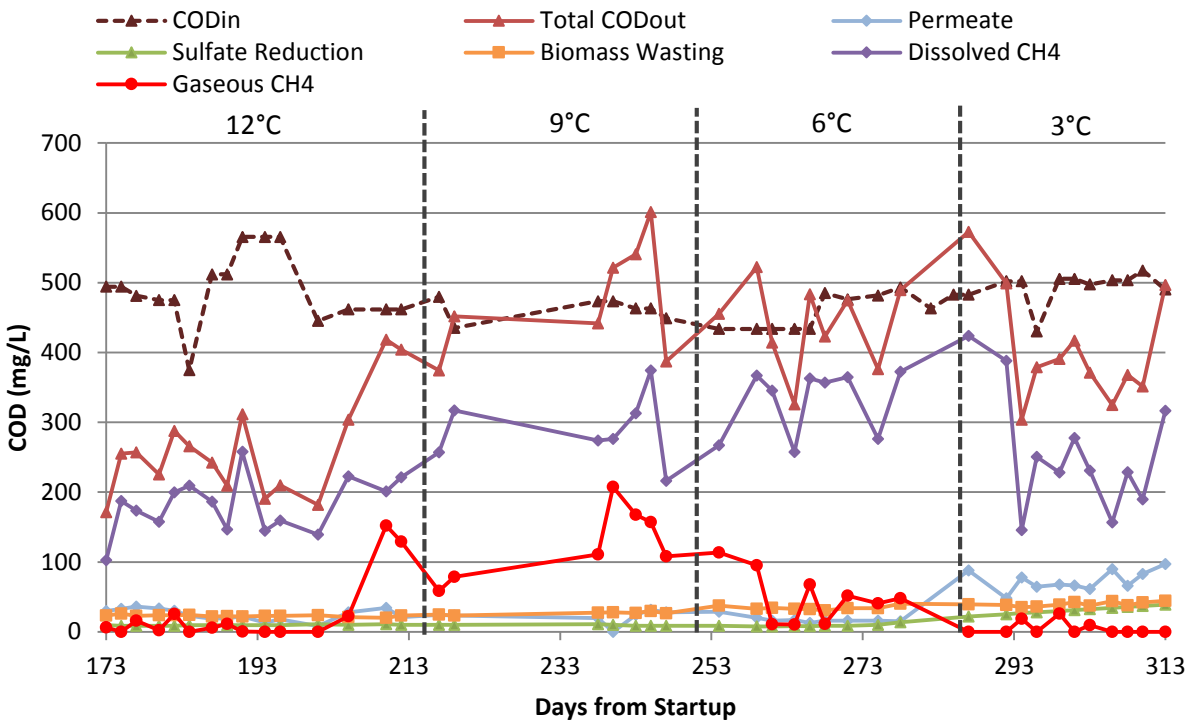

Figure S5-2. COD mass balance for days 173-313. Total COD_{out} is the summation of measured permeate COD, measured dissolved methane, measured gaseous methane, theoretical COD removal from measured sulfate reduction, and theoretical COD from measured biomass wasting. An issue with the biogas collection system during days 173-205 prevented accurate measurement of biogas production during that time.

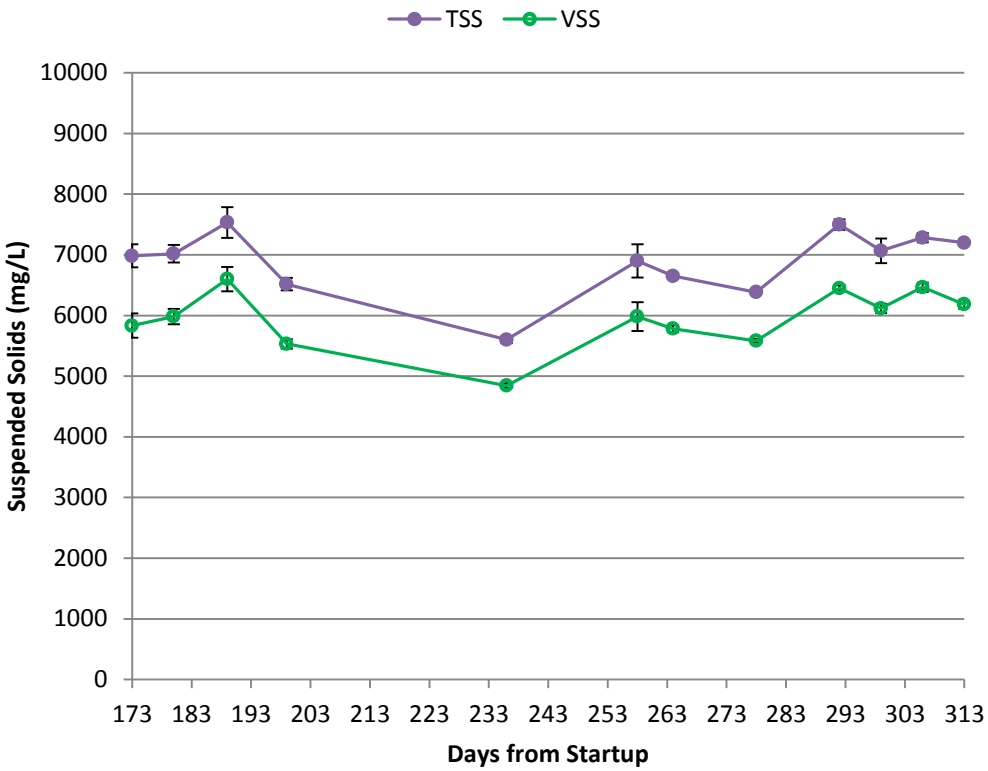

Figure S5-3. Total and suspended volatile solids (TSS, VSS) in the bioreactor during days 173-313. Error bars represent the standard deviation of triplicate measurements.

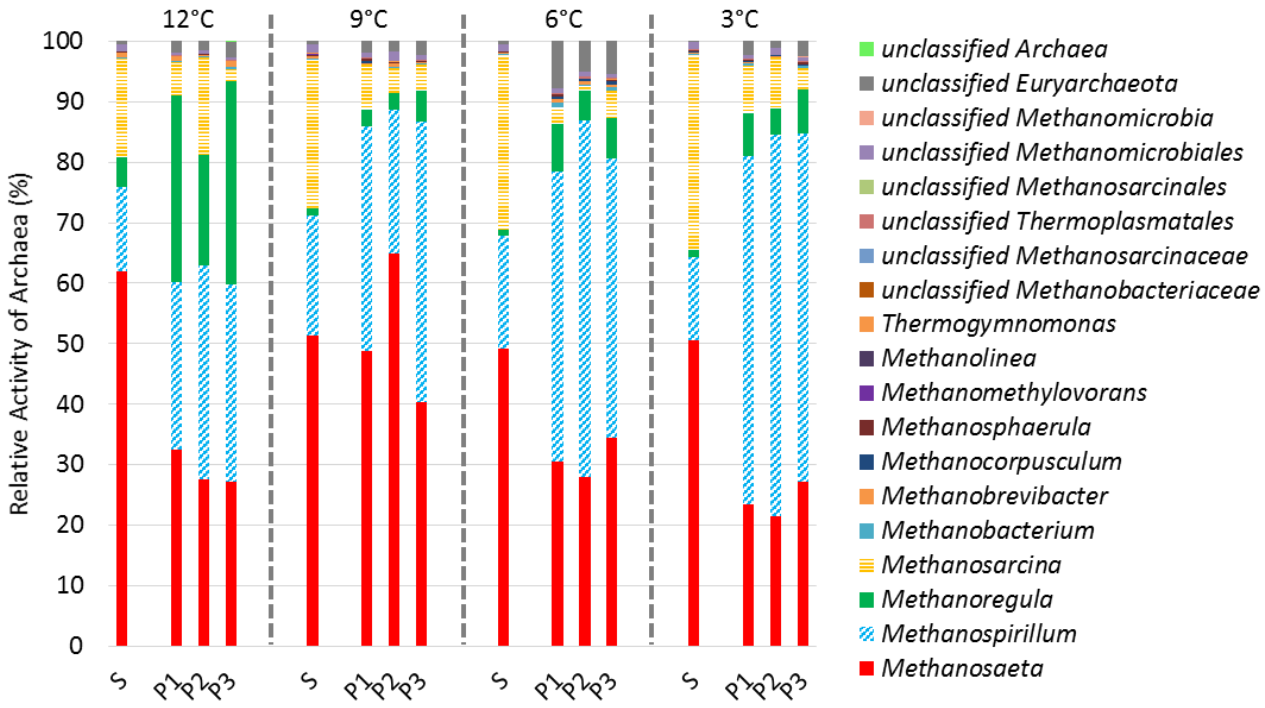

Figure S5-4. Relative activity of *Archaea* in suspended (S) and biofilm (P1, P2, and P3) biomass at operational temperatures of 12, 9, 6, and 3°C.

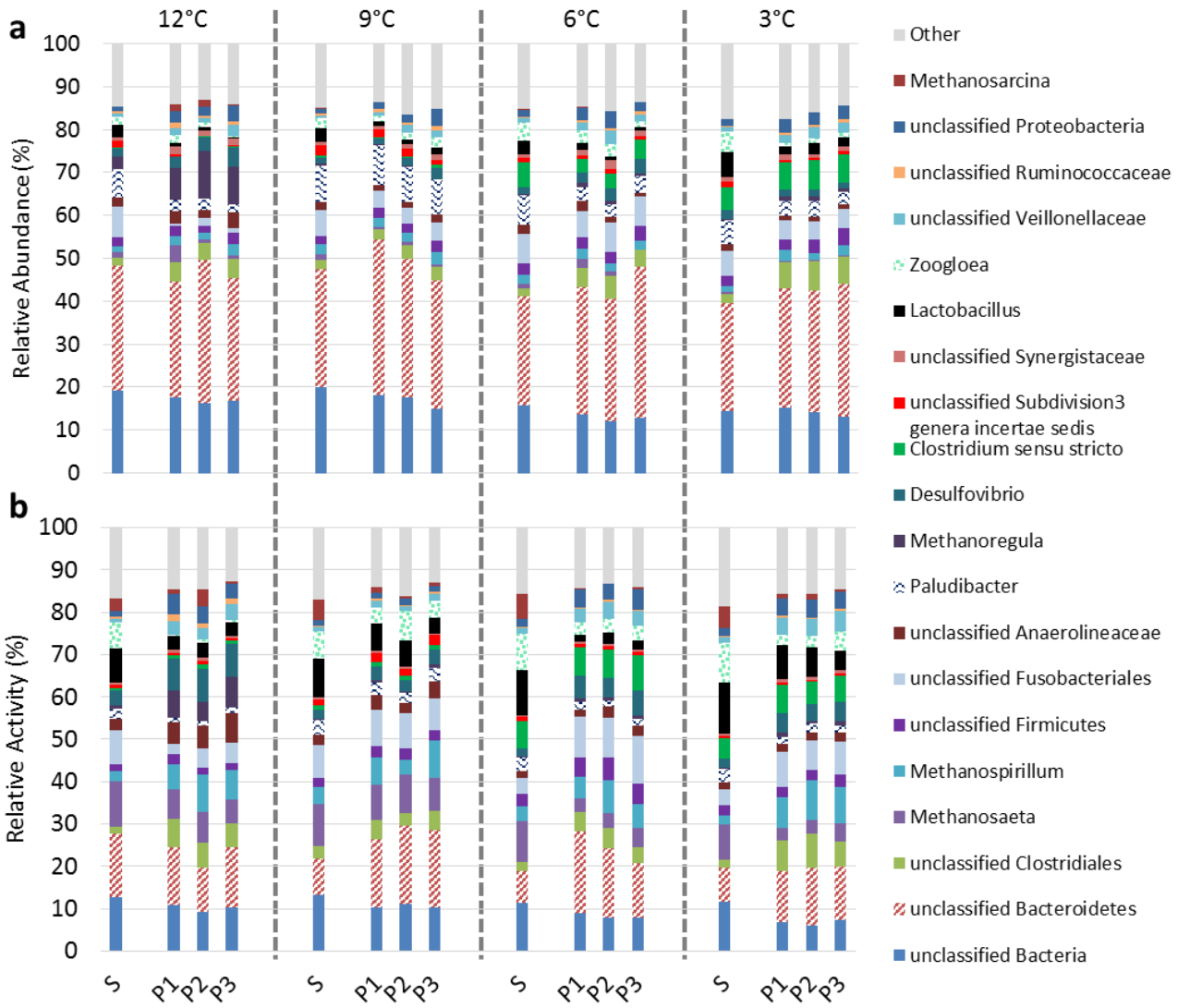

Figure S5-5. (a) Relative abundance based on 16S rDNA sequencing and (b) relative activity based on 16S rRNA sequencing of the top 20 phylotypes to total community in suspended (S) and biofilm (P1, P2, and P3) biomass at operational temperatures of 12, 9, 6, and 3°C.

Appendix D. Supplementary Information for Chapter 6

Table of Contents

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List of Figures

1) Treatment Scenarios

This section outlines the main assumptions and sources of data used for modeling the different treatment scenarios. It includes system boundaries and flow diagrams for the treatment scenarios not shown in the research article (CAS+AD and AeMBR+AD, Figure S6-1). The influent wastewater for each system was assumed to be medium strength domestic wastewater, as defined by Metcalf & Eddy (2003) (Table S6-1). Grit removal was assumed to remove 90% of inert solids in the influent.

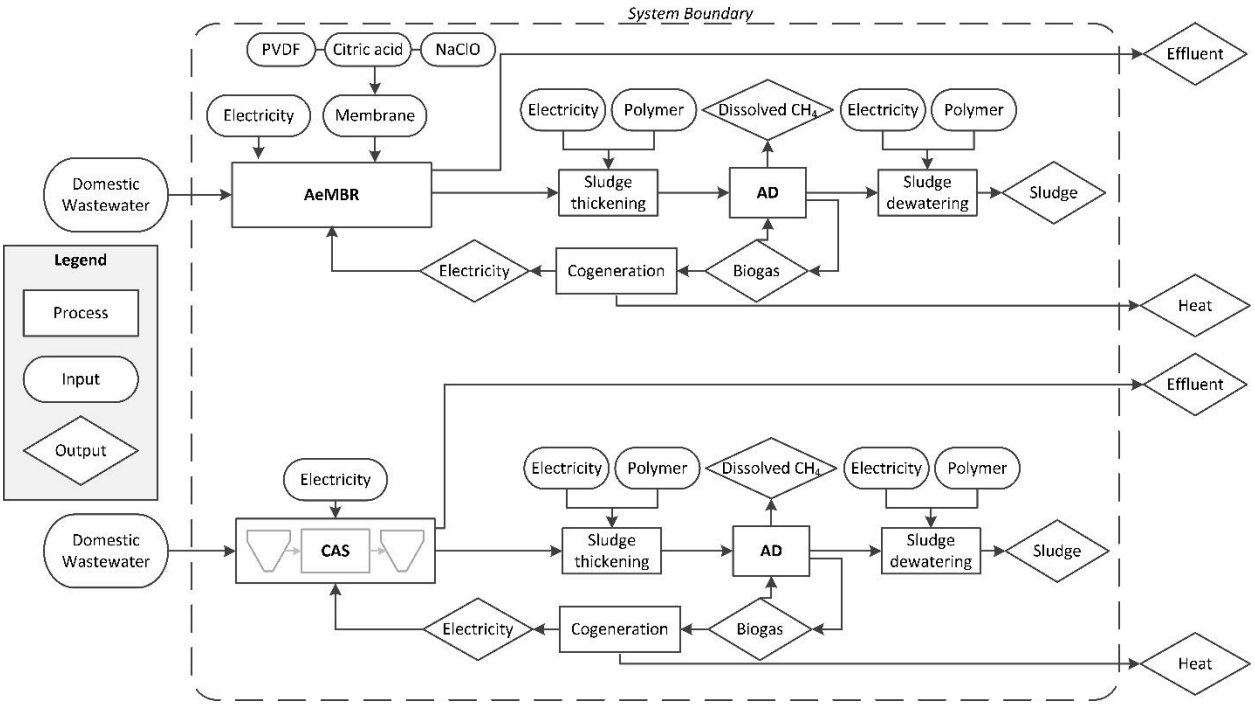


Figure S6-1. System boundary of CAS+AD and AeMBR+AD.

Table S6-1. Typical composition of untreated domestic wastewater.

		Concentration(Tchobanoglous et al. 2003)	
Contaminants	**Unit**	**Medium strength**	**High strength**
Solids, total (TS)	mg/L	720	1230
Dissolved, total (TDS)	mg/L	500	860
Fixed	mg/L	300	520
Volatile	mg/L	200	340
Suspended solids, total (TSS)	mg/L	210	400
Fixed	mg/L	50	85
Volatile	mg/L	160	315
Settleable solids	mg/L	10	20

Biochemical oxygen demand, 5-d	mg/L	190	350
Total organic carbon (TOC)	mg/L	140	260
Chemical oxygen demand (COD)	mg/L	430	800
Nitrogen (total as N)	mg/L	40	70
Organic	mg/L	15	25
Free ammonia	mg/L	25	45
Nitrites	mg/L	0	0
Nitrates	mg/L	0	0
Phosphorus (total as P)	mg/L	7	12
Organic	mg/L	2	4
Inorganic	mg/L	5	8
Chlorides	mg/L	50	90
Sulfate	mg/L	30	50

A. Anaerobic Membrane Bioreactor (AnMBR)

The process flow diagram for AnMBR is shown in Figure S6-2. AnMBR performance was based on published performance results from pilot and lab scale systems. Specifically, AnMBR COD removal was assumed to be 90% at 25°C (Chu et al. 2005, Ho and Sung 2009, Lew et al. 2009) and 85% at 15°C (Chu et al. 2005, Ho and Sung 2010, Smith et al. 2013) for medium and high strength domestic wastewater. It was not modeled using GPS-X, which uses deterministic models, because the current anaerobic models (e.g., ADM1) are not accurate for direct anaerobic treatment of domestic wastewater at ambient temperatures as they were designed for high strength wastewater and sludge treatment at mesophilic and thermophilic temperatures. The ratio of COD to BOD_5 in the effluent was assumed to be 3.04:1, based on bench-scale results (Smith et al. 2013). The kinetic and stoichiometric constants used to describe the biomass inventory are given in Table S6-2. The amount of sludge wasted to achieve a 200-day SRT was calculated based on equation 1 from Grady et al. (2011) The mixed liquor suspended solids (MLSS) concentration was calculated using equation 2.

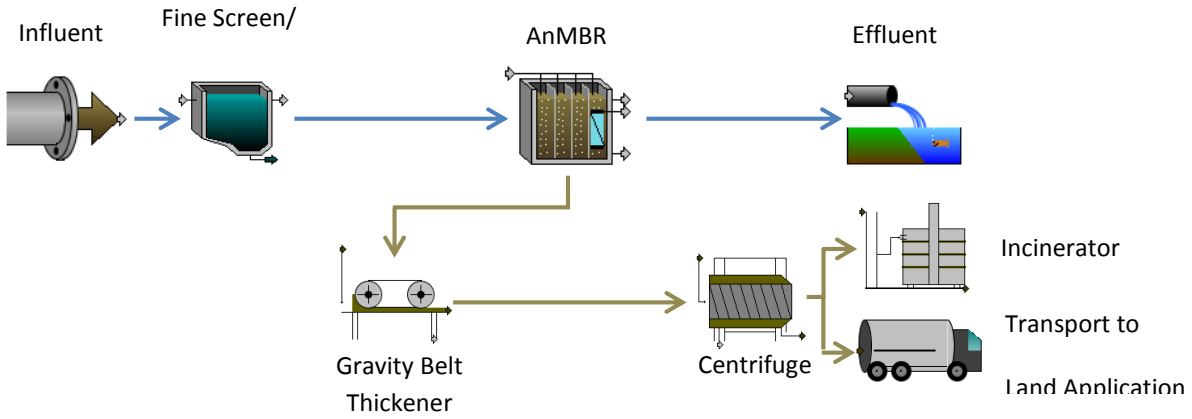


Figure S6-2. Process flow diagram for AnMBR.

Table S6-2. Kinetic parameters used for AnMBR biomass inventory.

Parameter	Symbol	Units	Value	Arrhenius	Reference
Decay coefficient	f_D	mgTSS mgTSS^{-1}	0.2	1	(Grady et al. 2011)
Decay rate	b_H	day^{-1}	0.02 (at 35 °C)	1.04	(Grady et al. 2011)
Yield	Y_{TSS}	mgTSS mgCOD^{-1}	0.076	1	(Hu and Stuckey 2006)

$$W_{TOTAL,T} = \frac{Q}{10^6}\left(X_{I,O} + \frac{(1+f_D b_H \theta)Y_T(S_{S,O}-S_S)}{(1+b_H\theta)}\right) \quad \text{(eq. 1)}$$

$W_{TOTAL,T}$ = Biomass wastage, kgTSS day^{-1}
Q = Influent flow, L day^{-1}
$X_{I,O}$ = Influent inert solids, mg L^{-1}
Y_T = Yield, mgTSS mgCOD^{-1}
θ = SRT, days
f_D = Decay coefficient, mgTSS mgTSS^{-1}
b_H = Decay rate, day^{-1}
$S_{S,O}$ = Influent soluble carbon, mg L^{-1}
S_S = Effluent soluble carbon, mg L^{-1}

$$X_{M,T} = \left(\frac{\theta}{\tau}\right)\left[X_{I,O} + \frac{(1+f_D b_H \theta)(Y_T)(S_{S,O}-S_S)}{1+b_H\theta}\right] \quad \text{(eq. 2)}$$

$X_{M,T}$ = MLSS, mgTSS L^{-1}
$X_{I,O}$ = Influent inert solids, mg L^{-1}
Y_T = Yield, mgTSS mgCOD^{-1}
θ = SRT, days
τ = HRT, days
f_D = Decay coefficient, mgTSS mgTSS^{-1}
b_H = Decay rate, day^{-1}
$S_{S,O}$ = Influent soluble carbon, mg L^{-1}
S_S = Effluent soluble carbon, mg L^{-1}

Biogas production from the AnMBR was calculated by subtracting the COD used for sulfate reduction and the COD associated with biomass from the total COD removed. Dissolved methane was calculated using Henry's law (equation 3) and assuming an oversaturation of 1.5 times (Smith et al. 2013).

$$CH_{4,dissolved} = \left(\frac{P_{CH_4}}{H_{CH_4}}\right)(M)\left(MW_{CH_4}\right)(OS)(1000) \quad \text{(eq. 3)}$$

$CH_{4,dissolved}$ = dissolved methane concentration, mg L^{-1}
P_{CH4} = partial pressure of methane, atm
H_{CH4} = Henry's constant for methane, atm
M = Molarity of solution, mol L^{-1}
MW_{CH4} = molecular weight of methane, g mol^{-1}
OS = Oversaturation (assumed 1.5)

The membranes were assumed to be GE ZeeWeed 500D hollow fiber membranes (HFMs). The membranes themselves were the only materials accounted for in the LCA as they were the only component of the membrane unit replaced at the end of the membranes' life. The membranes were made of polyvinylidene fluoride (PVDF), a thermoplastic material. Material quantities were estimated based on HFM dimensions, PVDF density, and membrane area required to achieve a given flux. ZeeWeed 500D HFM specifications are given in Table S6-2.

Table S6-3. GE ZeeWeed 500D hollow fiber membrane specifications.

Parameter	Value	Units
Outer diameter	0.0019	m
Inner diameter	0.0008	m
Density, PVDF	1.78	g $(cm^3)^{-1}$

Membrane cleaning requirements were based on GE recommendations for their ZeeWeed 500D units (Hong 2012). Membranes were cleaned in-situ by back flushing with 12% sodium hypochlorite and citric acid. Recovery cleans involved removing the membranes from the system and soaking them in a bath of 12% sodium hypochlorite followed by a soak in citric acid.

B. High Rate Activated Sludge with Anaerobic Digestion (HRAS+AD)

HRAS+AD was modeled using GPS-X (Hydromantis, Inc.). The process flow diagram is given in Figure S6-3. Kinetic and stoichiometric parameters used in the model are shown in Table S6-4 and were taken from Grady et al. (2011) The parameters were based on measurements made using biomass from CAS systems. HRAS, which operates at a significantly shorter SRT than CAS, might select for different organisms than CAS with slightly different kinetics and yields. However, because there is limited information published on kinetic parameters specific to HRAS, CAS parameters were used in the model. While this is a limitation in that it may not accurately capture sCOD uptake and storage, COD oxidation, and bioflocculation, the performance of the modeled HRAS was consistent with real systems' performances. The nitrifier parameters were of limited importance in the HRAS system as the SRT is 1.5 days, which is below the minimum SRT for nitrifying populations (ammonia and nitrite oxidizing bacteria). A sludge volume index (SVI) of 210 ml/mg, consistent with the selected SRT, was used for modeling secondary clarification. The kinetic parameters in Table S6-4 were also used to model the CAS and AeMBR. Effluent quality for HRAS+AD, CAS+AD, and AeMBR+AD were based on GPS-X outputs.

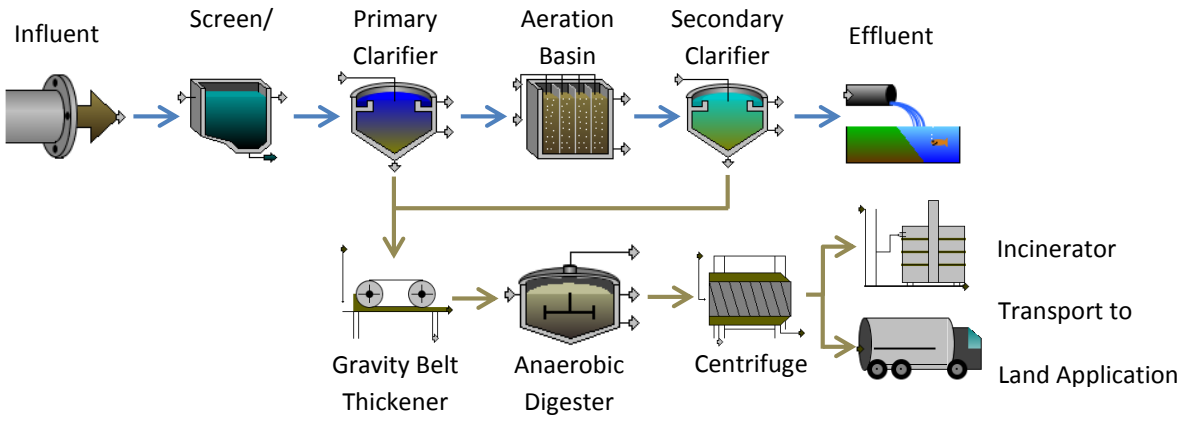


Figure S6-3. Process flow diagram for HRAS+AD.

Table S6-4. Kinetic and stoichiometric parameters for heterotrophs (H) and nitrifiers (N) used in GPS-X models (μ = specific growth rate; K = half saturation coefficient; Y = yield; b = decay rate; f_D = decay coefficient).

Parameter	Units	Value at 25°C	Arrhenius
$\mu_{H,\,max}$	day^{-1}	6.00	1.094
K_s	mg-COD L^{-1}	20.0	1
$Y_{H,T}$	mg-TSS mg-COD^{-1}	0.50	1
b_H	day^{-1}	0.396	1.029
f_D	mg-TSS mg-TSS^{-1}	0.20	
$\mu_{N,\,max}$	day^{-1}	0.77	1.114
K_{NH}	mg-N L^{-1}	1.93	1
$K_{O,N}$	mg- O_2 L^{-1}	0.74	1
$Y_{N,T}$	mg-N L^{-1}	0.20	1
b_N	day^{-1}	0.10	1.029

The anaerobic digester (AD) was designed with a retention time of 20 days and a side-depth of 6 m. AD temperature was assumed to be 35 °C. In the 15 °C scenario, the earth and air temperatures were assumed to be 10 and 0 °C, respectively. In the 25 °C scenario, the earth and air temperatures were assumed to be 10 and 17 °C, respectively. Heat loss coefficients for the walls, floor, and cover of the digester and the specific heat of sludge are given in Table S6-5 (all values taken from Metcalf & Eddy (Tchobanoglous et al. 2003)). The heat required to heat the digester to 35 °C was calculated based on the sludge capacity of the digester, the specific heat of the sludge, and the heat lost via the walls, roof, and floor of the digester. Waste heat from cogeneration was used to heat the digester. If additional heat was needed, biogas was used directly for heating the digester and was calculated assuming an energy content of biogas of 0.0338 m^3 MJ^{-1}.

Table S6-5. Heat loss coefficients and specific heat of sludge for AD design.

Parameter	Value	Units
Walls with insulation	0.7	W m^{-2} C^{-1}
Floor in contact with moist earth	2.85	W m^{-2} C^{-1}
Floating cover with insulation	0.95	W m^{-2} C^{-1}
Specific heat of sludge	4,200	J kg^{-1} C^{-1}

C. Conventional Activated Sludge with Anaerobic Digestion (CAS+AD)

The CAS+AD process flow diagram is presented in Figure S6-4. An SRT of 10 days was assumed, allowing for nitrification. The kinetic parameters used to model the CAS are given in Table S6-3. The AD used to digest the sludge was designed as described for HRAS+AD.

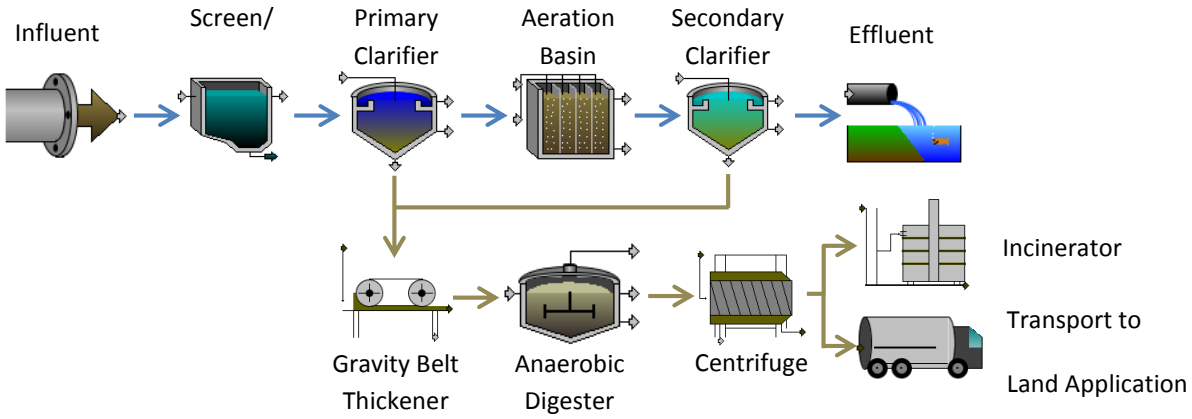


Figure S6-4. Process flow diagram for CAS+AD.

D. Aerobic Membrane Bioreactor with Anaerobic Digestion (AeMBR+AD)

AeMBR+AD process flow diagram is presented in Figure S6-5. The membrane materials and cleaning requirements were estimated as described for AnMBR. The SRT was assumed to be 10 days for the AeMBR, which was sufficient for nitrification to occur, to be consistent with CAS+AD. The kinetic parameters used to model the AeMBR are given in Table S6-3. The AD was designed as described for the HRAS+AD.

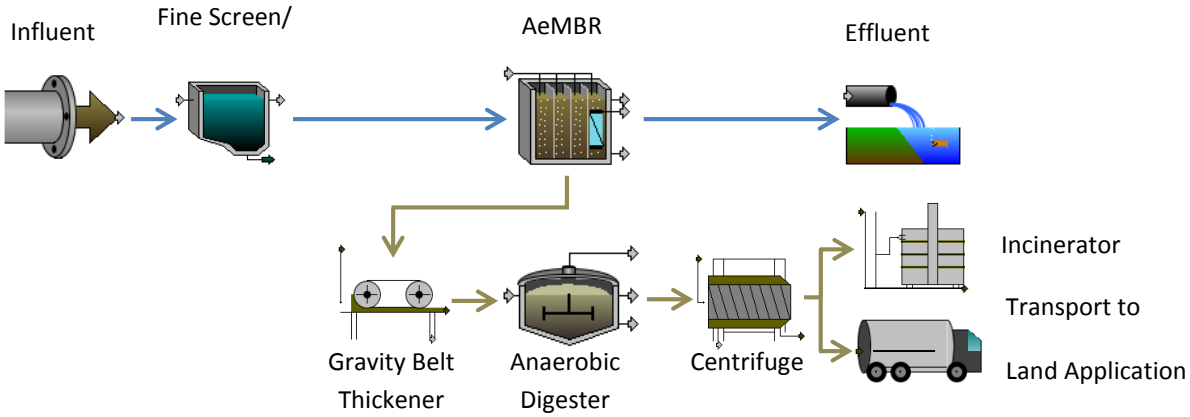


Figure S6-5. Process flow diagram for AeMBR+AD.

E. Summary of System Modeling

Table S6-6. Model Process Parameters and Effluent Quality for Baseline Scenario (15°C medium strength wastewater).

System	SRT	HRT	MLSS	$cBOD_5$	COD	Ammonia	Nitrate + Nitrite	TSS
	(d)	(hr)	(mg L^{-1})	(mg O_2 L^{-1})	(mg O_2 L^{-1})	(mg N L^{-1})	(mg N L^{-1})	(mg L^{-1})
CAS	10	8	1,380	10.1	53.9	0.5	26.9	18.7
HRAS	1.5	2	1,210	14.8	57.3	30.7	0.0	18.3
AeMBR	10	8	3,240	0.8	29.0	0.3	27.0	0.0
AnMBR	200	8	14,020	21.2*	64.5	40.0	0.0	0.0

SRT=solids retention time; HRT=hydraulic retention time; MLSS=mixed liquor suspended solids; Q=influent flow rate; SGD=specific gas demand; $cBOD_5$=carbonaceous five-day biochemical oxygen demand; COD=chemical oxygen demand; TSS=total suspended solids.
*BOD_5

2) Sludge Handling Scenarios

In all treatment scenarios, the sludge underwent gravity belt thickening and centrifuge dewatering. Centrifuge dewatering was assumed to produce a cake with 20% solids (Tchobanoglous et al. 2003). In the HRAS and CAS scenarios, primary sludge and thickened waste activated sludge (WAS) were blended before AD. Polymer (acrylonitrile) was dosed at 5 g per kg of dry solids in both the thickening and dewatering processes (Tchobanoglous et al. 2003).

A. Landfill

For HRAS+AD, CAS+AD, and AeMBR+AD, sludge was stabilized by AD and AnMBR sludge was lime stabilized.

B. Land application

Sludge was stabilized for each system as described in the landfill scenario to meet class B biosolids specifications. Nutrients present in land-applied biosolids were assumed to offset artificial nitrogen and phosphorus fertilizer use. Biosolids was assumed to offset 0.0196 g of artificial nitrogen fertilizer and 0.0274 g of artificial phosphorus fertilizer per g VSS of biosolids applied (Hospido et al. 2010). Electricity consumption and diesel for biosolids application was assumed to be 58.5 kWh and 0.73 kg per dry ton of biosolids (Hospido et al. 2005).

C. Incineration

Incineration was assumed to be an on-site fluidized bed incinerator. The ash produced was hauled to a landfill for disposal.

3) Emissions

A. Life Cycle Inventory

Energy calculations

Use-phase electrical energy requirements were calculated for each treatment scenario. Pumping energy requirements were calculated using equation 3.2 from Judd et al. (2010) Blower energy requirements were calculated using equation 5-56a from Metcalf & Eddy (Tchobanoglous et al. 2003). Gravity belt thickening and centrifuge dewatering energy requirements were calculated using equations 4 and 5, which were adapted from CAPDETWorks (Guest 2012). Mechanical mixing energy requirements in the AnMBR were based on anoxic reactor mixing requirements (8 kW/ 10^3 m^3, Metcalf & Eddy (Tchobanoglous et al. 2003)) and assuming that the mixer was only "on" a fraction of the time because some degree of mixing results from the production of biogas. Blend tank design and mixing energy were based on Qasim (1998) (section 16-9).

$$GBT_{EE}, kWh/day = \frac{\left(422{,}832\ \frac{kWh}{yr}\right)(GBT\ inf, MGD)^{0.9248}}{365.25\ days/yr} \quad \text{(eq. 4)}$$

$$CENT_{EE}, kWh/day = \frac{(5{,}024{,}825\ kWh/yr)(CENT\ inf, MGD) + 39{,}693\ kWh/yr}{365.25\ days/yr} \quad \text{(eq. 5)}$$

Unit Processes Included

Table S6-6 lists the unit processes considered in the life cycle inventory. Emissions data were taken from Ecoinvent (Frischknecht et al. 2005), U.S. LCI (Norris 2004), and ELCD (2010). Table S6-6 also indicates when modifications were made to the original database's emissions and where U.S. data were substituted for European data. Emissions data for PVDF production were not available in any life cycle inventory databases, so emissions for polyvinylidene chloride (PVDC) production, a similarly structured thermoplastic, were used instead. Citric acid, one of the membrane cleaning solutions, was also not found in any of the life cycle inventory databases. Organic chemical production (from the Ecoinvent database, which averages emissions from the top 20 organic chemicals produced) was used in its place. Land application of biosolids includes offset of artificial nitrogen and phosphorus fertilizer production, methane emissions, and diesel used during application (Hospido et al. 2010).

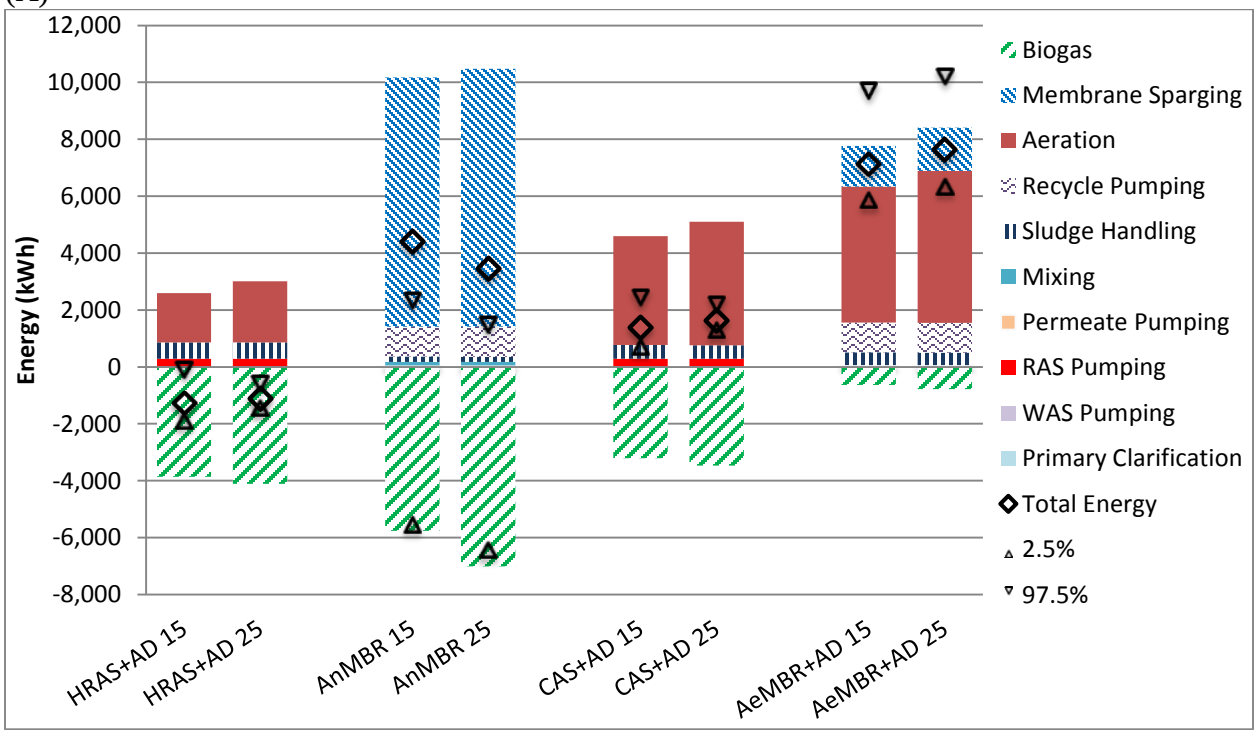
Table S6-7. Unit processes in life cycle inventory.

Unit Process	Source(s) of Emissions Data
Electricity generation and distribution (U.S. average)	U.S. LCI
Quicklime production	U.S. LCI
Single unit, diesel powered truck	U.S. LCI
Nitrogen fertilizer production	U.S. LCI
Phosphorus fertilizer production	U.S. LCI
Landfill of sludge, including landfill gas utilization	ELCD modified with U.S. electricity from U.S. LCI
Incineration process	Ecoinvent
Sodium hypochlorite production, 15% in water	Ecoinvent modified with U.S. electricity from U.S. LCI
Cogeneration with biogas engine	Ecoinvent
Heat produced using biogas (diffusion absorption heat pump 4kW)	Ecoinvent modified with U.S. electricity from U.S. LCI
Acrylonitrile (polymer) production	U.S. LCI
Land application of biosolids	Ecoinvent and U.S. LCI
Organic chemical production (top 20 averaged)	Ecoinvent
Polyvinylidene chloride (PVDC) production	Ecoinvent

B. Net energy balance

Net energy balance (NEB) for each treatment system at the two wastewater temperatures considered (15 and 25°C) for medium strength wastewater is shown in Figure S6-6 and for high strength wastewater in Figure S6-7.

(A)



(B)

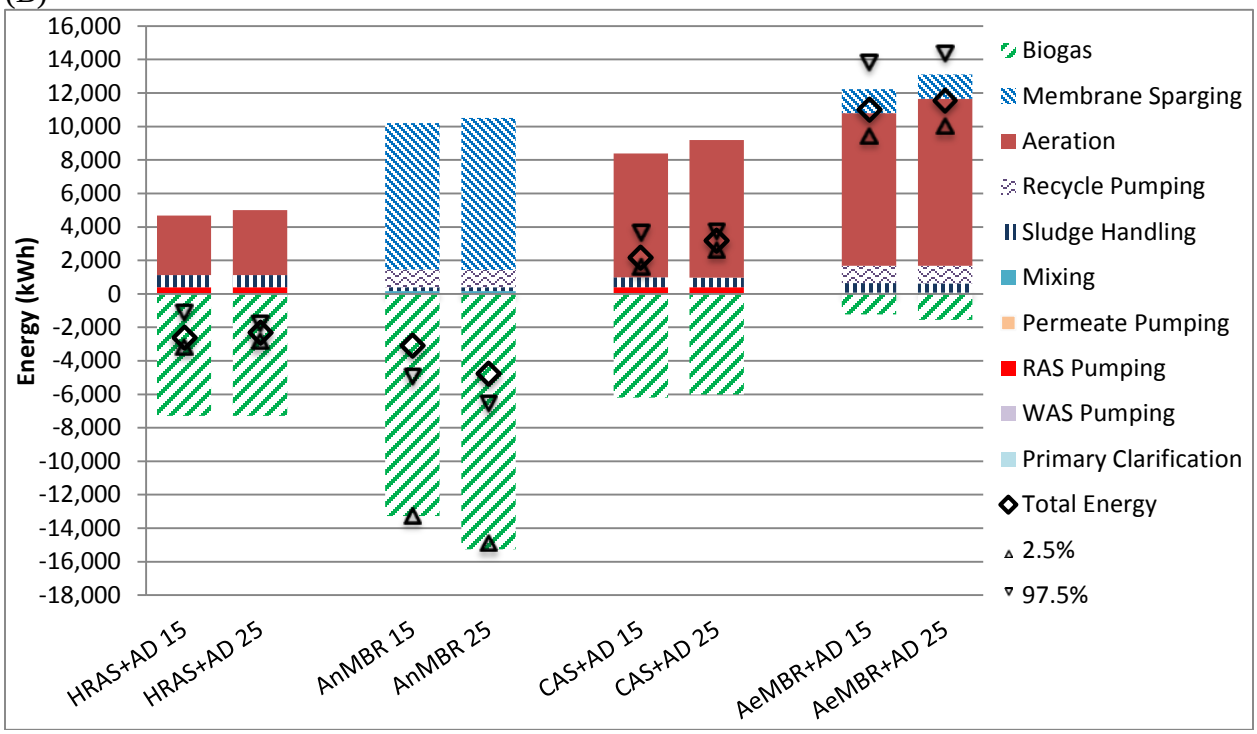
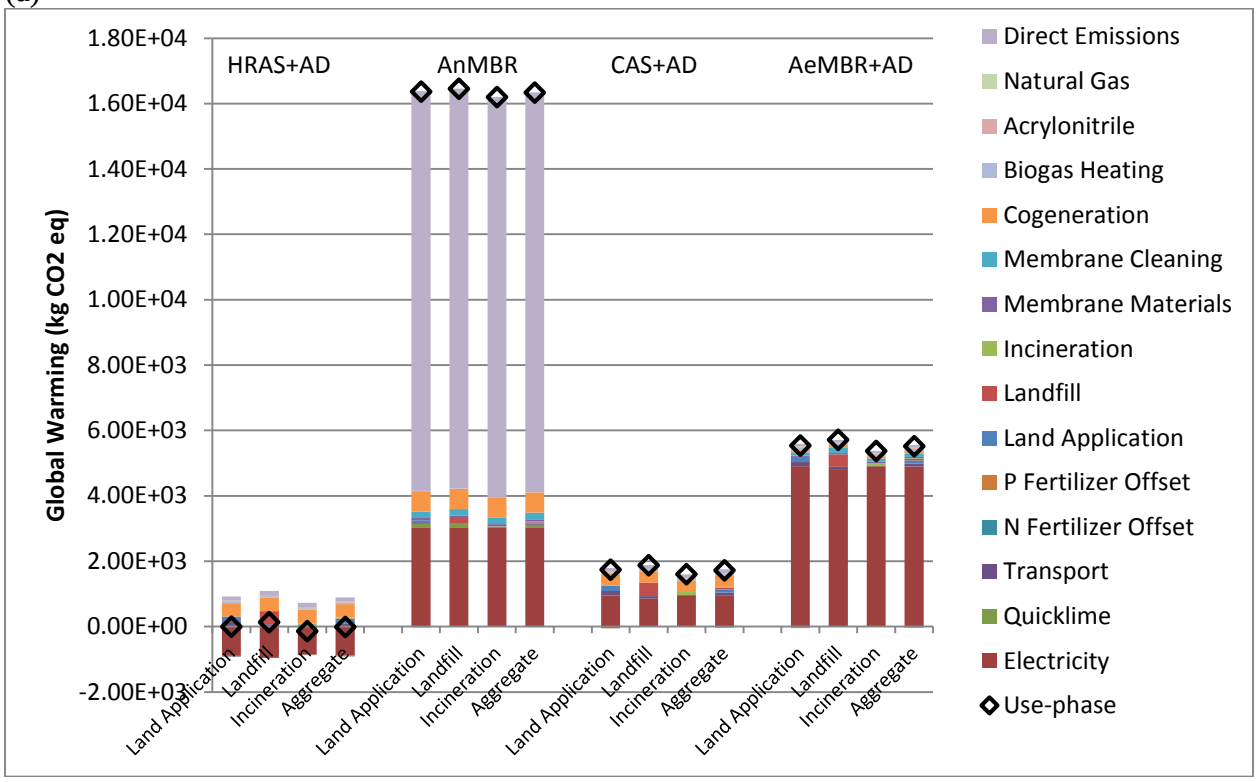


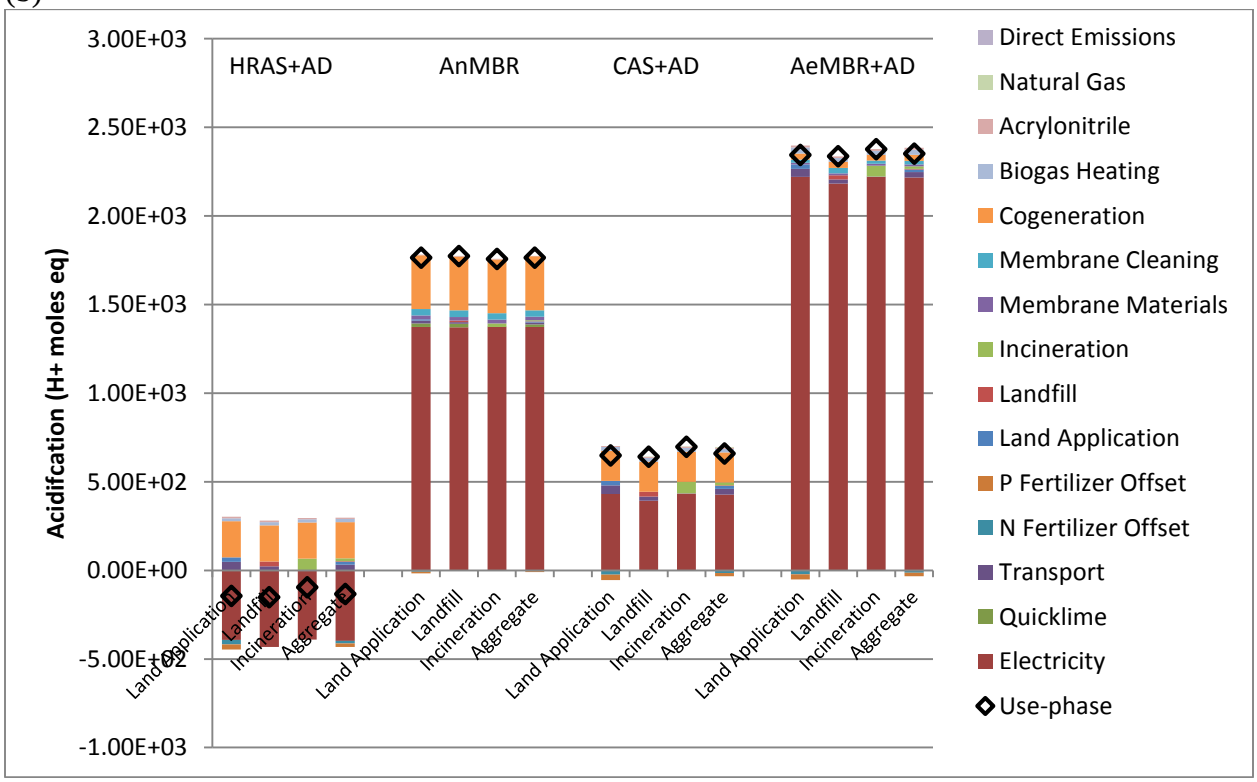
Figure S6-6. Net energy balance (NEB) for medium strength (A) and high strength (B) domestic wastewater at 15 and 25°C. Triangles (▽,△) represent the 95% confidence interval of net energy demand from the uncertainty analysis. For AnMBR, triangles represent uncertainty based on the developed parameter values.

C. Impact Assessment

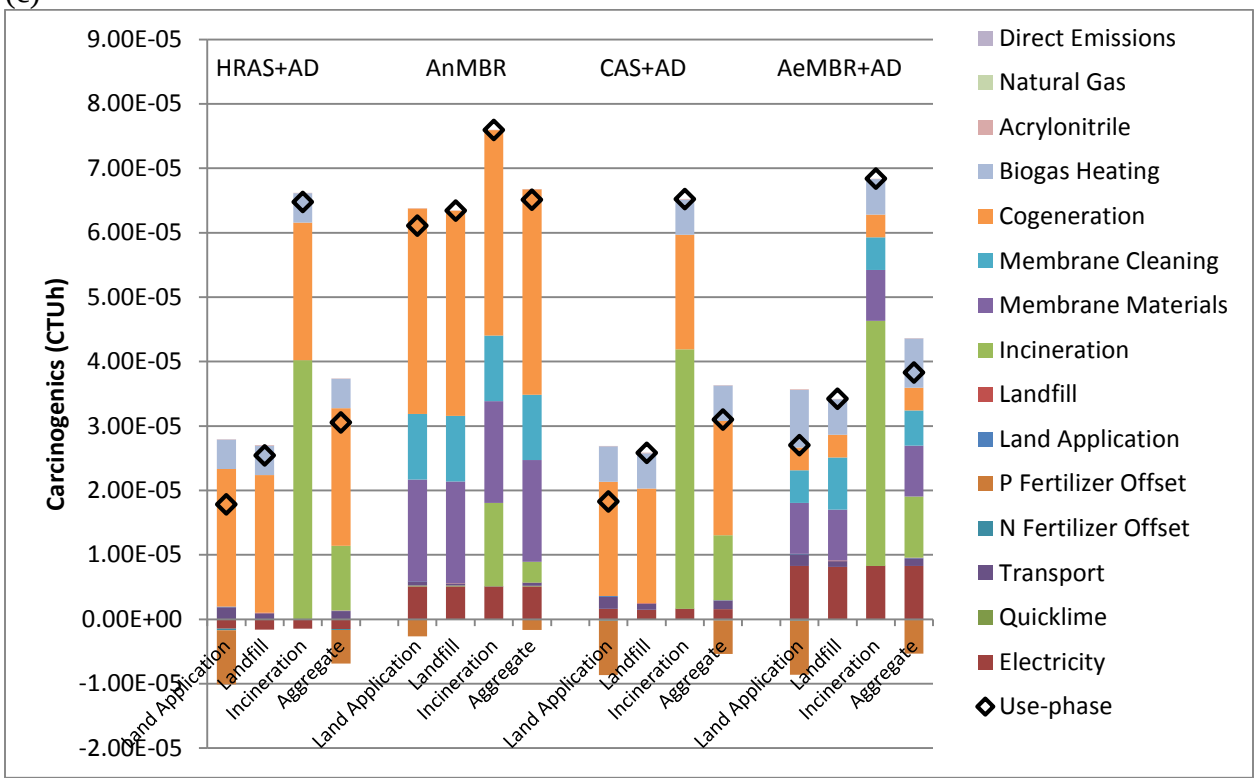
(a)



(b)



(c)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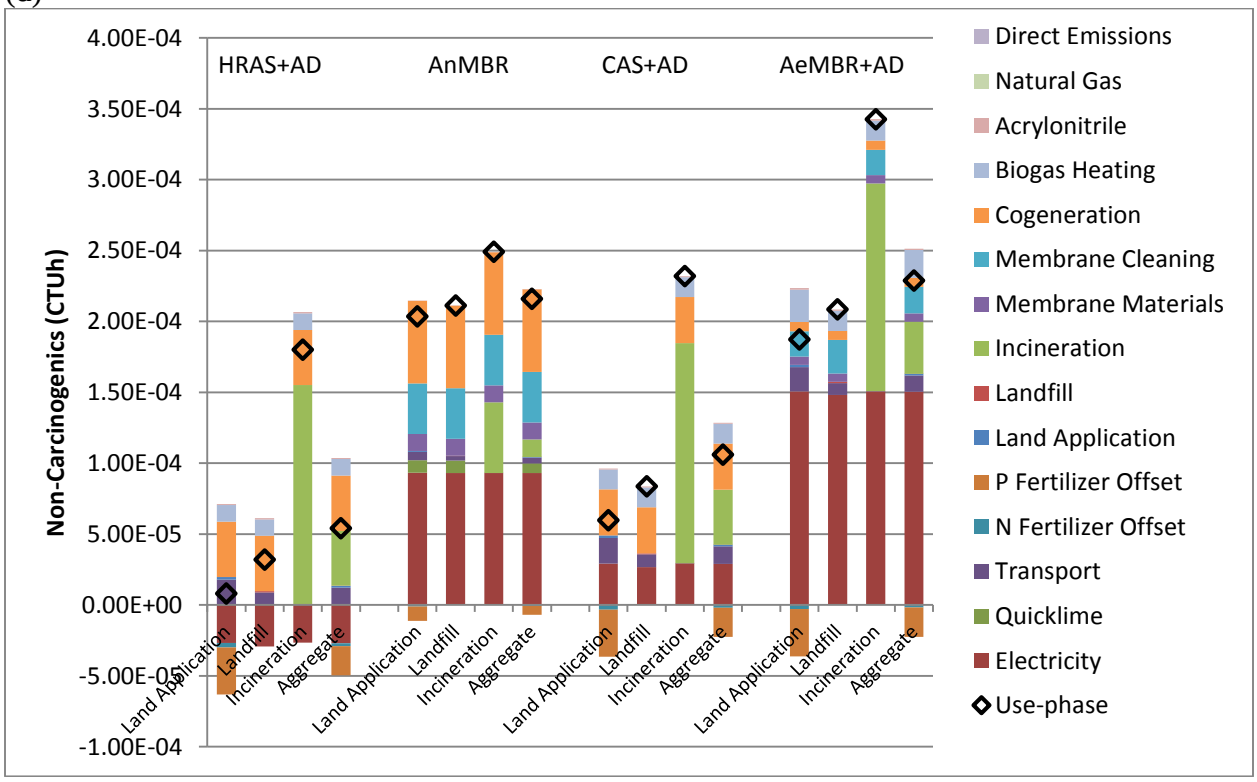
7.00E-05
6.00E-05
5.00E-05
4.00E-05
3.00E-05
2.00E-05
1.00E-05
0.00E+00
-1.00E-05
-2.00E-05
Carcinogenics (CTUh)
HRAS+AD
AnMBR
CAS+AD
AeMBR+AD
Land Application
Landfill
Incineration
Aggregate
Direct Emissions
Natural Gas
Acrylonitrile
Biogas Heating
Cogeneration
Membrane Cleaning
Membrane Materials
Incineration
Landfill
Land Application
P Fertilizer Offset
N Fertilizer Offset
Transport
Quicklime
Electricity
Use-phase

(d)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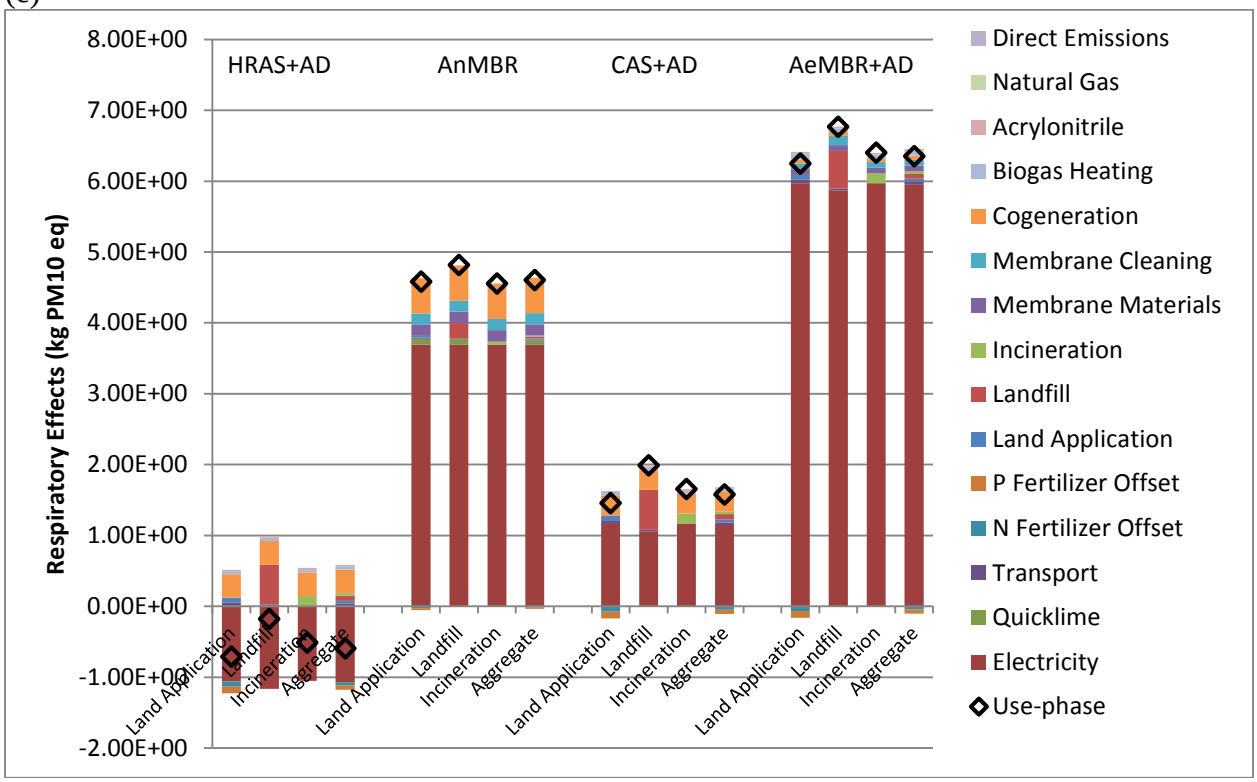
3.00E-04
2.50E-04
2.00E-04
1.50E-04
1.00E-04
5.00E-05
0.00E+00
-5.00E-05
-1.00E-04
Non-Carcinogenics (CTUh)
HRAS+AD
AnMBR
CAS+AD
AeMBR+AD
Land Application
Landfill
Incineration
Aggregate
Direct Emissions
Natural Gas
Acrylonitrile
Biogas Heating
Cogeneration
Membrane Cleaning
Membrane Materials
Incineration
Landfill
Land Application
P Fertilizer Offset
N Fertilizer Offset
Transport
Quicklime
Electricity
Use-phase

(e)


Respiratory Effects (kg PM10 eq)
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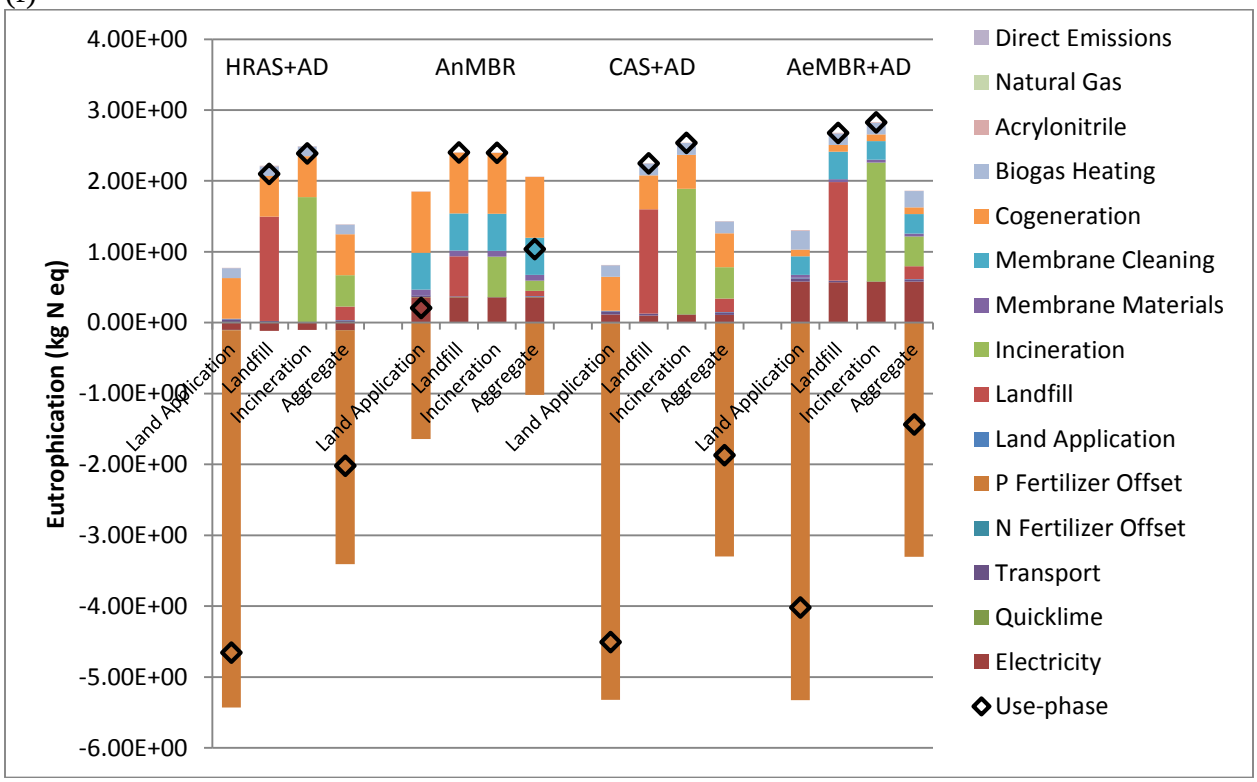
6.00E+00
5.00E+00
4.00E+00
3.00E+00
2.00E+00
1.00E+00
0.00E+00
-1.00E+00
-2.00E+00
HRAS+AD
AnMBR
CAS+AD
AeMBR+AD
Land Application
Landfill
Incineration
Aggregate
Direct Emissions
Natural Gas
Acrylonitrile
Biogas Heating
Cogeneration
Membrane Cleaning
Membrane Materials
Incineration
Landfill
Land Application
P Fertilizer Offset
N Fertilizer Offset
Transport
Quicklime
Electricity
Use-phase

(f)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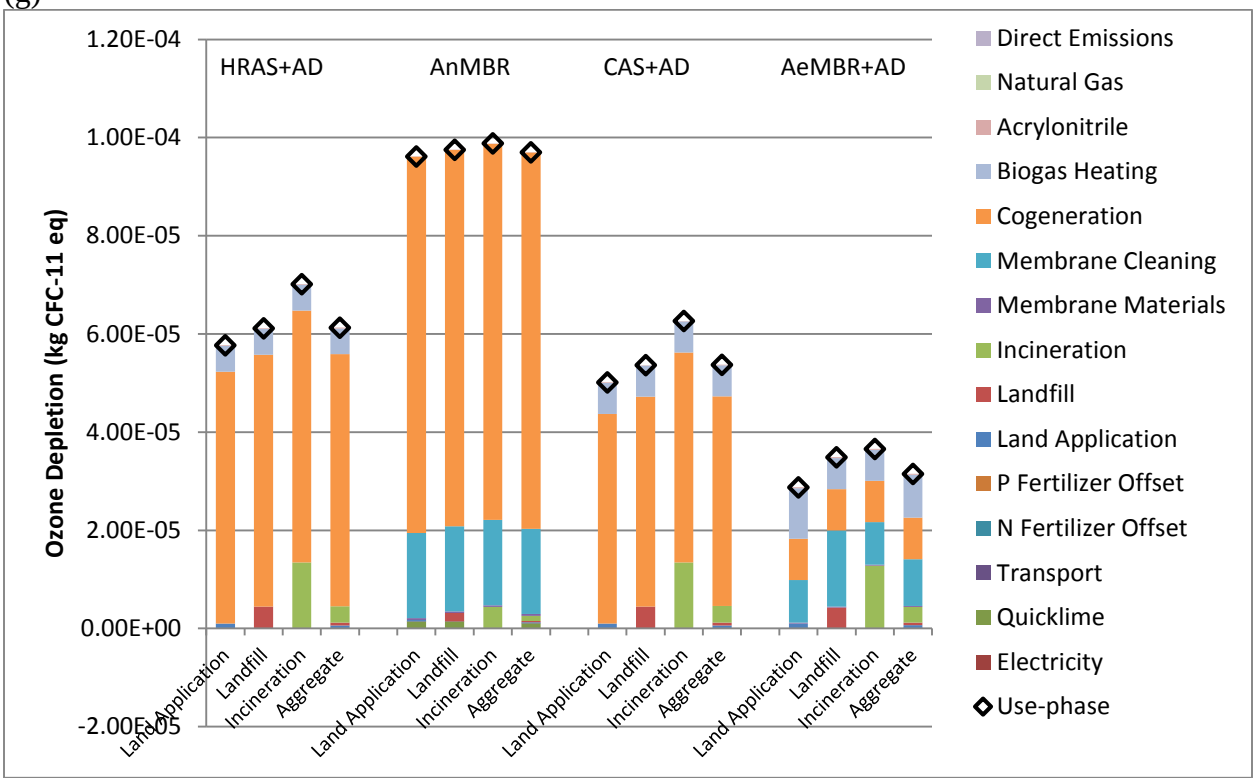
1.00E+00
0.00E+00
-1.00E+00
-2.00E+00
-3.00E+00
-4.00E+00
-5.00E+00
-6.00E+00
HRAS+AD
AnMBR
CAS+AD
AeMBR+AD
Land Application
Landfill
Incineration
Aggregate
Direct Emissions
Natural Gas
Acrylonitrile
Biogas Heating
Cogeneration
Membrane Cleaning
Membrane Materials
Incineration
Landfill
Land Application
P Fertilizer Offset
N Fertilizer Offset
Transport
Quicklime
Electricity
Use-phase

(g)


Ozone Depletion (kg CFC-11 eq)
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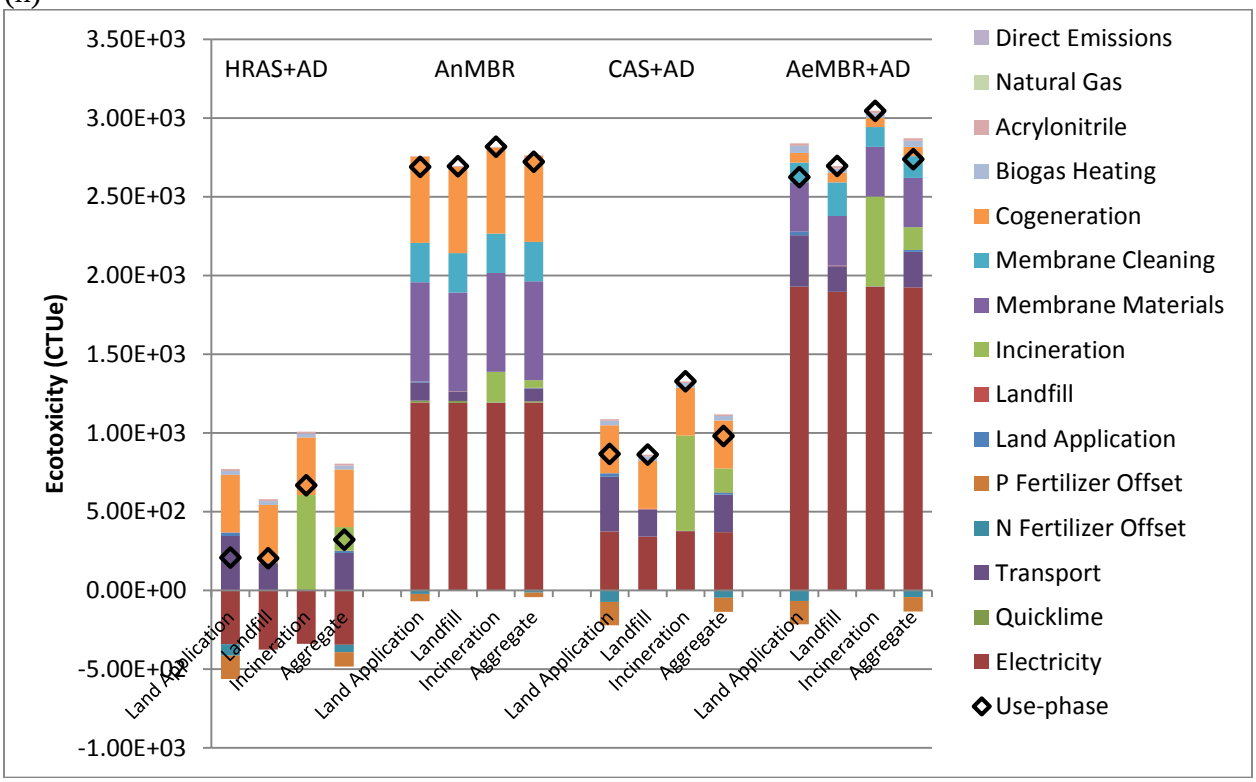
1.00E-04
8.00E-05
6.00E-05
4.00E-05
2.00E-05
0.00E+00
-2.00E-05
HRAS+AD
AnMBR
CAS+AD
AeMBR+AD
Land Application
Landfill
Incineration
Aggregate
Direct Emissions
Natural Gas
Acrylonitrile
Biogas Heating
Cogeneration
Membrane Cleaning
Membrane Materials
Incineration
Landfill
Land Application
P Fertilizer Offset
N Fertilizer Offset
Transport
Quicklime
Electricity
Use-phase

(h)


Ecotoxicity (CTUe)
3.50E+03
3.00E+03
2.50E+03
2.00E+03
1.50E+03
1.00E+03
5.00E+02
0.00E+00
-5.00E+02
-1.00E+03
HRAS+AD
AnMBR
CAS+AD
AeMBR+AD
Land Application
Landfill
Incineration
Aggregate
Direct Emissions
Natural Gas
Acrylonitrile
Biogas Heating
Cogeneration
Membrane Cleaning
Membrane Materials
Incineration
Landfill
Land Application
P Fertilizer Offset
N Fertilizer Offset
Transport
Quicklime
Electricity
Use-phase

(i)

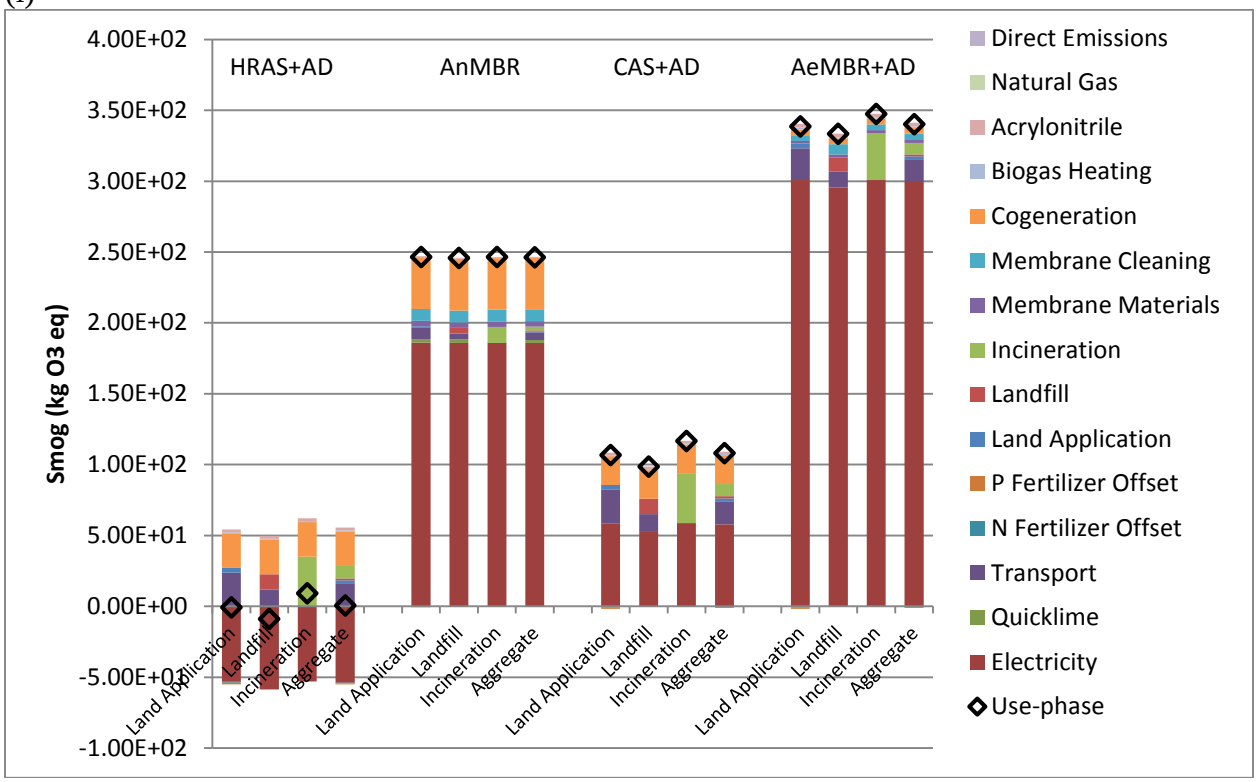


Figure S6-7. Environmental impacts of each sludge disposal scenario for medium strength wastewater at 15°C.

D. Uncertainty parameters

Table S6-8. Uncertainty parameters: ranges and data sources.

Uncertainty parameters	Units	System	Baseline value	Low value	High value	Distribution
Recycle flow	*Influent flow	AnMBR (C&F)	2	2	4	Uniform
	*Influent flow	AeMBR	2	2	4	Uniform
Flux	LMH	AnMBR (C)	10	7	17	Uniform
	LMH	AnMBR (F)	10	10	30	
	LMH	AeMBR	20	15	30	Uniform
Membrane lifetime	Years	AnMBR (C&F)	10	5	15	Triangular
	Years	AeMBR	10	5	15	Triangular
Dissolved methane recovered	%	AnMBR (F)	0	0	100	Uniform
Methane oversaturation		AnMBR (C&F)	1.5	1	2	Triangular
		HRAS-AD	5	1	10	Triangular
		AeMBR-AD	5	1	10	Triangular
		CAS-AD	5	1	10	Triangular
Specific gas demand	$m^3\ m^{-2}\ h^{-1}$	AnMBR (C)	0.23	0.10	0.50	Uniform
		AnMBR (F)	0.23	0.082	0.23	Uniform
Intermittent biogas sparging	% time on	AnMBR (C)	100	100	10000	Uniform
	% time on	AnMBR (F)	100	25	100	Uniform
	% time on	AeMBR	100	10	200	Uniform
Mixing	% time on	AnMBR (C&F)	10	0	100	Uniform
In-situ membrane cleaning	Times/month	AnMBR (C&F)	4.33	1.44	4.33	Uniform
frequency	Times/month	AeMBR	4.33	1.44	4.33	Uniform
Recovery cleaning frequency	Times/year	AnMBR (C&F)	1	1	2	Uniform
	Times/year	AeMBR	1	1	2	Uniform
Heating efficiency of biogas in AD		HRAS-AD	0.75	0.55	0.95	Triangular
		AeMBR-AD	0.75	0.55	0.95	Triangular
		CAS-AD	0.75	0.55	0.95	Triangular
Sludge transport distance (landfill)	km	(All)	100	10	160	Uniform
Sludge transport distance (land application)	km	(All)	50	10	160	Uniform
Sludge transport distance (incineration)	km	(All)	50	10	160	Uniform

Note: AnMBR (C) parameters were varied to reflect uncertainty in AnMBR in its current technological state. AnMBR (F) parameters were varied to reflect uncertainty related to potential future development in AnMBR technology.

E. Emissions Magnitudes

Table S6-9. The base case, mean, 2.5th, and 97.5th percentile values from each system's Monte Carlo simulation (medium strength wastewater, aggregate sludge practice).

			Acid.	Car.	Eco.	Eut.	GW	NC	OD	RE	Smog
CAS	15ºC	Base case	6.95E+02	3.64E-05	1.12E+03	-1.87+00	1.76E+03	1.29E-04	5.37E-05	1.69E+00	1.09E+02
		Mean	7.06E+02	3.67E-05	1.11E+03	-1.85E+00	1.79E+03	1.30E-04	5.39E-05	1.72E+00	1.10E+02
		2.50%	5.58E+02	3.17E-05	8.80E+02	-2.03E+00	1.42E+03	1.03E-04	5.21E-05	1.33E+00	8.56E+01
		97.50%	9.39E+02	4.44E-05	1.37E+03	-1.58E+00	2.33E+03	1.67E-04	5.68E-05	2.38E+00	1.40E+02
	25 ºC	Base case	7.59E+02	3.24E-05	1.17E+03	-1.94E+00	1.89E+03	1.21E-04	5.08E-05	1.85E+00	1.20E+02
		Mean	7.65E+02	3.26E-05	1.16E+03	-1.93E+00	1.91E+03	1.22E-04	5.09E-05	1.87E+00	1.20E+02
		2.50%	7.35E+02	3.15E-05	1.00E+03	-1.97E+00	1.78E+03	1.12E-04	5.06E-05	1.82E+00	1.08E+02
		97.50%	8.48E+02	3.53E-05	1.33E+03	-1.84E+00	2.11E+03	1.36E-04	5.19E-05	2.10E+00	1.33E+02
HRAS	15ºC	Base case	-9.85E+01	3.59E-05	4.61E+02	-2.02E+00	1.91E+01	7.69E-05	6.13E-05	-4.82E-01	1.94E+00
		Mean	-7.87E+01	3.65E-05	4.63E+02	-2.00E+00	7.12E+01	7.94E-05	6.15E-05	-4.22E-01	3.52E+00
		2.50%	-2.17E+02	3.19E-05	2.31E+02	-2.15E+00	-2.92E+02	5.40E-05	5.99E-05	-7.84E-01	-2.00E+01
		97.50%	1.76E+02	4.50E-05	7.35E+02	-1.69E+00	6.64E+02	1.20E-04	6.47E-05	3.01E-01	3.64E+01
	25 ºC	Base case	-5.48E+01	3.29E-05	5.01E+02	-2.09E+00	1.08E+02	7.07E-05	5.93E-05	-3.76E-01	9.28E+00
		Mean	-5.19E+01	3.29E-05	4.88E+02	-2.09E+00	1.22E+02	7.06E-05	5.94E-05	-3.64E-01	8.73E+00
		2.50%	-7.91E+01	3.19E-05	3.25E+02	-2.11E+00	-1.13E+01	6.16E-05	5.91E-05	-4.06E-01	-2.74E+00
		97.50%	1.69E+01	3.52E-05	6.53E+02	-2.01E+00	2.94E+02	8.22E-05	6.02E-05	-1.72E-01	2.06E+01
AeMBR	15ºC	Base case	2.39E+03	4.37E-05	2.87E+03	-1.44E+00	5.56E+03	2.51E-04	3.15E-05	6.46E+00	3.41E+02
		Mean	2.53E+03	4.24E-05	2.94E+03	-1.47E+00	5.87E+03	2.56E-04	2.88E-05	6.85E+00	3.60E+02
		2.50%	2.07E+03	3.70E-05	2.45E+03	-1.66E+00	4.85E+03	2.20E-04	2.61E-05	5.61E+00	2.97E+02
		97.50%	3.09E+03	5.00E-05	3.57E+03	-1.25E+00	7.10E+03	3.00E-04	3.26E-05	8.35E+00	4.36E+02
	25 ºC	Base case	2.53E+03	4.03E-05	2.98E+03	-1.20E+00	5.85E+03	2.51E-04	2.77E-05	6.83E+00	3.62E+02
		Mean	2.67E+03	3.89E-05	3.04E+03	-1.25E+00	6.15E+03	2.55E-04	2.47E-05	7.20E+00	3.79E+02
		2.50%	2.21E+03	3.31E-05	2.54E+03	-1.44E+00	5.12E+03	2.19E-04	2.17E-05	5.96E+00	3.16E+02
		97.50%	3.23E+03	4.68E-05	3.68E+03	-1.01E+00	7.41E+03	3.00E-04	2.89E-05	8.74E+00	4.57E+02
AnMBR (Current)	15ºC	Base case	1.76E+03	6.51E-05	2.72E+03	1.04E+00	1.63E+04	2.16E-04	9.70E-05	4.61E+00	2.46E+02
		Mean	2.47E+03	6.30E-05	3.20E+03	1.04E+00	1.78E+04	2.51E-04	9.06E-05	6.47E+00	3.40E+02

		2.50%	2.46E+02	4.74E-05	1.10E+03	3.47E-01	1.13E+04	9.38E-05	7.85E-05	4.60E-01	3.81E+01
		97.50%	6.01E+03	8.76E-05	6.61E+03	2.15E+00	2.64E+04	5.05E-04	1.04E-04	1.60E+01	8.20E+02
	25 **ºC**	Base case	1.54E+03	7.08E-05	2.58E+03	1.19E+00	1.38E+04	2.08E-04	1.13E-04	3.92E+00	2.14E+02
		Mean	2.26E+03	6.88E-05	3.08E+03	1.19E+00	1.53E+04	2.45E-04	1.07E-04	5.83E+00	3.10E+02
		2.50%	-1.95E+01	5.31E-05	9.28E+02	4.85E-01	9.08E+03	8.29E-05	9.61E-05	-3.20E-01	1.40E+00
		97.50%	5.93E+03	9.38E-05	6.58E+03	2.33E+00	2.39E+04	5.07E-04	1.19E-04	1.58E+01	8.08E+02
AnMBR (Future)	15**ºC**	Base case	1.76E+03	6.51E-05	2.72E+03	1.04E+00	1.63E+04	2.16E-04	9.70E-05	4.61E+00	2.46E+02
		Mean	-2.57E+02	4.99E-05	6.17E+02	2.99E-01	5.68E+03	6.06E-05	9.87E-05	-9.60E-01	-3.09E+01
		2.50%	-1.19E+03	4.00E-05	-2.65E+02	1.29E-02	-1.55E+03	-2.50E-06	8.18E-05	-3.50E+00	-1.57E+02
		97.50%	1.04E+03	6.47E-05	1.97E+03	7.80E-01	1.38E+04	1.57E-04	1.14E-04	2.59E+00	1.46E+02
	25 **ºC**	Base case	1.54E+03	7.08E-05	2.58E+03	1.19E+00	1.38E+04	2.08E-04	1.13E-04	3.92E+00	2.14E+02
		Mean	-5.00E+02	5.48E-05	4.58E+02	4.29E-01	4.09E+03	5.07E-05	1.13E-04	-1.68E+00	-6.51E+01
		2.50%	-1.41E+03	4.53E-05	-4.25E+02	1.38E-01	-2.16E+03	-1.25E-05	9.86E-05	-4.17E+00	-1.89E+02
		97.50%	8.37E+02	6.98E-05	1.87E+03	9.30E-01	1.12E+04	1.52E-04	1.27E-04	1.97E+00	1.17E+02

Table S6-10. The base case, mean, 2.5th and 97.5th percentile values from each system's Monte Carlo simulation (high strength wastewater, aggregate sludge practice).

			Acid.	Car.	Eco.	Eut.	GW	NC	OD	RE	Smog
CAS	15ºC	Base case	1.14E+03	5.77E-05	1.92E+03	-2.44E+00	2.85E+03	2.05E-04	9.11E-05	2.71E+00	1.85E+02
		Mean	1.18E+03	5.92E-05	1.94E+03	-2.38E+00	2.96E+03	2.11E-04	9.17E-05	2.85E+00	1.89E+02
		2.50%	1.09E+03	5.60E-05	1.61E+03	-2.48E+00	2.69E+03	1.88E-04	9.08E-05	2.66E+00	1.64E+02
		97.50%	1.44E+03	6.79E-05	2.28E+03	-2.07E+00	3.57E+03	2.53E-04	9.49E-05	3.58E+00	2.26E+02
	25 ºC	Base case	1.43E+03	5.76E-05	2.17E+03	-2.37E+00	3.50E+03	2.23E-04	8.85E-05	3.52E+00	2.25E+02
		Mean	1.43E+03	5.74E-05	2.14E+03	-2.37E+00	3.50E+03	2.21E-04	8.85E-05	3.52E+00	2.23E+02
		2.50%	1.39E+03	5.59E-05	1.85E+03	-2.41E+00	3.33E+03	2.06E-04	8.82E-05	3.47E+00	2.03E+02
		97.50%	1.47E+03	5.90E-05	2.43E+03	-2.33E+00	3.68E+03	2.36E-04	8.87E-05	3.57E+00	2.43E+02
HRAS	15ºC	Base case	-2.94E+02	5.87E-05	7.54E+02	-2.72E+00	-2.85E+02	1.17E-04	1.06E-04	-1.20E+00	-8.08E+00
		Mean	-2.51E+02	6.01E-05	7.61E+02	-2.67E+00	-1.78E+02	1.23E-04	1.06E-04	-1.07E+00	-4.48E+00
		2.50%	-3.38E+02	5.70E-05	4.32E+02	-2.78E+00	-4.62E+02	1.00E-04	1.05E-04	-1.25E+00	-3.00E+01
		97.50%	2.94E+01	6.94E-05	1.12E+03	-2.33E+00	4.73E+02	1.68E-04	1.10E-04	-2.78E-01	3.41E+01
	25 ºC	Base case	-1.90E+02	5.92E-05	8.50E+02	-2.71E+00	-5.28E+01	1.25E-04	1.06E-04	-9.17E-01	6.37E+00
		Mean	-1.94E+02	5.91E-05	8.20E+02	-2.71E+00	-5.12E+01	1.23E-04	1.06E-04	-9.20E-01	4.38E+00
		2.50%	-2.36E+02	5.74E-05	5.14E+02	-2.75E+00	-2.42E+02	1.07E-04	1.05E-04	-9.71E-01	-1.66E+01
		97.50%	-1.51E+02	6.07E-05	1.13E+03	-2.67E+00	1.44E+02	1.39E-04	1.06E-04	-8.69E-01	2.53E+01
AeMBR	15ºC	Base case	3.68E+03	6.13E-05	4.28E+03	-2.06E+00	8.57E+03	3.81E-04	4.48E-05	9.90E+00	5.30E+02
		Mean	3.82E+03	5.99E-05	4.33E+03	-2.10E+00	8.88E+03	3.85E-04	4.21E-05	1.03E+01	5.48E+02
		2.50%	3.35E+03	5.39E-05	3.76E+03	-2.31E+00	7.81E+03	3.43E-04	3.91E-05	8.99E+00	4.80E+02
		97.50%	4.39E+03	6.80E-05	5.01E+03	-1.85E+00	1.01E+04	4.33E-04	4.60E-05	1.18E+01	6.27E+02
	25 ºC	Base case	3.85E+03	5.91E-05	4.41E+03	-1.70E+00	8.92E+03	3.84E-04	4.35E-05	1.03E+01	5.53E+02
		Mean	3.99E+03	5.76E-05	4.47E+03	-1.74E+00	9.22E+03	3.87E-04	4.05E-05	1.07E+01	5.71E+02
		2.50%	3.52E+03	5.05E-05	3.89E+03	-1.98E+00	8.16E+03	3.44E-04	3.71E-05	9.43E+00	5.03E+02
		97.50%	4.57E+03	6.65E-05	5.15E+03	-1.47E+00	1.05E+04	4.37E-04	4.49E-05	1.23E+01	6.51E+02
AnMBR (Current)	15ºC	Base case	-1.50E+02	9.91E-05	1.47E+03	1.16E+00	1.21E+04	1.42E-04	1.98E-04	-9.52E-01	-1.54E+01
		Mean	5.68E+02	9.70E-05	1.96E+03	1.16E+00	1.36E+04	1.79E-04	1.92E-04	9.51E-01	8.01E+01

		2.50%	-1.65E+03	8.13E-05	-1.47E+02	4.70E-01	7.06E+03	2.10E-05	1.80E-04	-5.04E+00	-2.21E+02
		97.50%	4.12E+03	1.21E-04	5.35E+03	2.28E+00	2.21E+04	4.34E-04	2.05E-04	1.06E+01	5.63E+02
	25 **ºC**	Base case	-5.73E+02	1.08E-04	1.19E+03	1.40E+00	9.11E+03	1.25E-04	2.24E-04	-2.20E+00	-7.50E+01
		Mean	1.60E+02	1.06E-04	1.70E+03	1.41E+00	1.07E+04	1.63E-04	2.18E-04	-2.59E-01	2.25E+01
		2.50%	-2.13E+03	9.01E-05	-4.70E+02	6.95E-01	4.45E+03	-1.50E-07	2.07E-04	-6.46E+00	-2.89E+02
		97.50%	3.81E+03	1.31E-04	5.18E+03	2.55E+00	1.92E+04	4.24E-04	2.30E-04	9.61E+00	5.18E+02
AnMBR (Future)	15**ºC**	Base case	-1.50E+02	9.91E-05	1.47E+03	1.16E+00	1.21E+04	1.42E-04	1.98E-04	-9.52E-01	-1.54E+01
		Mean	-2.17E+03	8.38E-05	-6.40E+02	4.20E-01	1.42E+03	-1.29E-05	2.00E-04	-6.51E+00	-2.92E+02
		2.50%	-3.10E+03	7.39E-05	-1.53E+03	1.30E-01	-5.82E+03	-7.65E-05	1.83E-04	-9.05E+00	-4.19E+02
		97.50%	-8.81E+02	9.86E-05	7.26E+02	9.01E-01	9.65E+03	8.36E-05	2.15E-04	-2.99E+00	-1.16E+02
	25 **ºC**	Base case	-5.73E+02	1.08E-04	1.19E+03	1.40E+00	9.11E+03	1.25E-04	2.24E-04	-2.20E+00	-7.50E+01
		Mean	-2.61E+03	9.20E-05	-9.36E+02	6.40E-01	-6.08E+02	-3.20E-05	2.24E-04	-7.80E+00	-3.54E+02
		2.50%	-3.53E+03	8.24E-05	-1.82E+03	3.45E-01	-6.84E+03	-9.54E-05	2.10E-04	-1.03E+01	-4.79E+02
		97.50%	-1.29E+03	1.07E-04	4.60E+02	1.13E+00	6.46E+03	6.79E-05	2.38E-04	-4.19E+00	-1.74E+02

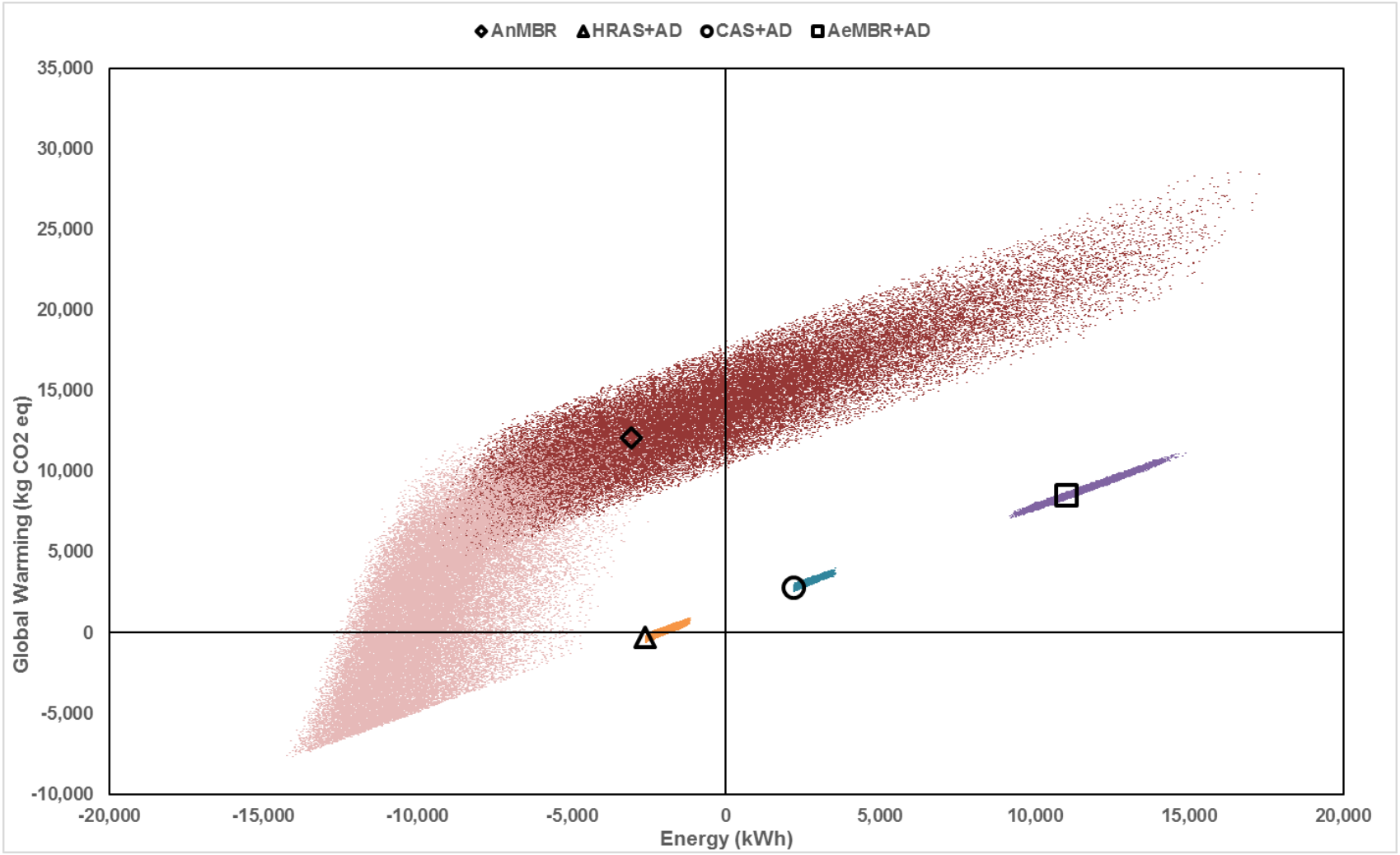


Figure S6-8. Global warming versus net energy balance (NEB) for HRAS+AD, AnMBR, CAS+AD, and AeMBR+AD for high strength domestic wastewater at 15°C. Open markers represent the baseline conditions. Colored dots indicate the values outputted by Monte Carlo simulations for HRAS+AD, AnMBR (current and future), CAS+AD, and AeMBR+AD.

F. Sensitivity Analysis

Table S6-11. Medium strength domestic wastewater at 15ºC and aggregate sludge handling: Emissions categories sensitive (|ρ|>0.6)* to a particular uncertainty parameter.

	Heating efficiency	Maintenance cleaning frequency	Membrane lifetime	Recycle ratio	CH_4 oversaturation	CH_4 recovery	Intermittent biogas sparging	Flux	Sludge transport distance for land application
Acid.	-CAS, -HRAS						AeMBR, AnMBR(C&F)		
Car.	-CAS, -HRAS							-AeMBR, -AnMBR(C&F)	
Eco.	-CAS, -HRAS						AeMBR, AnMBR(C)	-AnMBR(F)	CAS, HRAS
Eut.	-CAS, -HRAS						AnMBR(C)	-AnMBR(C&F)	
GW	-CAS, -HRAS					-AnMBR(F)	AeMBR, AnMBR(C)		
NC	-CAS, -HRAS						AeMBR, AnMBR(C&F)	-AnMBR(F)	
OD	-CAS, -HRAS	AeMBR			-AnMBR(C)	AnMBR(F)		-AeMBR	
RE	-CAS, -HRAS						AeMBR, AnMBR(C&F)		
Smog							AeMBR, AnMBR(C&F)		

Table S6-12. High strength domestic wastewater at 15ºC and aggregate sludge handling: Emissions categories sensitive (|ρ|>0.6)* to a particular uncertainty parameter.

	Heating efficiency	Maintenance cleaning frequency	Membrane lifetime	Recycle ratio	CH_4 oversaturation	CH_4 recovery	Intermittent biogas sparging	Flux	Sludge transport distance for land application
Acid.	-CAS, -HRAS			AeMBR			AeMBR, AnMBR(C&F)		
Car.	-CAS							-AeMBR, -AnMBR(C&F)	
Eco.							AeMBR, AnMBR(C&F)	-AnMBR(F)	CAS, HRAS, AeMBR
Eut.	-CAS, -HRAS						AnMBR(C)	-AnMBR(C&F)	
GW	-CAS			AeMBR		-AnMBR(F)	AeMBR, AnMBR(C)		
NC							AeMBR, AnMBR(C&F)		CAS, HRAS, AeMBR
OD	-CAS, -HRAS	AeMBR			-AnMBR(C)	AnMBR(F)			
RE	-CAS, -HRAS			AeMBR			AeMBR, AnMBR(C&F)		
Smog							AeMBR, AnMBR(C&F)		CAS, HRAS, AeMBR

Note: *ρ: rank correlation coefficient indicates ranking sensitivity of emissions categories to a particular uncertainty parameter. Negative and positive signs indicate correlation. Abbreviations: Acid.=acidification (H+ moles equivalent); Car.=carcinogenics (benzene equivalent); Eco.=ecotoxicity (kg 2,4-D equivalent); Eut.=eutrophication (kg nitrogen equivalent); GW=global warming (kg CO_2 equivalent); NC =noncarcinogenics (toluene equivalent); OD=ozone depletion (kg CFC-11 equivalent); RE=respiratory effects (kg PM2.5 equivalent); and Smog=Smog (kg NOX equivalent).

4) Life Cycle Costing

A. Costs Appended to CAPDETWorks Cost Estimates

Table S6-13. Supplemental costs appended to CAPDETWorks cost estimations

Supplemental Costs	Unit Cost	Unit	Lifetime (yr)	Reference
Quicklime	0.18	$/lb		(Hydromantis Inc. 2010)
Membranes	4,097,192	$/5MGD	10	(Hong 2012)
Tank cover	295,487	$/each	40	
Gas safety	76,000	$/each	20	(Hydromantis Inc. 2010)
PLC & cleaning CIP	135,000	$/each	20	(Hong 2012)
Permeate pump	81,000	$/each	20	(Hong 2012)
RAS pump (MBRs)	99,900	$/each	20	(Hong 2012)
Piping (blower & RAS; MBRs)	135,000	$/each	40	(Hong 2012)
Membrane labor, installation, & commissioning	405,000	$/each	40	(Hong 2012)
CHP	800	$/kW	20	(Chambers and Potter 2002)
CHP maintenance	0.0134	$/kWh/yr		(Chambers and Potter 2002)
Hypochlorite	$0.75	/gallon		(US Peroxide 2012)
Citric acid	1.98	$/kg		(Brinckerhoff 2001)

B. Construction and Equipment Cost Estimates

Table S6-14. Construction and equipment costs for land application and landfill scenarios.

	HRAS	AnMBR	CAS	AeMBR
Blower System	$55,300.00	$772,000.00	$509,000.00	$677,000.00
Equalization Basin	$0.00	$340,000.00	$0.00	$340,000.00
Preliminary Treatment	$347,000.00	$493,000.00	$347,000.00	$493,000.00
Primary Clarification	$338,000.00	$0.00	$338,000.00	$0.00
Secondary Clarification	$379,000.00	$0.00	$375,000.00	$0.00
Secondary Treatment	$901,000.00	$1,290,000.00	$1,930,000.00	$1,260,000.00
Anaerobic Digester	$2,100,000.00	$0.00	$2,020,000.00	$1,610,000.00
Gravity Belt Thickener	$1,840,000.00	$676,000.00	$1,300,000.00	$1,300,000.00
Centrifuge	$1,920,000.00	$357,000.00	$1,380,000.00	$829,000.00
Hauling and Landfill	$293,000.00	$247,000.00	$291,000.00	$269,000.00
Membrane System	$0.00	$8,204,873.64	$0.00	$4,564,274.71
CHP	$166,426.46	$283,738.39	$140,507.38	$31,571.41
Cover and Gas Safety	$0.00	$387,792.66	$0.00	$0.00
Mixer	$0.00	$8,950.00	$0.00	$0.00
Blend Tank	$39,400.00	$0.00	$24,400.00	$0.00
Other	$14,900,000.00	$14,140,000.00	$15,200,000.00	$16,140,000.00
Total	**$23,279,126.46**	**$27,200,354.70**	**$23,854,907.38**	**$27,513,846.13**

Table S6-15. Construction and equipment costs for incineration scenario.

	HRAS	AnMBR	CAS	AeMBR
Blower System	$55,300.00	$772,000.00	$509,000.00	$677,000.00
Equalization Basin	$0.00	$340,000.00	$0.00	$340,000.00
Preliminary Treatment	$347,000.00	$493,000.00	$347,000.00	$493,000.00
Primary Clarification	$338,000.00	$0.00	$338,000.00	$0.00
Secondary Clarification	$379,000.00	$0.00	$375,000.00	$0.00
Secondary Treatment	$901,000.00	$1,290,000.00	$1,930,000.00	$1,260,000.00
Anaerobic Digester	$2,100,000.00	$0.00	$2,020,000.00	$1,610,000.00
Gravity Belt Thickener	$1,840,000.00	$676,000.00	$1,300,000.00	$1,300,000.00
Centrifuge	$1,920,000.00	$357,000.00	$1,380,000.00	$829,000.00
Incinerator	$1,730,000.00	$1,570,000.00	$1,730,000.00	$1,610,000.00
Hauling and Landfill	$239,000.00	$238,000.00	$239,000.00	$238,000.00
Membrane System	$0.00	$8,204,873.64	$0.00	$4,564,274.71
CHP	$166,426.46	$283,738.39	$140,507.38	$31,571.41
Cover and Gas Safety	$0.00	$387,792.66	$0.00	$0.00
Mixer	$0.00	$8,950.00	$0.00	$0.00
Blend Tank	$39,400.00	$0.00	$24,400.00	$0.00
Other	$16,200,000.00	$15,240,000.00	$16,400,000.00	$17,340,000.00
Total	**$26,255,126.46**	**$29,861,354.70**	**$26,732,907.38**	**$30,292,846.13**

C. Present Worth, Construction, and Yearly Cost Estimates

Table S6-16. Present worth, construction, and yearly cost estimates for land application scenario (8% discount rate).

System	Present Worth	Project	Operation (/yr)	Maint. (/yr)	Material (/yr)	Chemical (/yr)	Energy (/yr)	Total (/yr)
HRAS 25_Landapp	32,675,208.42	23,279,126.46	453,000.00	125,117.06	193,000.00	28,500.00	-70,027.29	729,589.77
AnMBR 25_Landapp	37,784,698.89	27,200,354.70	299,000.00	110,502.01	141,000.00	201,711.29	69,643.00	821,856.31
CAS 25_Landapp	36,350,189.99	23,854,907.38	489,000.00	142,984.05	195,000.00	24,000.00	119,253.38	970,237.42
AeMBR 25_Landapp	40,652,433.90	27,513,846.13	386,000.00	95,516.77	165,000.00	112,876.39	260,795.82	1,020,188.97
HRAS 15_Landapp	32,777,576.61	23,279,126.46	453,000.00	123,887.12	193,000.00	28,500.00	-60,848.63	737,538.48
AnMBR 15_Landapp	38,300,035.07	27,200,354.70	299,000.00	104,405.28	141,000.00	202,324.97	115,141.04	861,871.29
CAS 15_Landapp	36,455,406.45	23,854,907.38	489,000.00	141,719.89	195,000.00	24,000.00	128,687.42	978,407.30
AeMBR 15_Landapp	40,711,818.92	27,513,846.13	386,000.00	94,803.26	165,000.00	112,876.39	266,120.47	1,024,800.12

Table S6-17. Present worth, construction, and yearly cost estimates for landfill scenario (8% discount rate).

System	Present Worth	Project	Operation (/yr)	Maint. (/yr)	Material (/yr)	Chemical (/yr)	Energy (/yr)	Total (/yr)
HRAS 25_Landfill	39,603,885.75	23,279,126.46	432,000.00	125,117.06	752,000.00	28,500.00	-70,027.29	1,267,589.77
AnMBR 25_Landfill	38,286,963.61	27,200,354.70	296,000.00	110,502.01	183,000.00	201,711.29	69,643.00	860,856.31
CAS 25_Landfill	42,892,509.85	23,854,907.38	470,000.00	142,984.05	722,000.00	24,000.00	119,253.38	1,478,237.42
AeMBR 25_Landfill	44,103,893.98	27,513,846.13	375,000.00	95,516.77	444,000.00	112,876.39	260,795.82	1,288,188.97
HRAS 15_Landfill	39,706,253.94	23,279,126.46	432,000.00	123,887.12	752,000.00	28,500.00	-60,848.63	1,275,538.48
AnMBR 15_Landfill	38,802,299.79	27,200,354.70	296,000.00	104,405.28	183,000.00	202,324.97	115,141.04	900,871.29
CAS 15_Landfill	42,997,726.31	23,854,907.38	470,000.00	141,719.89	722,000.00	24,000.00	128,687.42	1,486,407.30
AeMBR 15_Landfill	44,163,279.00	27,513,846.13	375,000.00	94,803.26	444,000.00	112,876.39	266,120.47	1,292,800.12

Table S6-18. Present worth, construction, and yearly cost estimates for incineration scenario (8% discount rate).

System	Present Worth	Project	Operation (/yr)	Maint. (/yr)	Material (/yr)	Chemical (/yr)	Energy (/yr)	Total (/yr)
HRAS 25_Incineration	39,075,623.48	26,255,126.46	474,000.00	148,117.06	190,000.00	28,500.00	154,872.71	995,489.77
AnMBR 25_Incineration	40,990,997.17	29,861,354.70	320,000.00	121,402.01	125,000.00	190,152.78	107,643.00	864,197.79
CAS 25_Incineration	42,512,228.50	26,732,907.38	511,000.00	165,984.05	191,000.00	24,000.00	333,253.38	1,225,237.42
AeMBR 25_Incineration	45,405,720.58	30,292,846.13	409,000.00	112,816.77	152,000.00	112,876.39	386,795.82	1,173,488.97
HRAS 15_Incineration	39,177,991.67	26,255,126.46	474,000.00	146,887.12	190,000.00	28,500.00	164,051.37	1,003,438.48
AnMBR 15_Incineration	41,498,430.13	29,861,354.70	320,000.00	115,305.28	125,000.00	190,152.78	153,141.04	903,599.10
CAS 15_Incineration	42,617,444.96	26,732,907.38	511,000.00	164,719.89	191,000.00	24,000.00	342,687.42	1,233,407.30
AeMBR 15_Incineration	45,465,105.60	30,292,846.13	409,000.00	112,103.26	152,000.00	112,876.39	392,120.47	1,178,100.12

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