Anaerobic Membrane Bioreactors to Treat Municipal Wastewater at Ambient Temperatures

Matthew D. Seib
Marquette University

ANAEROBIC MEMBRANE BIOREACTORS TO TREAT MUNICIPAL WASTEWATER AT AMBIENT TEMPERATURES

by

Matthew D. Seib

A Dissertation submitted to the Faculty of the Graduate School,
Marquette University,
in Partial Fulfillment of the Requirements for
the Degree of Doctor of Philosophy

Milwaukee, Wisconsin

December 2015

ABSTRACT
ANAEROBIC MEMBRANE BIOREACTORS TO TREAT
MUNICIPAL WASTEWATER AT AMBIENT TEMPERATURES

Matthew D. Seib

Marquette University, 2015

Anaerobic biotechnology is viewed as a sustainable alternative to aerobic biotechnology for municipal wastewater recovery. However, anaerobic processes have not been successful in cold climates. Past examples have not been able to meet low organic effluent concentrations, or have failed due to biomass washout resulting from low temperature operation and short hydraulic residence time.

Recently, the anaerobic membrane bioreactor (AnMBR) has been shown to achieve low effluent organic concentrations and maintain stable anaerobic biomass. However, shortcomings have included high energy demands for membrane operation and poor understanding of microbial community structures within AnMBRs. This dissertation describes efforts to improve AnMBRs by developing a low energy membrane operation strategy and describes the microbial relationships responsible for organic removal.

Two different AnMBR configurations were operated at both 10 and 25^{o}C. The AnMBRs achieved over 94% organic removal with average permeate five-day biochemical oxygen demand (BOD_5) concentrations remaining at 10 mg/L or less while treating synthetic or real primary effluent municipal wastewater. The AnMBRs utilized either ceramic or polymeric external tubular membranes that were operated at crossflow velocities (CFV) ranging from 0.018 to 0.3 m/s, which is below the typical CVF range of 2 to 5 m/s. Use of fluidized granular activated carbon (GAC) within the membranes at very low CFV extended membrane run time between cleanings by 55 to 120% and resulted in energy demands of 0.07 to 0.15 kWh/m^3, which represents a 98% energy savings compared to historical energy requirements.

Additionally, Illumina sequencing and statistical techniques were used to characterize the microbial consortia within each AnMBR. Results indicated a large portion of the microbial communities were composed of only 5 out of over 700 uniquely identified operational taxonomic units. Unique microbial community structures were observed in each bioreactor during synthetic wastewater operation, ostensibly due to selective pressures including bioreactor configuration and temperature. A significant shift in all AnMBR microbial populations was observed when switching from synthetic to real wastewater, suggesting that continual bioreactor seeding with influent wastewater microbiota impacts bioreactor community composition. Sequencing and results of activity assays also indicated that hydrogenotrophic methanogenesis emerged as the dominant pathway in each AnMBR.

ACKNOWLEDGEMENTS

Matthew D. Seib

I would like to thank Dr. Dan Zitomer for graciously acting as my adviser and mentor during the course of my doctoral studies. I am grateful for your patience, generosity, accessibility, and insightfulness during my time at Marquette. I have learned many lessons from you both in the realm of academia and beyond, and could not attest enough to your value during this journey. To my committee – Dr. Brooke Mayer, Dr. James Maki, and Dr. Michael Switzenbaum– I thank you for your insights and willingness to participate in this project.

Kevin Berg, you helped me maintain sanity. Without your tireless work ethic I would not have been able to enjoy time off, weekends, or holidays. The extra hands and mind you provided made this project a success. Thanks also to Nick Benn, Mike Billings, and Andrew Patek for lending a hand. You guys kept things moving.

Special thanks to Glen Trickle, Alan Merry, John Koch, and Xylem, Inc. for financial, material, and consulting support during my research. Without you my research would not have been possible or as well guided – especially when it came to membrane operation and maintenance. I also thank Aleksandra Lazic and Taylor Jordan for their presence during the course of many meetings and conference calls.

To everyone I crossed paths in the Water Quality Center – I am so glad to have met you all. Your thoughts, help, humor, and friendship made coming to “work” everyday a pleasure. Mike Dollhpf, my day to day efforts in the lab would not have been possible without your constant support. Ben Bocher, thanks for the chats and career advice. Kaushik Venkiteshwaran, your insistence to challenge everything helped sharpen me as a scientist and engineer. Dan Carey, thanks for bringing humor to the lab, your assistance with microbial work, and for

watering my plant. To Tom Hoffman, Yiran Tong, John Ross, Mike Syverson, Alan Williams, John Kusowski, Emily Gorsalitz, Paige Peters, Carlan Johnson and Yu Yang: thank you for tolerating my office tangents and for letting me join in on yours. Thanks also to Joe Heffron for unintentionally acting as my compendium of interesting knowledge.

I am also grateful for the help from others within the Marquette community. Dr. Chris Foley, as department chair the support and resources you provided are greatly appreciated. Dr. Prince Mathai, I could not imagine a person to work with more who is more diligent, good natured, and humble as you. Dave Newman, thanks for the tough love and for letting me keep my research in your fridge. Tom Silman, your help in the DLC was much needed at the beginning of this project. Pam Golanowski, your prompt attention to paperwork kept things moving. Barb Ploszay, thanks for working through the nuances of my fellowship, scholarship, and research funds. Dr. Lars Olson, thank you so much for lending me a space to write this document.

Much thanks also goes to the members of the various committees that awarded financial assistance during the course of my doctoral studies. To the Federation of Environmental Technologists for your scholarship. To the Jobling Fellowship for their scholarship. To the Marquette University Forward Thinking Committee for their research award. To FACILIS for the opportunity to travel to Milan, Italy to meet and work with some great scientists. Special thanks to the Water Environment Federation for the special award of the Canham Scholarship. Special thanks also to the Environmental Protection Agency for the prestigious STAR Fellowship and for the reviewer comments that motivated me to succeed.

A very special thank you to Megan for your support, for sharing me with the lab, and for accompanying me on late night visits to check on things. Thank you as well to friends and family who have offered support, interest, and encouragement along the way.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 INTRODUCTION

"The candidate's potential for long term success in research is not clear. The proposal seems to lack significant scientific curiosity about fundamental understandings. The candidate's scientific curiosity and acumen seem limited. There is a lack of vision related to a career in environmental engineering research."

2012 EPA STAR Fellowship Reviewer

1.1 THE NEED FOR SUSTAINABILITY

As defined by the Brundtland Commission in 1987, “sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs” (Brundtland 1987). It is evident that the activities of the present generation cannot be sustained. Great effort needs to be made to improve human stewardship of natural resources in order to meet the present and future needs of the world.

Better stewardship is especially relevant when considering improvements to water recovery technology. While the introduction of wastewater treatment in the early part of the last century was a great leap forward for environmental protection, it has evolved into a resource intensive process. There is a need, therefore, to replace existing wastewater treatment processes with technology that considers resource utilization and recovery in tandem with remediation of environmentally harmful wastes.

1.2 WATER-ENERGY-FOOD NEXUS

Traditional wastewater treatment such as the activated sludge process is an example of energy intensive treatment. When first developed, the primary objective of these technologies was to mitigate nuisances such as odors and environmental hazards from waste, and little attention was given to minimizing resource consumption while achieving this goal. This is still seen today in the way most municipal wastewater is treated. Organic material is degraded aerobically, which requires a large energy input for aeration. Nutrients such as nitrogen and phosphorus are removed, rather than recovered. Treated water is released rather than re-utilized for irrigation or other beneficial reuse. Clearly, the focus has been on remediation, not

resource recovery, which is needed for sustainable supplies of water, food (nutrients), and energy.

Within the boundaries of the treatment facility, sustainable design means increasing energy efficiency, reducing energy and chemical inputs, utilizing thermal and chemical energy in the waste, reducing waste products such as biosolids, and minimizing facility footprint (Foresti et al. 2006; Novotny 2011). These improvements can translate directly to reduced costs and resource consumption while maintaining the same level of waste remediation. Recovery of nutrients such as nitrogen, phosphorus, and potassium along with renewable energy generation from produced biogas translates into benefits that extend beyond the traditional boundary of a water recovery facility. Recovered ammonia-nitrogen could be used to offset agricultural nutrient demands currently satisfied by the energy intensive and fairly inefficient Haber-Bosch process (Smith et al. 2012). Likewise, recovered phosphorus and potassium could be used to offset mining of finite mineral deposits (Novotny 2011; Batstone et al. 2015). Renewable energy production from biogas alleviates energy demand that would otherwise typically be satisfied with fossil fuels, which reduces societal carbon footprint (Mo & Zhang 2013).

1.3 BRINGING SUSTAINABILITY TO WASTEWATER RECLAMATION

Anaerobic biotechnology has been highlighted as a sustainable alternative to traditional aerobic technologies for municipal wastewater treatment (van Lier 2008; Novotny 2011). The primary benefit gained from an anaerobic process is the elimination of aeration energy, which accounts for over 50% of the energy demand in aerobic activated sludge systems (WEF 2009). In addition, anaerobic treatment converts most organic waste into methane, a self-distilling organic molecule which can be utilized as a renewable, carbon-neutral fuel for facility energy demands. Anaerobic treatment also produces roughly 10% of the biosolids typically yielded from

aerobic activated sludge, meaning disposal costs and need for storage facilities are greatly reduced (Speece 1996; McCarty & Bae 2011). Lastly, nutrients such as nitrogen and phosphorus are not removed, but instead converted to soluble forms such as ammonia and phosphate, making these agriculturally significant resources available for recovery.

While anaerobic treatment has been hailed as an improvement compared to traditional aerobic technology, there are difficulties that need to be overcome for widespread implementation. Firstly, anaerobic treatment is typically carried out at mesophilic or thermophilic temperatures, whereas incoming wastewater temperature in cold and/or temperate climates varies from 3-27^{o}C (Metcalf & Eddy 2003). Operation at mesophilic temperatures is not feasible given the high energy demand for heating, meaning processes need to be run at ambient temperatures below the optimum temperature for most anaerobic trophic groups. This translates to reduced rates of microbial metabolism, which makes high organic removal difficult and systems susceptible to biomass washout. Also, since nutrients are not removed, additional steps are often necessary to recover nutrients before discharge. Additionally, dissolved methane in the effluent needs to be captured to prevent this greenhouse gas from being released to the atmosphere. Combined, these factors have discouraged the implementation of anaerobic biotechnology for municipal wastewater reclamation.

In recent years, renewed attention in anaerobic biotechnology has led to the development of the anaerobic membrane bioreactor (AnMBR). This new process shows great promise for overcoming traditional barriers to widespread adoption of anaerobic biotechnology due to advantages gained from membrane filtration. The membrane provides a barrier that allows for the decoupling of hydraulic and solids retention times (HRT, SRT), which allows for greater process control, prevents biomass washout, and can reduce facility footprint by reducing tank sizes. Additionally, recalcitrant particulate matter that is difficult to hydrolyze is contained

within the bioreactor long enough to be broken down. Lastly, the membrane produces a high quality permeate virtually free of suspended solids, making the final product suitable for reuse applications. Early tests have demonstrated that various AnMBR configurations treating low-strength wastewaters can effectively remove chemical oxygen demand (COD) to less than 40 mg/L , even at temperatures as low as 6^{o}C (Ho & Sung 2009; Shin et al. 2014; Smith et al. 2015).

1.4 MORE WORK NEEDED FOR ANMBRS

Although AnMBRs show great promise for improving process stability and performance of anaerobic treatment systems, they can actually require even more energy than aerobic treatment unless great care is taken to reduce energy demands. In order for an AnMBR process to be more sustainable, it has to require less energy than the 0.3 to 0.6 kWh/m^3 typically required for activated sludge (Metcalf & Eddy 2003). While AnMBRs eliminate aeration and can generate renewable energy from produced methane, traditional membrane operation strategies are energy intensive. Historically, the energy demand of AnMBRs using submerged membranes with biogas sparging range from 0.25 to 3.4 kWh/m^3, whereas external membranes using crossflow velocity (CFV) have had demands of 0.23 to 10 kWh/m^3 (Liao et al. 2006; Le-Clech et al. 2006; Martin et al. 2011). Whereas energy demand for activated sludge is inclusive of demand to remove both organics and nutrients, the AnMBR energy demands described above are for organics removal alone. Additional processes are required for nutrient and dissolved methane removal, which in turn further increase overall process energy requirements, but these steps also offer the potential to produce useable products such as nutrients for fertilizer or methane for energy production. Combined, these energy demands typically result in higher energy usage than activated sludge, even with energy generation from methane.

The additional treatment steps needed for AnMBRs to remove dissolved methane and nutrients are known, but not yet well developed. Dissolved methane removal can be achieved via air stripping (McCarty et al. 2011) where the off gas potentially can be used for biogas combustion. Nutrient removal poses a more difficult challenge since most removal technologies have been developed for use with aerobic systems. Implementing a nitrification/denitrification scheme would be counterproductive given the absence of oxygen in AnMBR permeate and due to the fact that eliminating aeration is one of the primary objectives of using anaerobic technology. Methods such as Anammox or ion exchange (Williams et al. 2015) are not well established in mainstream wastewater applications and, especially in the case of Anammox, may prove challenging to achieve very low effluent concentrations. New nutrient removal technologies, therefore, require further investigation to understand the best uses with anaerobic technology along with better defining energy demands for these technologies.

A greater understanding of the microbial community structures present in AnMBRs is also required in addition to further process development. While much is already understood about the anaerobic degradation pathway and the steps of methanogenesis, technologists do not possess a full understanding of the different microbial consortia responsible for healthy bioreactor operation. Characterizing microbial communities within AnMBRs would be useful to identify advantageous bioreactor configurations as well as help select bioreactor inoculum for start-up.

1.5 RESEARCH OBJECTIVES AND HYPOTHESIS

The objective of this research was to understand and develop an AnMBR that requires less energy than existing configurations in order to improve the sustainability of secondary wastewater reclamation processes. To accomplish this, both an established and uncommon

bioreactor configuration were each joined with tubular, external crossflow membranes operated outside of traditional operating conditions. In addition, membranes were operated with and without fluidized granular activated carbon (GAC) to extend membrane operation between cleanings, and minimize energy inputs while achieving effluent five day biochemical oxygen demand (BOD_5) concentrations less than 10 mg/L. This project also sought to determine the impact of GAC abrasive and adsorptive characteristics on membrane fouling. Lastly, this project used molecular methods to observe the microbial community composition within multiple AnMBRs.

The main research hypothesis is that AnMBRs treating synthetic or real primary effluent wastewater at low/ambient temperatures (10°C and 25°C) will achieve effluent BOD_5 less than 10 mg/L while using significantly less energy than reported in previous studies or for activated sludge. Additionally, unique microbial communities will emerge inside different AnMBRs based on reactor configuration and temperature when seeded with the same inoculum.

1.6 REFERENCES

Batstone, D.J., Hülsen, T., Mehta, C.M., Keller, J., 2015. Platforms for energy and nutrient recovery from domestic wastewater: A review. Chemosphere 140, 2–11. doi:10.1016/j.chemosphere.2014.10.021

Brundtland, G.H., 1987. Our Common Future World Commission on Environment and Development, Oxford paperbacks, Oxford paperbacks. Oxford University Press.

Foresti, E., Zaiat, M., Vallero, M., 2006. Anaerobic processes as the core technology for sustainable domestic wastewater treatment: Consolidated applications, new trends, perspectives, and challenges. Rev. Environ. Sci. Biotechnol. 5, 3–19. doi:10.1007/s11157-005-4630-9

Ho, J., Sung, S., 2009. Anaerobic membrane bioreactor treatment of synthetic municipal wastewater at ambient temperature. Water Environ. Res. 81, 922–928. doi:10.2175/106143009X407339

Le-Clech, P., Chen, V., Fane, T.A.G., 2006. Fouling in membrane bioreactors used in wastewater

treatment. J. Memb. Sci. 284, 17–53. doi:10.1016/j.memsci.2006.08.019

Liao, B.Q., Kraemer, J.T., Bagley, D.M., 2006. Anaerobic membrane bioreactors: Applications and research directions. Crit. Rev. Environ. Sci. Technol. 36, 489–530. doi:10.1080/10643380600678146

Martin, I., Pidou, M., Soares, a., Judd, S., Jefferson, B., 2011. Modelling the energy demands of aerobic and anaerobic membrane bioreactors for wastewater treatment. Environ. Technol. 32, 921–932. doi:10.1080/09593330.2011.565806

McCarty, P.L., Bae, J., 2011. Model to couple anaerobic process kinetics with biological growth equilibrium thermodynamics. Environ. Sci. Technol. 45, 6838–6844. doi:10.1021/es2009055

McCarty, P.L., Bae, J., Kim, J., 2011. Domestic wastewater treatment as a net energy producer-can this be achieved? Environ. Sci. Technol. 45, 7100–7106. doi:10.1021/es2014264

Metcalf & Eddy, 2003. Wastewater Engineering, Treatment and Reuse, 4th ed.

Mo, W., Zhang, Q., 2013. Energy-nutrients-water nexus: Integrated resource recovery in municipal wastewater treatment plants. J. Environ. Manage. 127, 255–267. doi:10.1016/j.jenvman.2013.05.007

Novotny, V., 2011. Water and energy link in the cities of the future - Achieving net zero carbon and pollution emissions footprint. Water Sci. Technol. 63, 184–190. doi:10.2166/wst.2011.031

Shin, C., McCarty, P.L., Kim, J., Bae, J., 2014. Pilot-scale temperate-climate treatment of domestic wastewater with a staged anaerobic fluidized membrane bioreactor (SAF-MBR). Bioresour. Technol. 159, 95–103. doi:10.1016/j.biortech.2014.02.060

Smith, A.L., Skerlos, S.J., Raskin, L., 2015. Anaerobic membrane bioreactor treatment of domestic wastewater at psychrophilic temperatures ranging from 15 °C to 3 °C. Environ. Sci. Water Res. Technol. 1, 56–64. doi:10.1039/C4EW00070F

Smith, A.L., Stadler, L.B., Love, N.G., Skerlos, S.J., Raskin, L., 2012. Perspectives on anaerobic membrane bioreactor treatment of domestic wastewater: A critical review. Bioresour. Technol. 122, 149–159. doi:10.1016/j.biortech.2012.04.055

Speece, R., 1996. Anaerobic Biotechnology for Industrial Wastewaters. Archae Press, Nashville, TN.

van Lier, J.B., 2008. High-rate anaerobic wastewater treatment: Diversifying from end-of-the-pipe treatment to resource-oriented conversion techniques. Water Sci. Technol. 57, 1137–1148. doi:10.2166/wst.2008.040

WEF, 2009. Energy Conservation in Water and Wastewater Treatment Facilities, WEF Manual of

Practice No. 32. WEF Press, Alexandria, VA.

Williams, A.T., Zitomer, D.H., Mayer, B.K., 2015. Ion exchange-precipitation for nutrient recovery from dilute wastewater. Environ. Sci. Water Res. Technol. 17–19. doi:10.1039/C5EW00142K

2 LITERATURE REVIEW

"When we try to pick out anything by itself, we find it hitched to everything else in the Universe"

John Muir
My First Summer in the Sierra, 1911

2.1 INTRODUCTION

Anaerobic membrane bioreactors (AnMBRs) are seen as one avenue to advance the sustainability of municipal wastewater reclamation (Smith et al. 2013). The necessity for membrane use comes from the need to retain slow growing anaerobic biomass and maintain long solids retention times (SRT) in order to achieve maximum organic removal (Skouteris et al. 2012). Including a membrane has also been found to be necessary to achieve the very low final COD concentrations required for discharge directly to the environment. The following is a review of the key parameters to consider for AnMBR design along with a description of milestones and research needs for AnMBR technology advancement.

2.2 ANAEROBIC BIOTECHNOLOGY DESIGN CONCERNS

2.2.1 ANAEROBIC DEGRADATION AND METHANOGENESIS

Anaerobic biological conversion of organic molecules to methane is a complex process that relies on a variety of microbial groups and specific environmental conditions. Figure 2.1 shows a simplified example of the four stages that occur within the methanogenic pathway. First, during hydrolysis, organisms break down large organic molecules into short chain soluble intermediates (Dhaked et al. 2010). These soluble organics are then broken down into volatile fatty acids or converted directly to acetate or H_2 and CO_2 by fermenting organisms (Speece 1983). The volatile fatty acids are then broken down further during acetogenesis where another group of organisms produces acetate and H_2 (McCarty & Smith 1986). Lastly, methanogenic organism produce CH_4 from acetate or H_2 and CO_2 (Rittmann & McCarty 2001). Historically,

methane produced during methanogenesis has been thought to come primarily through acetate (72%), whereas only 28% comes from direct synthesis using H_2 and CO_2 (Speece 1996).

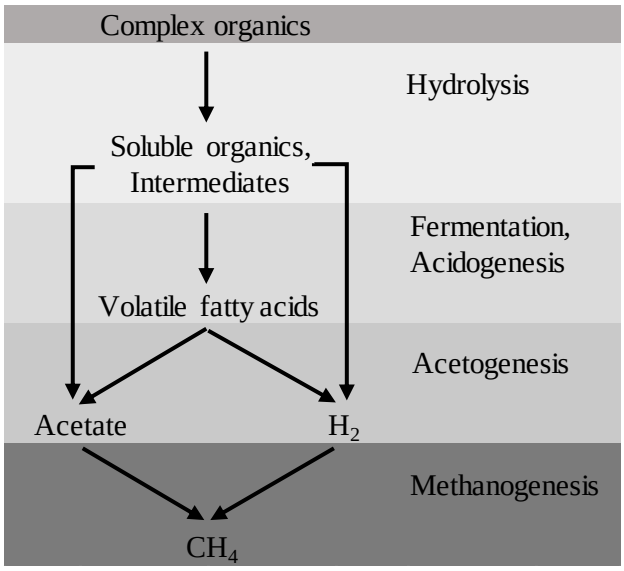

Figure 2.1 Stages of the methanogenic pathway.

The complex microbial degradation pathway leading to methanogenesis is built on an interdependence among different trophic groups known as series metabolism (Speece 2008). In this process, CH_4 production rate is limited by the slowest trophic group involved. In anaerobic solids digestion this can be the organisms related to hydrolysis (Metcalf & Eddy 2003), but in high-rate anaerobic systems the rate limiting organisms are often the acetogens responsible for converting volatile acids into acetate and H_2 as well as methanogens (Speece 2008). In the latter example, if fermentation proceeds faster than acetogenesis, acids will accumulate, which consumes system alkalinity and can lead to a decrease in pH, resulting in inhibition and decrease or cessation of CH_4 production.

Efficient function of anaerobic microbial consortia is due to several factors including temperature, pH, alkalinity, presence of inhibitory compounds, and microbial physical proximity.

Of these, physical proximity appears to be particularly important considering the syntrophic relationships that must exist for methanogenesis. Thermodynamically, methanogenesis of complex organics will not proceed without very low H_2 partial pressure (McCarty & Smith 1986) between H_2 producing and consuming species. In order to facilitate low H_2 partial pressure, these H_2 associated species need to remain in very close proximity in order to efficiently consume H_2 or accomplish direct electron transfer (Summers et al. 2010; Morita et al. 2015). These phenomena are favored within biofilms, which explains why high-rate anaerobic biotechnologies are all based on presence of biofilms.

2.2.2 BIOREACTOR CONFIGURATIONS

Anaerobic biotechnology has been practiced for over a hundred years, with most of the improvements coming within the last five decades (Foresti et al. 2006; Speece 2008). During this time, anaerobic biotechnology development first emphasized flocculant biomass technology before following improvements that considered biofilms, flocculant systems with membrane filtration, and now biofilm systems with membrane filtration. The first improvement came when the completely stirred tank reactor (CSTR) was introduced, which enabled uniform microbe/substrate distribution compared to the relatively static environment present in a septic tank. Then in the 1950s the anaerobic contact process was introduced which utilized a settler to recycle solids to the bioreactor (Schroepfer et al. 1955; Speece 1983). In the 1960s and 1970s the first biofilm technology was introduced with the anaerobic filter (AF), which was developed in both upflow (Young & McCarty 1969) and downflow (van den Berg & Lenz 1979) modes. In the early 1980s the upflow anaerobic sludge blanket (UASB) and fluidized bed reactor (FBR) were the first high-rate anaerobic technologies to be developed (Lettinga et al. 1980; Switzenbaum & Jewell 1980). Modifications to these high-rate systems followed, including the

anaerobic baffled reactor (ABR) (Bachmann et al. 1985), expanded granular sludge bed (EGSB) (Lettinga & Pol 1986), and downflow fluidized bed reactor (Garcia-Calderon et al. 1998). Lastly, the anaerobic membrane bioreactor (AnMBR) was introduced as an improvement to the anaerobic contact process in the early 1980's (Sutton et al. 1983; Speece 1983).

2.2.3 TEMPERATURE

In general, microorganisms are separated into one of four categories based on temperature for optimal growth and temperature range of metabolism. These categories include: psychrophilic (<20^{o}C), mesophilic (20-45^{o}C), thermophilic (45-80^{o}C), and hyperthermophilic (>80^{o}C). Growth rate decreases at low temperature, which has been well documented in anaerobic systems (van den Berg 1977; van Lier et al. 1997; Lettinga et al. 1999). Additionally, chemical and biochemical reactions typically require more energy to proceed at lower temperatures, making microbial substrate utilization at low temperatures more thermodynamically challenging (Lettinga et al. 2001). However, some reactions such as those involving hydrogenotrophic sulfate reduction, hydrogenotrophic methane production, and formation of acetate from hydrogen and bicarbonate are less thermodynamically taxing at lower temperatures (Lettinga et al. 2001). These facts suggest that while most methane production is derived from acetate at mesophilic and thermophilic temperatures, methane production may be primarily derived from H_2 at psychrophilic temperatures (Enright et al. 2009; McKeown, Scully, Mahony, et al. 2009; Madden et al. 2010; Bialek et al. 2011). The methanogenic organisms responsible for methane production are found in all three categories, but most are mesophilic with an optimum temperature of 35^{o}C (Lin et al. 1987).

While low temperature operation of anaerobic bio-systems is possible, mesophilic temperatures typically have been employed to create an environment for optimal

methanogenic organism growth and activity. Reduced microbial metabolism and thermodynamics at psychrophilic temperatures means that growth of biomass within reactors is slow. Traditionally, this has led to problems including biomass washout and an inability to recover quickly from upsets (Switzenbaum 1995; Lettinga et al. 2001). Additionally, complete substrate utilization is often not achieved at low temperatures, meaning anaerobic biotechnology has not been viewed as a reliable means of meeting national pollutant discharge elimination system (NDPES) permit requirements of approximately 30 mg/L BOD_5 or less (Federal Register 2011) in treated municipal wastewater effluent (Seghezzo et al. 1998).

In addition to biological impacts, temperature also creates physical and chemical concerns for engineered bio-systems. As temperature decreases, methane solubility increases, meaning that more methane will be dissolved at psychrophilic compared to mesophilic temperatures. This creates recovery challenges and discharge concerns as methane is a damaging greenhouse gas (McCarty et al. 2011; Smith et al. 2012). Additionally, as temperature decreases, water density and viscosity increase, meaning hydraulic headloss and energy requirements/pumping costs increase.

2.2.4 SOLIDS RETENTION

Long solids retention time (SRT) traditionally has been the primary control method to maintain stable operation for anaerobic biotechnology. Ensuring sufficient volatile solids retention gives anaerobic biomass the time required to reproduce in order to prevent biomass washout. Longer SRT is required at lower temperatures due to reduced microorganism substrate utilization rate (McCarty 1964), and in general, longer SRTs are understood to increase organic removal (Metcalf & Eddy 2003).

Traditional SRT control has been employed in a variety of ways. The simplest method is to use a tank large enough to achieve the desired residence time based on average flow. In this scenario hydraulic residence time (HRT) and SRT are coupled, meaning there is no ability to actively control SRT with variations in HRT. Another method, the anaerobic contact process, uses a settling tank after the bioreactor to decouple HRT and SRT by returning settled solids back to the bioreactor. In this way, SRT can be increased beyond HRT. Newer bioreactor configurations such as the UASB, EGSB, FBR, AF, etc., decouple HRT and SRT by forming biofilms that remain in the bioreactor instead of relying on a settling tank. Membrane filtration is the latest method to decouple HRT and SRT, theoretically allowing for infinite solids retention. This complete decoupling of HRT and SRT with membranes allows for very good process control of solids.

2.2.5 LOW STRENGTH WASTEWATER

Anaerobic biotechnology is typically used to treat high-strength industrial wastewaters or digest solids from primary sludge and waste activated sludge in municipal wastewater treatment plants (Metcalf & Eddy 2003). This is in part because anaerobic biotechnology can tolerate much higher loading rates than aerobic technology (Speece 1996). In contrast, anaerobic biotechnology typically has not been used for low strength wastes, such as municipal wastewater, in cold regions because the very low effluent organic concentrations required for municipal wastewater treatment in developed countries have been difficult to achieve (Lettinga et al. 2001). Additionally, municipal wastewater does not contain enough organic material to produce methane in the quantities necessary to heat wastewater to mesophilic temperatures in cold climates (Martin et al. 2011). These factors have historically discouraged the use of

anaerobic biotechnology for municipal wastewater treatment in colder climates including those of the US and Europe.

2.2.6 NUTRIENT DEMAND AND REMOVAL

Anaerobic bioprocesses do not appreciably remove nutrients (nitrogen and phosphorus). This is because very little nitrogen or phosphorus are incorporated into biomass due to low anaerobic biomass yields of 0.05-0.1 g VSS/g COD_r (Metcalf & Eddy 2003). These low yields mean that biomass nitrogen and phosphorus demands for anaerobic systems are typically met with constituents in the influent waste. Potential nutrient deficiency concerns center around micronutrients such as nickel, cobalt, molybdenum, etc. (Speece 2008). Nitrification and denitrification also do not occur in anaerobic systems; the result being that nitrogen and phosphorus leave anaerobic systems primarily as soluble NH_3 or PO_4^{-3}.

Since nutrients are not appreciably removed during anaerobic treatment, subsequent removal/recovery processes are needed, especially for municipal wastewater applications. This is to prevent nutrients being discharged in the effluent stream, which may adversely impact surface waters (Seghezzo et al. 1998). Nutrient recovery from anaerobic processes may be more sustainable than aerobic nutrient removal that typically requires high aeration (for nitrification) and pumping energy (for recycle streams) and converts nitrogen to nitrogen gas that is wasted and not captured for reuse. At present, agricultural nutrients are produced using very energy intensive methods such as the Haber-Bosch process for nitrogen, or are mined from finite reserves in the case of phosphorus and potassium. Capturing these nutrients rather than removing them from wastewater could significantly reduce the environmental footprint associated with agricultural fertilizer production (Rittmann et al. 2011; Williams et al. 2015).

In anaerobic municipal wastewater treatment applications, nutrient removal/recovery technologies are best used after secondary treatment. This is because organics in influent wastewater may inhibit/complicate nutrient extraction. Therefore, in anaerobic treatment scenarios, nutrient removal processes should be amenable to the anaerobic effluent, which is virtually free of both carbon and oxygen. This means traditional aerobic nutrient removal such as enhanced biological phosphorus removal and nitrification/denitrification are not optimum.

Partial nitritation/nitrification coupled with Anammox has been suggested as an autotrophic biological process to remove nitrogen (van de Graaf et al. 1996; Stuckey 2012), but control of this process is considered challenging and mesophilic temperatures are believed necessary (Smith et al. 2012). Rather, physical/chemical processes such as ion exchange (Aiyuk et al. 2006; Williams et al. 2015) or struvite precipitation (Mo & Zhang 2013) appear more appropriate. This is especially true since most of the N and P entering the AnMBRs are converted to soluble NH_3 or PO_4^{-3}, making physical/chemical recovery more feasible. However, struvite precipitation requires the addition of magnesium and would only remove a portion of the nitrogen. Nutrient recovery and concentration using ion exchange may be particularly attractive as the concentrated nutrients could be utilized in agricultural applications to offset atmospheric or mineral fertilizer production (Rittmann et al. 2011; Williams et al. 2015).

2.2.7 DISSOLVED METHANE

Along with nutrient removal, dissolved methane in anaerobic effluents is a significant concern. Low temperature increases the soluble fraction of methane gas; meaning less methane is actually available for traditional direct capture (Rebac et al. 1999; Kim et al. 2011). In fact, at 15^{o}C soluble methane concentration is roughly 150% of what it is at 35^{o}C (Smith et al. 2012). Dissolved methane is not available for traditional direct capture from biogas, which translates to

lost energy production potential if the dissolved methane is not recovered (Rebac et al. 1999; Kim et al. 2011; McCarty et al. 2011). In addition to maximizing energy production potential, dissolved methane must be captured to prevent release to the atmosphere as it has a global warming potential much greater than carbon dioxide (Solomon et al. 2007).

Air stripping has been proposed as a way to recover dissolved methane (McCarty et al. 2011), where the offgas from this process could potentially be directly used for combustion of bioreactor headspace gas for energy production as opposed to being flared. Air stripping would also help aerate AnMBR permeate to increase dissolved oxygen concentration for discharge. Since the membrane permeate is free of dissolved oxygen, reaeration steps may be required to satisfy minimum dissolved oxygen concentrations for receiving waters. This may be achieved simply by cascading the effluent or with a small aeration basin, but special attention should be given to potential concerns with sulfurous gasses (von Sperling 2007; van Haandel & van der Lubbe 2012) and odors (Switzenbaum 1995).

2.2.8 MICROBIAL COMMUNITY

Design of engineered biological wastewater treatment processes are typically based on empirical criteria that do not consider the impact of microbial consortia on treatment performance (Connaughton et al. 2006). Engineers have viewed bioreactors as “black boxes” without considering the relationship between microbiology and process function (Collins, McHugh, et al. 2006; van der Gast et al. 2006; Sanz & Köchling 2007; Madden et al. 2010; Siggins et al. 2011b; McKeown et al. 2012). An understanding of important microbial players within bioreactors is important for managing the potentials and limitations of biologically-driven processes such as hydrolysis, fermentation, and methanogenesis (McKeown et al. 2012; Vanwonterghem et al. 2014). Linking microbial community structure with system function could

be used to match inoculum biomass to specific operating conditions, including operation temperature or type of waste (Collins et al. 2003; McKeown et al. 2012; Petropoulos et al. 2013), or development of bioaugmentation to increase process efficiency (Schauer-Gimenez et al. 2010; Tale et al. 2011; Bocher et al. 2015). Additionally, community structure analysis could allow for early detection of process upset conditions by identifying adverse changes in the microbial consortia that could, for example, indicate potential for disintegration of granules in upflow anaerobic sludge blanket digesters (Collins, McHugh, et al. 2006; Madden et al. 2010).

Although microorganisms in biological systems are responsible for successful treatment (Lettinga et al. 2001), there is a lack of knowledge describing microbial consortia in anaerobic wastewater treatment systems. Currently, most studies have described anaerobic microbial communities in digesters treating high strength wastes. Anaerobic systems treating dilute or municipal wastewater have been given little attention. Thus far, key findings for anaerobic systems include: community structure is effected by selective pressures such as temperature, substrate, and bioreactor configuration (Fernandez et al. 2000; O'Reilly et al. 2009; Bialek et al. 2011; Bialek et al. 2012), bacterial communities are statistically more even and diverse than archaeal communities in anaerobic systems (Rivière et al. 2009; Regueiro et al. 2012; Chaganti et al. 2012; O'Reilly et al. 2010), and hydrogenotrophic methanogenesis increases at psychrophilic temperatures (McKeown et al. 2012).

2.3 MEMBRANE TECHNOLOGY AND DESIGN CONCERNS

2.3.1 CONFIGURATION AND OPERATION

In wastewater applications, membrane configurations are categorized as submerged or external. Submerged configurations immerse the membrane module directly into the bioreactor

liquor and rely on hydrostatic head and/or vacuum to draw permeate through the membrane. External configurations keep the membrane module outside the bioreactor where bioreactor liquor is pumped across the membrane surface and drawn through either by positive pressure or vacuum depending on module type. Submerged membranes eliminate the need for recycle pumping associated with external configurations. However, submerged membranes are typically operated with biogas sparging to maintain membrane flux, which also requires significant energy to operate biogas blowers.

Over time, both configuration types require chemical cleaning to remove foulants that cannot be removed via surface shear from biogas sparing or liquid pumping velocity. To chemically clean submerged membranes, the bioreactor may need to be opened to remove the membrane module, which may create safety concerns and disrupt biomass in anaerobic applications. External configurations, on the other hand, can be isolated from the bioreactor and cleaned in place, which protects biomass from disruption and makes routine cleaning less disruptive to the bioreactor biomass.

2.3.2 MATERIAL AND MODULE TYPES

Membranes are generally categorized by two material types: organic and inorganic. Material of construction has many implications on module type, fouling behavior, permeability/flux maintenance strategies, and cost. Organic membranes used in wastewater applications have been made from a variety of polymers including polyvinylideneflouride (PVDF), polyethersulphone (PES), polyethylene (PE), polypropylene (PP), polytetrafluoroethylene (PTFE), polysulfone (PSF), and polyacrylonitrile (PAN) (Meng et al. 2009; Lin et al. 2013). Inorganic membranes used in wastewater applications have typically been made from

aluminum, zirconium, or titanium oxides (Meng et al. 2009; Zitomer et al. 2005), but metal such as stainless steel has also been used (Zhang et al. 2005).

More examples exist of polymeric membranes being used in wastewater treatment compared to inorganic examples. This is primarily due to cost, as polymeric membranes are much less expensive (Cornel & Krause 2008). Additionally, polymerics are more versatile when it comes to module type as they are able to be formed into flat sheets, tubes, or hollow fibers. Flat sheet and hollow fiber types can be located internally or externally, whereas tubular types are located externally. Inorganic materials are more rigid and are typically formed into tubes, but have also been used as flat sheets. While inorganic membranes are more expensive and restrictive on shape, they can be used in more extreme conditions, including higher temperatures and harsh chemical conditions, meaning cleaning procedures can be more vigorous (Meng et al. 2009).

2.3.3 FOULING MECHANISMS

As in any filtration application, membrane filtration capacity, or permeability, will diminish over time due to buildup of foulants on the membrane surface. Membrane foulants are separated into three categories: removable, irremovable, and irreversible (Meng et al. 2009). Removable fouling refers to fouling that can be eliminated by physical means such as backwashing or inducing surface shear via liquid crossflow or gas sparging. Irremovable fouling refers to fouling that occurs within pores and has to be removed by chemical cleaning. Irreversible fouling cannot be removed by either physical or chemical cleaning.

Fouling can be due to organic and/or inorganic foulants found in the incoming waste stream or biofoulants formed during microbial breakdown of the organic fraction of the waste. Organic fouling is the result of organic material accumulating on or adsorbing to the membrane.

Pore clogging can be caused by cellular debris and colloidal particles, whereas cake formation is a biological buildup of solids on the membrane surface (Liao et al. 2006) primarily due to biopolymers secreted by microorganisms typically referred to as extracellular polymeric substances (EPS) (Skouteris et al. 2012). EPS becomes a more significant factor at lower flux (Cho & Fane 2002) and longer SRT (Barker et al. 2000; Huang et al. 2011). Inorganic fouling occurs on the membrane surface or in membrane pores and is typically the result of struvite precipitation (Choo & Lee 1996b; Norddahl & Rohold 2000; Yoon et al. 1999) or other inorganic precipitate foulants such as $K_2NH_4PO_4$ and $CaCO_3$ (Nagata et al. 1989; Norddahl & Rohold 2000).

2.3.4 EFFECTS OF HRT AND SRT ON ORGANIC FOULING

Two important factors affecting membrane fouling are HRT and SRT. Higher EPS production and greater suspended biomass concentration are associated with a longer SRT (Barker et al. 2000; Huang et al. 2011), which reduces flux and increases fouling rate, especially in CSTR systems. Several studies have shown HRT to have little effect on treatment performance (Lew et al. 2009; Chu et al. 2005; Huang et al. 2011; Ho & Sung 2009; Baek et al. 2010). However, an increase in EPS concentration inside an AnMBR at low HRT has also been observed, suggesting a lower boundary for HRT may be needed to avoid fouling (Salazar-Peláez et al. 2011).

2.3.5 SURFACE CHARACTERISTICS

Membrane surface chemistry is known to affect membrane fouling, especially hydrophobicity. Cake layer formation occurs more easily on hydrophobic membrane material, as compared to hydrophilic membrane material (Meng et al. 2009). In general, this means fouling from cake layer deposition is more significant in polymeric membranes than ceramic (inorganic)

membranes (Sutton et al. 2004) as most polymerics tend to be hydrophobic. However, desired membrane hydrophobicity is not certain. Choo et al. (2000) showed a hydrophilic membrane could operate at higher flux whereas Choo and Lee (1996) indicated that hydrophobic membranes have a lower amount of fouling (Sutton et al. 2004)**.** Membrane surface charge is also important for preventing fouling and is strongly affected by reactor liquid ionic strength and pH (Sutton et al. 2004)**.** However, membrane surface charge appears to become negligible in high ionic strength solutions (Fane et al. 1983).

2.3.6 FOULING PREVENTION AND CLEANING STRATEGIES

Membrane fouling during long-term operation is inevitable. Several strategies can be used to maintain or regain flux lost from fouling depending on membrane configuration and module type. These strategies are further broken down by the type of foulant to be controlled. For removable foulants, control can be based on operational strategy and addition of materials in order to discourage cake layer formation using physical phenomena. For reversible foulants, chemical cleaning procedures are used to remove material clogging membrane pores. Control strategies for removable foulants are conducted either continuously or at short intervals, whereas procedures for reversible foulants occur only periodically.

2.3.6.1 REMOVALBE FOULANT CONTROL

2.3.6.1.1 CROSSFLOW VELOCITY

One method of preventing fouling cake layer formation on membranes is to induce hydraulic shear across the membrane surface by pumping liquid across the membrane surface with sufficient crossflow velocity (CFV). This strategy is typically used for external tubular

membranes with CFVs of 2-5 m/s recommended to maintain flux (Liao et al. 2006; Le-Clech et al. 2006). Historical use of CFV has been criticized because of the high energy demand required to maintain sufficient CFV (Liao et al. 2006; Martin et al. 2011) and because the high pumping rate can lead to cell lysis and therefore increase microbial polymeric substances depending on pump selection (Choo & Lee 1996b).

2.3.6.1.2 GAS SPARGING

Gas sparging is another method of producing surface shear to prevent fouling cake formation. This process involves passing coarse bubbles along the membrane with sufficient superficial velocity to disrupt/remove the fouling cake layer. This strategy is typically used for submerged flat sheet and hollow fiber membranes with superficial gas velocities reported ranging from <1 to 70 m/h (Jeison & van Lier 2006; Martin et al. 2011). Using gas slugs in tubular membranes has also been shown to enhance membrane flux (Mercier et al. 1997; Cheng et al. 1999; Taha et al. 2006). Traditionally, using gas sparging with submerged membranes has required less energy for operation than systems using CFV (Liao et al. 2006; Martin et al. 2011).

2.3.6.1.3 BACKFLUSHING

Backflushing entails reversing flow through the membrane material so that permeate is sent back to the retentate side. In addition to permeate, gas can also be used as the flushing medium (Le-Clech et al. 2006). Backflushing can be used to physically remove foulants that have become entrapped in membrane pores which cannot be removed with surface shear. Effectiveness of backflushing is related to both frequency and duration of backflushing events (Le-Clech et al. 2006). In general, longer run-time between backflushing and longer duration of

backflushing events have been found to be more efficient than more frequent, shorter backflushing regimes (Jiang et al. 2005).

2.3.6.1.4 RELAXATION

Relaxation is a flux enhancement procedure that involves periodically reducing transmembrane pressure (TMP) to zero for a short period of time (Sutton et al. 2004). During the relaxation period, back transport of irremovable foulants is facilitated by diffusion of foulants away from the membrane surface (Le-Clech et al. 2006). During relaxation, foulant removal can be enhanced with gas scouring along the membrane surface (Chua et al. 2002).

2.3.6.1.5 MECHANICAL ABRASION & ADSOPRTION

Physical/mechanical abrasion and/or adsorption can also be used to prevent fouling cake layer formation on membrane surfaces. Abrasion can be achieved through the use of a fluidized abrasive material along the membrane surface. Long-term operation at constant flux with the use of different polymeric beads and no chemical cleaning has been achieved in several studies (Siembida et al. 2010; Krause et al. 2009). Additionally, Kim et al. (2011) reduced membrane fouling with the use of fluidized granular activated carbon (GAC), which is thought to achieve both physical abrasion and adsorption of EPS or other soluble foulants. With regard to adsorbing vs. non-adsorbing particles, Aslam et al. (2014) found physical abrasion to be the primary avenue for flux maintenance, especially when GAC became saturated. Ion exchange resins have also been used for abrasion and adsorption/control of VFA concentrations within submerged AnMBR configurations (Stuckey 2012). Powdered activated carbon (PAC) has also been shown to increase membrane flux by adsorbing membrane foulants (Hu & Stuckey 2007; Akram & Stuckey 2008).

2.3.6.2 REVERSIBLE FOULANT CONTROL

Chemical cleaning is the common approach for removal of irremovable membrane foulants that cannot be removed by creating shear across the membrane surface or by backwashing. Acidic agents such as HCl and H_2SO_4 are commonly used to remove inorganic foulants (Choo et al. 2000; Ross et al. 1992; Lee et al. 2001) while alkaline chemicals such as NaOH are used for biological foulants (Lee et al. 2001). Caustic hypochlorite and ozone aeration have also been used to remove organic foulants (Kim et al. 1998; Ross et al. 1992). Exact chemical cleaning procedures are specific to individual membranes.

2.3.7 NOMINAL PORE SIZE

Microfiltration (pore size >0.05 μm) and ultrafiltration (0.002 < pore size < 0.05 μm) membranes are typical for AnMBR applications (Liao et al. 2006)**.** For micro and ultrafiltration membranes, permeate flux will be higher with a larger pore size; however larger pore sizes also tend to foul faster (Saw et al. 1985). Although, Hernandez et al. (2002) showed that a membrane with a nominal pore size of 10 μm fouled several times more quickly than one with a pore size of 100 μm. This suggests that there may be different fouling mechanisms governing large pore size membranes compared to micro and ultrafiltration membranes. While no optimum nominal pore size has been found, the goal has been to optimize the relationship between particle removal with a sufficiently small pore size and energy consumption, which increases as pore size decreases (Sutton et al. 2004).

2.3.8 RELATIONSHIP BETWEEN TMP, FLUX, AND TEMPERATURE

Flux is defined as the amount of liquid passing through a defined membrane surface area per unit time (L/m^2-hr). A higher flux rate translates to lower operating costs and fewer membranes. However, fouling rate increases with increased flux, which in turn demands shorter cleaning intervals to restore desired flux (Wen et al. 1999). Transmembrane pressure (TMP) is defined as the difference in pressure across the membrane. Flux is proportional to TMP at low pressure conditions and is not affected by cross-flow velocity, but is affected by low solids concentrations in CSTRs (Beaubien et al. 1996). However, at high TMP, flux is dictated by mass transfer, meaning cross-flow velocity and solids concentration govern flux (Beaubien et al. 1996).

The relationship between fluid viscosity and flux as temperature changes is inversely proportional (Stephenson et al. 2000). This means that as temperature is decreased flux will decrease. For every 1^{o}C rise in temperature there is a 2% increase in flux (Ross et al. 1992). Additionally, low temperature (<20 ^{o}C) is associated with decreased biologic activity and increased production of soluble microbial products (SMPs) (Sutton et al. 2004). Decreased biological activity translates to longer HRTs to achieve biologic treatment. Increased viscosity and SMPs result in increased TMP and quicker flux decrease from biological/organic membrane fouling.

2.4 ANAEROBIC MEMBRANE BIOREACTOR (ANMBR) TECHNOLOGY

2.4.1 MEMBRANE BIOREACTOR CONFIGURATIONS

Previous AnMBR studies reveal two main approaches for configuring AnMBRs. CSTRs have been used to maintain flocculant biomass (Ho & Sung 2010) with submerged membranes or external crossflow membranes. However, with high suspended solids concentrations,

membrane fouling potential is increased. A more recent approach is to use attached growth technologies such as the UASB or FBR to maintain biofilms and minimize bulk liquid suspended solids, thus reducing solids sent to the membrane (Shin et al. 2014). External membranes (flat sheet, hollow fiber or tubular) are used with these attached growth bioreactors so that membrane fouling control methods do not disrupt biofilm formation in the bioreactor. Biofilm configurations are typically more efficient than flocculant systems because biofilms facilitate enhanced interspecies substrate degradation and mass transfer and may accomplish electron transfer directly between individual cells (McCarty & Smith 1986; Morita et al. 2015); making biofilm technologies the suggested path forward (McHugh et al. 2005; Sutton et al. 2004; Rittmann & McCarty 2001; McCarty et al. 2011; Kim et al. 2011).

2.4.2 SUMMARY OF EXISTING ANMBR TECHNOLOGIES

While examples of AnMBRs exist going back to the early 1980's, it was not until the late 1990's that an AnMBR was first used to treat domestic wastewater (Wen et al. 1999). Since then there have been multiple studies examining different aspects of AnMBRs treating low-strength wastewaters (Table 2.1). These studies have employed different bioreactor technologies including UASB, FBR, EGSB, and CSTR. Additionally, both submerged and crossflow membrane configurations have been employed, with different module types and membrane materials being utilized. Together, these examples demonstrate something previously thought unattainable is now possible: anaerobic biotechnology can effectively treat low-strength waste with very little organic content remaining in the effluent, even at low temperatures.

Table 2.1 Summary of existing AnMBR studies treating dilute wastewaters.

Source	Reactor	Membrane Configuration	Module Type	Membrane Material	Pore Size (µm)	TMP (kPa)	Flux (L/m²h)	Fouling Control	Temp (°C)	Substrate[1,2]	Influent TCOD (mg/L)	Effluent TCOD (mg/L)	HRT (h)	SRT (day)
(Wen et al. 1999)	UASB	Submerged	hollow fiber	PE	0.03	Up to 70	5-10	R; cleaning with 5% NaOCl	14-25	W	100-2600	<35	4-6	150
(Chu et al. 2005)	EGSB	Submerged	hollow fiber	PE	0.1		>~10	BF; R; cleaning with 0.03% NaOCl	11 - 25	S	383-849	10 - 96	3.5-5.7	145
(Hu & Stuckey 2006)	Complete mix	Submerged	hollow fiber & flat sheet	polyethylene chloride	0.4	<0.05-50	1.25-15	BS	35	S	460	23 - 27	48	∞
												29 - 34	24	
												32 - 38	12	
												40 - 45	6	
												43 - 48	3	
(Baek & Pagilla 2006)	Complete mix	External	tubular	PVDF	0.1 (200 kDa)	<0.68 - 68.9		CFV; cleaning with NaOH and chlorine	32	W	84 (SCOD)	25 (SCOD)	48	19 - 233
												37 (SCOD)	24	
												37 (SCOD)	16	
												24 (SCOD)	12	
(Saddoud et al. 2007)	Jet flow	External			100 kDa	100-200	3.5-13	CFV	37	W	685	87	15-60	
(Ho et al. 2007)	Complete mix	External	tubular	PP and PTFE	12, 10	6.9-20.7	5 - 12	BF	25	S	500 - 1000	30	18	
(Ho & Sung 2009)	Complete mix	External	tubular	PTFE	1	<55	5-8	CFV; BF; cleaning with NaOCl	25	S	500	<40	6-12	90-360
(Lew et al. 2009)	Complete mix	External	hollow fiber		0.2	<19.6	3.75-11.25	BF; cleaning with NaOH, H_2O_2, HCl	25	W	540	65	4.5-12	∞
(An et al. 2009)	UASB	External	tubular	Poly-acrylonitrile			10.5	BF; R; cleaning with NaOCl, H_2SO4	ambient	W	58-348			
Ho and Sung 2010	Complete mix	External	tubular	PTFE	1	6.9-55.2	5	BF	25	S	500	~25	12	∞
									15			~75		
(Gao et al. 2010)	Upflow anaerobic reactor	External	flat sheet	PVDF coated with PEBAX, polyetherimide	100 kDa, 30 kDa		8-12	CFV	30	S	500	<20	24	50

Source	Reactor	Membrane Configuration	Module Type	Membrane Material	Pore Size (µm)	TMP (kPa)	Flux (L/m^2h)	Fouling Control*	Temp (°C)	Substrate**	Influent TCOD (mg/L)	Effluent TCOD (mg/L)	HRT (h)	SRT (day)
(Herrera-Robledo et al. 2010)	UASB	External	tubular		40 kDa	400-620	1.5-8	CFV	20-25	W	646	106	3	
(Huang et al. 2011)	Complete mix	External	flat sheet	PES	0.45		5.3	BS; R	25-30	S	550	<20	8-12	30
							6.4					<20		60
							7.9					<20		∞
(Salazar-Peláez et al. 2011)	UASB	External	tubular	PVDF	100 kDa	103	>20	CVF; cleaning with NaOCl		S	350	40 - 65	4-12	∞
(Herrera-Robledo et al. 2011)	UASB	External	tubular	PVDF	100 kDa	87	120-130, 45-50	Cleaning with NaOCl	22	W	445	33	6	180
(Calderón et al. 2011)	UASB	External	tubular	FPVD	100 kDa			Cleaning with NaOCl		W	425	33	6	
(Kim et al. 2011)	FBR+ AnMBR	Submerged	hollow fiber	PVDF	0.1	5-35	4-10	GAC fluidization; BF; cleaning with NaOCl/NaOH	35	S	513	7	4.2-5.9	∞
(Dagnew et al. 2011)	Complete mix	External	hollow fiber	PVDF	0.04	6-13	17	BS; R; chemical cleaning	22	W	224	47	8.5	80-100
(Martinez-Sosa et al. 2011)	Complete mix	External	flat sheet	PES	0.038	20-25	7	CFV; GS; BF; R	20-35	W & S	630	<90		
(Giménez et al. 2011)	Complete mix	External	hollow fiber	PVDF	0.05	8	10	BS; BF; R			445	77		
(Smith et al. 2013)	Complete mix	Submerged	flat sheet	PES	0.2	10	7-8.5	BS; BF	15	S	440	36	16-24	300
(Shin et al. 2014)	FBR+ AnMBR	External	hollow fiber	PVDF	0.03	6-56	4.1-7.5	GAC fluidization, R	8-30	W	198-362	23	4.6-6.8	6.2-36
(Smith et al. 2015)	Complete mix	Submerged	flat sheet	PES	0.2	10	1.5-3	BS; BF	3-15	S	440	70	17-29	300

*R = relaxation, CFV = crossflow velocity, BS = biogas sparging, R = relaxation, BF = backflushing

**W = real wastewater, S = synthetic low-strength wastewater

2.4.3 SUMMARY OF OBSERVED FOULING CONTROL STRATEGIES

Most of the studies listed in Table 2.1 have relied on traditional membrane fouling control strategies. These include one or several measures such as CFV, gas sparging, backflushing, relaxation, and chemical cleaning with basic and/or acidic solutions. Protocols for fouling control strategies vary between these studies, especially with regards to chemical cleaning. Frequency of chemical cleaning ranged from every 6-8 h to weekly/bi-weekly to monthly/semi-monthly (Baek & Pagilla 2006; Zhang et al. 2007; Ho & Sung 2009; Salazar-Peláez et al. 2011). Only two examples were found where activated carbon was used to control fouling via adsorption (Hu & Stuckey 2007) and abrasion/adsorption (Kim et al. 2011; Shin et al. 2014) in AnMBRs treating low-strength wastewater.

2.4.4 SUMMARY OF OBSERVED ENERGY REQUIREMENTS

Historical energy consumption for lab and pilot-scale AnMBRs has been reported across a wide range for both submerged and external configurations. For submerged AnMBRs, Liao et al. (2006) reported energy demands of 0.25-1.0 kWh/m^3, while estimates from other studies range from 0.69-3.41 kWh/m^3 (Martin et al. 2011). External crossflow AnMBRs typically have required much more energy due to high CFV required to maintain flux. Liao et al. (2006) reported external crossflow energy demands of 3-7.3 kWh/m^3 and Le-Clech et al. (2006) indicated demands as high as 10 kWh/m^3. However, low CFV side-stream examples were found with estimated CFV energy demands ranging from 0.23-0.48 kWh/m^3 (Martin et al. 2011). These findings indicate that in general submerged configurations require less energy for operation than crossflow configurations, but energy demand is also highly dependent on operation strategy and membrane selection, as indicated by the wide ranges reported for each

configuration. These findings also suggest that historical membrane operational strategies are more energy intensive than the 0.3-0.6 kWh/m^3 typically required for activated sludge aeration (Metcalf & Eddy 2003).

Recently, several strategies to reduce membrane fouling rate and reduce membrane energy demands have been investigated. Efforts have centered on ways to minimize membrane fouling through membrane surface modification (Hilal et al. 2005; Stuckey 2012), use of adsorbents such as activated carbon (Hu & Stuckey 2007; Akram & Stuckey 2008; Kim et al. 2011), or physical scouring mechanisms (Krause et al. 2010; Siembida et al. 2010). Other operational factors such as backflushing and relaxation (Liao et al. 2006; Berube & Hall 2006; Meng et al. 2009) have also been evaluated. While these efforts have revealed much, they have done little to address a more significant underlying challenge – the relatively fixed energy cost of providing membrane flux control via gas sparging or CFV. For example, Smith et al. (2014) compared energy requirements for high rate and conventional aerobic systems against a submerged AnMBR using typical gas sparging rates and concluded that AnMBR energy demands exceeded those of typical aerobic technologies due to gas sparging requirements– even when accounting for energy produced from methane. Only two studies have been found that describe the successful implementation of fluidized granular activated carbon (GAC) in conjunction with a submerged hollow fiber membrane as a means to reduce energy demands to as low as 0.058 kWh/m^3 by eliminating gas sparging in favor of a relatively lower CFV (Kim et al. 2011; Shin et al. 2014). While important, this example was limited to a submerged configuration that only employed one kind of membrane.

2.4.5 FEASIBILITY OF ENERGY NET POSITIVE OPERATION

New low-energy scenarios using AnMBRs are emerging despite the traditionally high energy demand associated with these systems. Now, a goal for municipal wastewater recovery is to develop a process that achieves net positive energy performance (McCarty et al. 2011; Shoener et al. 2014). Recent reports by McCarty et al. (2011) and Scherson and Criddle (2014) have concluded that net positive energy operation of municipal wastewater facilities is feasible if activated sludge is replaced with anaerobic biotechnology. However, the conclusions of these studies are questionable. McCarty et al. (2011) assumed a low-energy AnMBR without nutrient removal, which eliminates important energy demands for tertiary treatment. Scherson and Criddle (2014) modeled flocculant anaerobic biomass systems and indicated that net positive energy operation was feasible while heating wastewater from 15 to 35^{o}C despite a heating requirement for water of 1.17 kWh/m^{3}, which would greatly exceed energy gained from methane combustion. Despite the limitations of these studies, others have concluded that energy positive treatment is technically possible if anaerobic biotechnology is used for organic removal and phototrophic biotechnology is used for nutrient recovery (Shoener et al. 2014). In addition, Shoener et al. (2014) also indicate that further work is needed for technologies such as AnMBRs to eliminate parasitic losses from processes such as biogas sparging and mixing.

2.4.6 RESEARCH NEEDS

Additional work is needed in several areas in order to advance AnMBR technology as a viable alternative to existing aerobic processes. One of the primary research needs involves developing low energy membrane operation strategies in order to eliminate unnecessary energy demands. Work on energy reduction strategies can also be extended to other process elements including efficiency improvements to bioreactor configuration and hydraulic design to minimize losses from piping. Another need is to develop effective, low energy nutrient and dissolved

methane recovery methods to be used on AnMBR permeate. Lastly, more research is needed to define and understand the microbial community structure found within AnMBRs in order to understand the impacts of reactor selection and operation strategy on biomass performance.

2.5 REFERENCES

Aiyuk, S., Forrez, I., Lieven, D.K., van Haandel, A., Verstraete, W., 2006. Anaerobic and complementary treatment of domestic sewage in regions with hot climates - A review. Bioresour. Technol. 97, 2225–2241. doi:10.1016/j.biortech.2005.05.015

Akram, A., Stuckey, D.C., 2008. Flux and performance improvement in a submerged anaerobic membrane bioreactor (SAMBR) using powdered activated carbon (PAC). Process Biochem. 43, 93–102. doi:10.1016/j.procbio.2007.10.020

An, Y.Y., Yang, F.L., Bucciali, B., Wong, F.S., 2009. Municipal wastewater treatment using a UASB coupled with cross-flow membrane filtration. J. Environ. Eng. 135, 86–91. doi:10.1061/(ASCE)0733-9372(2009)135:2(86)

Aslam, M., McCarty, P.L., Bae, J., Kim, J., 2014. The effect of fluidized media characteristics on membrane fouling and energy consumption in anaerobic fluidized membrane bioreactors. Sep. Purif. Technol. 132, 10–15. doi:10.1016/j.seppur.2014.04.049

Bachmann, A., Beard, V.L., McCarty, P.L., 1985. Performance characteristics of the anaerobic baffled reactor. Water Res. 19, 99–106.

Baek, S., Pagilla, K., Kim, H., 2010. Lab-scale Study of an Anaerobic Membrane Bioreactor (AnMBR) for Dilute Municipal Wastewater Treatment. Biotechnol. Bioprocess 15, 704–708.

Baek, S.H., Pagilla, K.R., 2006. Aerobic and anaerobic membrane bioreactors for municipal wastewater treatment. Water Environ. Res. 78, 133–140. doi:10.2175/106143005X89599

Barker, D.J., Salvi, S.M.L., Langenhoff, A.A.M., Stuckey, D.C., 2000. SOLUBLE MICROBIAL PRODUCTS IN ABR TREATING LOW-STRENGTH WASTEWATER. J. Environ. Eng. 126, 239.

Beaubien, A., Baty, M., Jeannot, F., Francoeur, E., Manem, J., 1996. Design and operation of anaerobic membrane bioreactors: development of a filtration testing strategy. J. Memb. Sci. 109, 173–184.

Berube, P., Hall, E., 2006. Parameters governing permeate flux in an anaerobic membrane bioreactor treating low-strength municipal wastewaters: A literature review. Water Environ. Res. 78, 887. doi:10.2175/106143005X72858

Bialek, K., Kim, J., Lee, C., Collins, G., Mahony, T., O'Flaherty, V., 2011. Quantitative and

qualitative analyses of methanogenic community development in high-rate anaerobic bioreactors. Water Res. 45, 1298–1308. doi:10.1016/j.watres.2010.10.010

Bialek, K., Kumar, A., Mahony, T., Lens, P.N.L., O'Flaherty, V., 2012. Microbial community structure and dynamics in anaerobic fluidized-bed and granular sludge-bed reactors: Influence of operational temperature and reactor configuration. Microb. Biotechnol. 5, 738–752. doi:10.1111/j.1751-7915.2012.00364.x

Bocher, B.T.W., Cherukuri, K., Maki, J.S., Johnson, M., Zitomer, D.H., 2015. Relating methanogen community structure and anaerobic digester function. Water Res. 70, 425–435. doi:10.1016/j.watres.2014.12.018

Calderón, K., Rodelas, B., Cabirol, N., González-López, J., Noyola, A., 2011. Analysis of microbial communities developed on the fouling layers of a membrane-coupled anaerobic bioreactor applied to wastewater treatment. Bioresour. Technol. 102, 4618–4627. doi:10.1016/j.biortech.2011.01.007

Chaganti, S.R., Lalman, J. a., Heath, D.D., 2012. 16S rRNA gene based analysis of the microbial diversity and hydrogen production in three mixed anaerobic cultures. Int. J. Hydrogen Energy 37, 9002–9017. doi:10.1016/j.ijhydene.2012.02.146

Cheng, T.W., Yeh, H.M., Wu, J.H., 1999. Effects of gas slugs and inclination angle on the ultrafiltration flux in tubular membrane module. J. Memb. Sci. 158, 223–234. doi:10.1016/S0376-7388(99)00013-7

Cho, B., Fane, A., 2002. Fouling transients in nominally sub-critical flux operation of a membrane bioreactor. J. Memb. Sci. 209, 391–403.

Choo, K.H., Kang, I.J., Yoon, S.H., Park, H., Kim, J.H., Adlya, S., Lee, C.H., 2000. Approaches to membrane fouling control in anaerobic membrane bioreactors. Water Sci. Technol. 41, 363–371.

Choo, K.H., Lee, C.H., 1996a. MEMBRANE FOULING MECHANISMS IN THE MEMBRANE-COUPLED ANAEROBIC BIOREACTOR. Water Res. 30, 1771–1780.

Choo, K.H., Lee, C.H., 1996b. Effect of anaerobic digestion broth composition on membrane permeability. Water Sci. Technol. 34, 173–179.

Chu, L.-B., Yang, F.-L., Zhang, X.-W., 2005. Anaerobic treatment of domestic wastewater in a membrane-coupled expended granular sludge bed (EGSB) reactor under moderate to low temperature. Process Biochem. 40, 1063–1070. doi:10.1016/j.procbio.2004.03.010

Chua, H.C., Arnot, T.C., Howell, J. a., 2002. Controlling fouling in membrane bioreactors operated with a variable throughput. Desalination 149, 225–229. doi:10.1016/S0011-9164(02)00764-6

Collins, G., McHugh, S., Connaughton, S., Enright, A.-M., Kearney, A., Scully, C., Mahony, T.,

Madden, P., O'Flaherty, V., 2006. New low-temperature applications of anaerobic wastewater treatment. J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng. 41, 881–95. doi:10.1080/10934520600614504

Collins, G., Woods, A., McHugh, S., Carton, M.W., O'Flaherty, V., 2003. Microbial community structure and methanogenic activity during start-up of psychrophilic anaerobic digesters treating synthetic industrial wastewaters. FEMS Microbiol. Ecol. 46, 159–170. doi:10.1016/S0168-6496(03)00217-4

Connaughton, S., Collins, G., O'Flaherty, V., 2006. Development of microbial community structure and actvity in a high-rate anaerobic bioreactor at 18°C. Water Res. 40, 1009–1017. doi:10.1016/j.watres.2005.12.026

Cornel, P., Krause, S., 2008. Advanced Membrane Technology and Applications, in: Li, N., Fane, A., Ho, W.W.S., Matsuura, T. (Eds.), Advanced Membrane Technology and Applications. John Wiley & Sons, Inc., Hoboken, pp. 217–238.

Dagnew, M., Parker, W., Seto, P., Waldner, K., Hong, Y., Bayly, R., Cumin, J., 2011. Weftec 2011, in: Proceedings of the Water Environment Federation. Water Environment Federation, Los Angeles, California, pp. 4931–4941.

Dhaked, R.K., Singh, P., Singh, L., 2010. Biomethanation under psychrophilic conditions. Waste Manag. 30, 2490–2496. doi:10.1016/j.wasman.2010.07.015

Enright, A.M., McGrath, V., Gill, D., Collins, G., O'Flaherty, V., 2009. Effect of seed sludge and operation conditions on performance and archaeal community structure of low-temperature anaerobic solvent-degrading bioreactors. Syst. Appl. Microbiol. 32, 65–79. doi:10.1016/j.syapm.2008.10.003

Fane, A., Fell, C., Suki, A., 1983. The effect of pH and ionic environment on the ultrafiltration of protein solutions with retentive membranes. J. Memb. Sci. 16, 195–210.

Federal Register, 2011. Secondary Treatment Regulations.

Fernandez, A.S., Hashsham, S. a., Dollhopf, S.L., Raskin, L., Glagoleva, O., Dazzo, F.B., Hickey, R.F., Criddle, C.S., Tiedje, J.M., 2000. Flexible community structure correlates with stable community function in methanogenic bioreactor communities perturbed by glucose. Appl. Environ. Microbiol. 66, 4058–4067. doi:10.1128/AEM.66.9.4058-4067.2000

Foresti, E., Zaiat, M., Vallero, M., 2006. Anaerobic processes as the core technology for sustainable domestic wastewater treatment: Consolidated applications, new trends, perspectives, and challenges. Rev. Environ. Sci. Biotechnol. 5, 3–19. doi:10.1007/s11157-005-4630-9

Gao, D.-W., Zhang, T., Tang, C.-Y.Y., Wu, W.-M., Wong, C.-Y., Lee, Y.H., Yeh, D.H., Criddle, C.S., 2010. Membrane fouling in an anaerobic membrane bioreactor: Differences in relative

abundance of bacterial species in the membrane foulant layer and in suspension. J. Memb. Sci. 364, 331–338. doi:10.1016/j.memsci.2010.08.031

Garcia-Calderon, D., Buffiere, P., Moletta, R., Elmaleh, S., 1998. Anaerobic digestion of wine distillery wastewater in down-flow fluidized bed. Water Res. 32, 3593–3600. doi:10.1016/S0043-1354(98)00134-1

Giménez, J.B., Robles, a, Carretero, L., Durán, F., Ruano, M. V, Gatti, M.N., Ribes, J., Ferrer, J., Seco, a, 2011. Experimental study of the anaerobic urban wastewater treatment in a submerged hollow-fibre membrane bioreactor at pilot scale. Bioresour. Technol. 102, 8799–806. doi:10.1016/j.biortech.2011.07.014

Hernández, A.E., Belalcazar, L.C., Rodríguez, M.S., Giraldo, E., 2002. Retention of granular sludge at high hydraulic loading rates in an anaerobic membrane bioreactor with immersed filtration. Water Sci. Technol. 45, 169–74.

Herrera-Robledo, M., Cid-León, D.M., Morgan-Sagastume, J.M., Noyola, a., 2011. Biofouling in an anaerobic membrane bioreactor treating municipal sewage. Sep. Purif. Technol. 81, 49–55. doi:10.1016/j.seppur.2011.06.041

Herrera-Robledo, M., Morgan-Sagastume, J.M., Noyola, A., 2010. Biofouling and pollutant removal during long-term operation of an anaerobic membrane bioreactor treating municipal wastewater. Biofouling 26, 23–30. doi:10.1080/08927010903243923

Hilal, N., Ogunbiyi, O.O., Miles, N.J., Nigmatullin, R., 2005. Methods employed for control of fouling in MF and UF membranes: A comprehensive review. Sep. Sci. Technol. doi:10.1081/SS-200068409

Ho, J., Sung, S., 2010. Methanogenic activities in anaerobic membrane bioreactors (AnMBR) treating synthetic municipal wastewater. Bioresour. Technol. 101, 2191–6. doi:10.1016/j.biortech.2009.11.042

Ho, J., Sung, S., 2009. Anaerobic membrane bioreactor treatment of synthetic municipal wastewater at ambient temperature. Water Environ. Res. 81, 922–928. doi:10.2175/106143009X407339

Ho, J.H., Khanal, S.K., Sung, S., 2007. Anaerobic membrane bioreactor for treatment of synthetic municipal wastewater at ambient temperature. Water Sci. Technol. 55, 79. doi:10.2166/wst.2007.130

Hu, A.Y., Stuckey, D.C., 2007. Activated carbon addition to a submerged anaerobic membrane bioreactor: effect on performance, transmembrane pressure, and flux. J. Environ. Eng. 133, 73. doi:10.1061/(ASCE)0733-9372(2007)133:1(73)

Hu, A.Y., Stuckey, D.C., 2006. Treatment of Dilute Wastewaters Using a Novel Submerged Anaerobic Membrane Bioreactor. J. Environ. Eng. 132, 190–198. doi:10.1061/(ASCE)0733-

9372(2006)132:2(190)

Huang, Z., Ong, S.L., Ng, H.Y., 2011. Submerged anaerobic membrane bioreactor for low-strength wastewater treatment: effect of HRT and SRT on treatment performance and membrane fouling. Water Res. 45, 705–13. doi:10.1016/j.watres.2010.08.035

Jeison, D., van Lier, J.B., 2006. On-line cake-layer management by trans-membrane pressure steady state assessment in Anaerobic Membrane Bioreactors for wastewater treatment. Biochem. Eng. J. 29, 204–209. doi:10.1016/j.bej.2005.11.017

Jiang, T., Kennedy, M.D., Guinzbourg, B.F., Vanrolleghem, P. a., Schippers, J.C., 2005. Optimising the operation of a MBR pilot plant by quantitative analysis of the membrane fouling mechanism. Water Sci. Technol. 51, 19–25.

Kim, J., Kim, K., Ye, H., Lee, E., Shin, C., McCarty, P.L., Bae, J., 2011. Anaerobic fluidized bed membrane bioreactor for wastewater treatment. Environ. Sci. Technol. 45, 576–581. doi:10.1021/es1027103

Kim, J., Somiya, I., Kishimoto, N., 1998. Flux recovery by ozone aeration on anaerobic membrane bioreactor for carbon recovery. Proc. Environ. Eng. Res. 35, 265–272.

Krause, S., Zimmermann, B., Meyer-Blumenroth, U., Lamparter, W., Siembida, B., Cornel, P., 2010. Enhanced membrane bioreactor process without chemical cleaning. Water Sci. Technol. 61, 2575–80. doi:10.2166/wst.2010.183

Krause, S., Zimmermann, B., Siembida, B., Cornel, P., 2009. Mechanical Cleaning Process for Enhanced MBR-Performance, in: Proceedings of the Water Environment Federation, WEFTEC 2009. Water Environment Federation, pp. 5633–5643.

Le-Clech, P., Chen, V., Fane, T.A.G., 2006. Fouling in membrane bioreactors used in wastewater treatment. J. Memb. Sci. 284, 17–53. doi:10.1016/j.memsci.2006.08.019

Lee, S.M., Jung, J.Y., Chung, Y.C., 2001. Novel method for enhancing permeate flux of submerged membrane system in two-phase anaerobic reactor. Water Res. 35, 471–7.

Lettinga, G., Pol, L.H., 1986. Advanced reactor design, operation and economy. Water Sci. Technol. 18, 99–108.

Lettinga, G., Rebac, S., Parshina, S., Nozhevnikova, a, van Lier, J.B., Stams, a J., 1999. High-rate anaerobic treatment of wastewater at low temperatures. Appl. Environ. Microbiol. 65, 1696–1702. doi:10.2166/wst.2008.040

Lettinga, G., Rebac, S., Zeeman, G., 2001. Challenge of psychrophilic anaerobic wastewater treatment. Trends Biotechnol. 19, 363–70. doi:10.1016/S0167-7799(01)01701-2

Lettinga, G., Velsen, A.F.M. van, Hobma, S.W., Zeeuw, W. de, Klapwijk, A., 1980. Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment,

especially for anaerobic treatment. Biotechnol. Bioeng. 22, 699–734.

Lew, B., Tarre, S., Beliavski, M., Dosoretz, C., Green, M., 2009. Anaerobic membrane bioreactor (AnMBR) for domestic wastewater treatment. Desalination 243, 251–257. doi:10.1016/j.desal.2008.04.027

Liao, B.Q., Kraemer, J.T., Bagley, D.M., 2006. Anaerobic membrane bioreactors: Applications and research directions. Crit. Rev. Environ. Sci. Technol. 36, 489–530. doi:10.1080/10643380600678146

Lin, C.Y., Noike, T., Sato, K., Matsumoto, J., 1987. Temperature characteristics of the methanogenesis process in anaerobic digestion. Water Sci. Technol. 19, 299–310.

Lin, H., Peng, W., Zhang, M., Chen, J., Hong, H., Zhang, Y., 2013. A review on anaerobic membrane bioreactors: Applications, membrane fouling and future perspectives. Desalination 314, 169–188. doi:10.1016/j.desal.2013.01.019

Madden, P., Chinalia, F. a., Enright, A.M., Collins, G., O'Flaherty, V., 2010. Perturbation-independent community development in low-temperature anaerobic biological wastewater treatment bioreactors. Biotechnol. Bioeng. 105, 79–87. doi:10.1002/bit.22507

Martin, I., Pidou, M., Soares, a., Judd, S., Jefferson, B., 2011. Modelling the energy demands of aerobic and anaerobic membrane bioreactors for wastewater treatment. Environ. Technol. 32, 921–932. doi:10.1080/09593330.2011.565806

Martinez-Sosa, D., Helmreich, B., Netter, T., Paris, S., Bischof, F., Horn, H., 2011. Pilot-scale anaerobic submerged membrane bioreactor (AnSMBR) treating municipal wastewater: the fouling phenomenon and long-term operation. Water Sci. Technol. 64, 1804–11. doi:10.2166/wst.2011.745

McCarty, P.L., 1964. Anaerobic Waste Treatment Fundamentals. Public Work. 95, 107–112.

McCarty, P.L., Bae, J., Kim, J., 2011. Domestic wastewater treatment as a net energy producer-can this be achieved? Environ. Sci. Technol. 45, 7100–7106. doi:10.1021/es2014264

McCarty, P.L., Smith, D.P., 1986. Anaerobic wastewater treatment. Environ. Sci. Technol. 20, 1200–1206. doi:10.1021/es00154a002

McHugh, S., Collins, G., Mahony, T., O'Flaherty, V., 2005. Biofilm reactor technology for low temperature anaerobic waste treatment: microbiology and process characteristics. Water Sci. Technol. 52, 107–113.

McKeown, R.M., Hughes, D., Collins, G., Mahony, T., O'Flaherty, V., 2012. Low-temperature anaerobic digestion for wastewater treatment. Curr. Opin. Biotechnol. 23, 444–451. doi:10.1016/j.copbio.2011.11.025

McKeown, R.M., Scully, C., Mahony, T., Collins, G., O'Flaherty, V., 2009. Long-term (1,243 days),

low-temperature (4-15 degrees C), anaerobic biotreatment of acidified wastewaters: bioprocess performance and physiological characteristics. Water Res. 43, 1611–20. doi:10.1016/j.watres.2009.01.015

Meng, F., Chae, S.-R., Drews, A., Kraume, M., Shin, H.-S., Yang, F., 2009. Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material. Water Res. 43, 1489–512. doi:10.1016/j.watres.2008.12.044

Mercier, M., Fonade, C., Lafforgue-Delorme, C., 1997. How slug flow can enhance the ultrafiltration flux in mineral tubular membranes. J. Memb. Sci. 128, 103–113. doi:10.1016/S0376-7388(96)00317-1

Metcalf & Eddy, 2003. Wastewater Engineering, Treatment and Reuse, 4th ed.

Mo, W., Zhang, Q., 2013. Energy-nutrients-water nexus: Integrated resource recovery in municipal wastewater treatment plants. J. Environ. Manage. 127, 255–267. doi:10.1016/j.jenvman.2013.05.007

Morita, M., Malvankar, N.S., Franks, A.E., Summers, Z.M., Giloteaux, L., Rotaru, A.E., Rotaru, C., Lovleya, D.R., 2015. Potential for direct interspecies electron transfer in methanogenic wastewater digester aggregates. MBio 2. doi:10.1128/ mBio.00159-11

Nagata, N., Herouvis, K.J., Dziewulski, D.M., Belfort, G., 1989. Cross-flow membrane microfiltration of a bacteriol fermentation broth. Biotechnol. Bioeng. 34, 447–466.

Norddahl, B., Rohold, L., 2000. The Biorek® concept for the conversion of organic effluent to energy, concentrated fertilizer and potable water, in: Kyritsis, S. (Ed.), 1st World Conference on Biomass for Energy and Industry: Proceedings of the Conference Held in Sevilla, Spain, 5-9 June 2000, Volume 2. James and James Ltd, Sevilla, pp. 1529–1532.

O'Reilly, J., Lee, C., Chinalia, F., Collins, G., Mahony, T., O'Flaherty, V., 2010. Microbial community dynamics associated with biomass granulation in low-temperature (15 °C) anaerobic wastewater treatment bioreactors. Bioresour. Technol. 101, 6336–6344. doi:10.1016/j.biortech.2010.03.049

O'Reilly, J., Lee, C., Collins, G., Chinalia, F., Mahony, T., O'Flaherty, V., 2009. Quantitative and qualitative analysis of methanogenic communities in mesophilically and psychrophilically cultivated anaerobic granular biofilims. Water Res. 43, 3365–3374. doi:10.1016/j.watres.2009.03.039

Petropoulos, E., Dolfing, J., Bowen, E., Davenport, R., Curtis, T., 2013. Anaerobic digestion of domestic wastewater at low temperatures (4 , 8 and 15 o C) in reactors with psychrophilic inocula . 13th World Congr. Anaerob. Dig. 1, 1–4.

Rebac, S., Lier, J. van, Lens, P., Stams, A., Dekkers, F., Swinkels, K., Lettinga, G., 1999. Psychrophilic anaerobic treatment of low strength wastewaters. Water Sci. Technol. 39,

203–310.

Regueiro, L., Veiga, P., Figueroa, M., Alonso-Gutierrez, J., Stams, A.J.M., Lema, J.M., Carballa, M., 2012. Relationship between microbial activity and microbial community structure in six full-scale anaerobic digesters. Microbiol. Res. 167, 581–589. doi:10.1016/j.micres.2012.06.002

Rittmann, B.E., Mayer, B., Westerhoff, P., Edwards, M., 2011. Capturing the lost phosphorus. Chemosphere 84, 846–853. doi:10.1016/j.chemosphere.2011.02.001

Rittmann, B.E., McCarty, P.L., 2001. Environmental Biotechnology: Principles and Applications. McGraw-Hill.

Rivière, D., Desvignes, V., Pelletier, E., Chaussonnerie, S., Guermazi, S., Weissenbach, J., Li, T., Camacho, P., Sghir, A., 2009. Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. ISME J. 3, 700–14. doi:10.1038/ismej.2009.2

Ross, W., Barnard, J., Strohwald, N., Grobler, C., Sanetra, J., 1992. Practical application of the ADUF process to the full-scale treatment of a maize-processing effluent. Water Sci. Technol. 25, 27–39.

Saddoud, A., Ellouze, M., Dhouib, A., Sayadi, S., 2007. Anaerobic membrane bioreactor treatment of domestic wastewater in Tunisia. Desalination 207, 205–215. doi:10.1016/j.desal.2006.08.005

Salazar-Peláez, M.L., Morgan-Sagastume, J.M., Noyola, A., 2011. Influence of hydraulic retention time on fouling in a UASB coupled with an external ultrafiltration membrane treating synthetic municipal wastewater. Desalination 277, 164–170. doi:10.1016/j.desal.2011.04.021

Sanz, J.L., Köchling, T., 2007. Molecular biology techniques used in wastewater treatment: An overview. Process Biochem. 42, 119–133. doi:10.1016/j.procbio.2006.10.003

Saw, C.B., Anderson, G.K., James, A., Le, M.S., 1985. A membrane technique for biomass retention in anaerobic waste treatment, in: University, P. (Ed.), Proceedings of the 40th Industrial Waste Conference : May 14, 15, 16, 1985 / Purdue University West Lafayette, Indiana. Butterworths, pp. 805–811.

Schauer-Gimenez, A.E., Zitomer, D.H., Maki, J.S., Struble, C. a., 2010. Bioaugmentation for improved recovery of anaerobic digesters after toxicant exposure. Water Res. 44, 3555–3564. doi:10.1016/j.watres.2010.03.037

Scherson, Y.D., Criddle, C.S., 2014. Recovery of freshwater from wastewater: Upgrading process configurations to maximize energy recovery and minimize residuals. Environ. Sci. Technol. 48, 8420–8432. doi:10.1021/es501701s

Schroepfer, G.J., Fullen, W.J., Johnson, A.S., Ziemke, N.R., Anderson, J.J., 1955. The anaerobic

contact process as applied to packing house wastes. Sew. Ind. Wastes 27, 460–467.

Seghezzo, L., Zeeman, G., Lier, J. van, 1998. A review: the anaerobic treatment of sewage in UASB and EGSB reactors. Bioresource 65, 175–190.

Shin, C., McCarty, P.L., Kim, J., Bae, J., 2014. Pilot-scale temperate-climate treatment of domestic wastewater with a staged anaerobic fluidized membrane bioreactor (SAF-MBR). Bioresour. Technol. 159, 95–103. doi:10.1016/j.biortech.2014.02.060

Shoener, B.D., Bradley, I.M., Cusick, R.D., Guest, J.S., 2014. Energy positive domestic wastewater treatment: the roles of anaerobic and phototrophic technologies. Environ. Sci. Process. Impacts 1204–1222. doi:10.1039/c3em00711a

Siembida, B., Cornel, P., Krause, S., Zimmermann, B., 2010. Effect of mechanical cleaning with granular material on the permeability of submerged membranes in the MBR process. Water Res. 44, 4037–4046. doi:10.1016/j.watres.2010.05.016

Siggins, A., Enright, A.M., O'Flaherty, V., 2011. Temperature dependent (37-15C) anaerobic digestion of a trichloroethylene-contaminated wastewater. Bioresour. Technol. 102, 7645–7656. doi:10.1016/j.biortech.2011.05.055

Skouteris, G., Hermosilla, D., López, P., Negro, C., Blanco, Á., 2012. Anaerobic membrane bioreactors for wastewater treatment: A review. Chem. Eng. J. 198-199, 138–148. doi:10.1016/j.cej.2012.05.070

Smith, A.L., Skerlos, S.J., Raskin, L., 2015. Anaerobic membrane bioreactor treatment of domestic wastewater at psychrophilic temperatures ranging from 15 °C to 3 °C. Environ. Sci. Water Res. Technol. 1, 56–64. doi:10.1039/C4EW00070F

Smith, A.L., Skerlos, S.J., Raskin, L., 2013. Psychrophilic anaerobic membrane bioreactor treatment of domestic wastewater. Water Res. 47, 1655–1665. doi:10.1016/j.watres.2012.12.028

Smith, A.L., Stadler, L.B., Cao, L., Love, N.G., Raskin, L., Skerlos, S.J., 2014. Navigating wastewater energy recovery strategies: A life cycle comparison of anaerobic membrane bioreactor and conventional treatment systems with anaerobic digestion. Environ. Sci. Technol. 48, 5972–5981. doi:10.1021/es5006169

Smith, A.L., Stadler, L.B., Love, N.G., Skerlos, S.J., Raskin, L., 2012. Perspectives on anaerobic membrane bioreactor treatment of domestic wastewater: A critical review. Bioresour. Technol. 122, 149–159. doi:10.1016/j.biortech.2012.04.055

Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L., 2007. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA.

Speece, R., 2008. Anaerobic Biotechnology and Odor/Corrosion Control for Municipalities and Industries. Archae Press, Nashville, TN.

Speece, R., 1996. Anaerobic Biotechnology for Industrial Wastewaters. Archae Press, Nashville, TN.

Speece, R., 1983. Anaerobic biotechnology for industrial wastewater. Environ. Sci. Technol. 17, 416A–427A.

Stephenson, T., Judd, S., Jefferson, B., Brindle, K., 2000. Membrane Bioreactors for Wastewater Treatment. IWA Publishing, London.

Stuckey, D.C., 2012. Recent developments in anaerobic membrane reactors. Bioresour. Technol. 122, 137–148. doi:10.1016/j.biortech.2012.05.138

Summers, Z.M., Fogarty, H.E., Leang, C., Franks, A.E., Malvankar, N.S., Lovley, D.R., 2010. Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. Science 330, 1413–1415. doi:10.1126/science.1196526

Sutton, P.M., Berube, P., Hall, E.R., 2004. Membrane bioreactors for anaerobic treatment of wastewaters. Water Environment Research Foundation, Alexandria, VA.

Sutton, P.M., Li, A., Evans, R.R., Korchin, S., 1983. Dorr-Oliver's fixed film and suspended growth anaerobic systems for industrial wastew ater treatment and energy recovery, in: Proceedings 37th Industrial Waste Conference. Ann Arbor Science, Layfayette, IN, pp. 667–675.

Switzenbaum, M.S., 1995. Obtacles in the implementation of anaerobic treatment technology. Bioresour. Technol. 53, 255–262.

Switzenbaum, M.S., Jewell, W.J., 1980. Anaerobic attached-film expanded-bed reactor treatment. J. (Water Pollut. Control Fed. 52, 1953–1965.

Taha, T., Cheong, W.L., Field, R.W., Cui, Z.F., 2006. Gas-sparged ultrafiltration using horizontal and inclined tubular membranes-A CFD study. J. Memb. Sci. 279, 487–494. doi:10.1016/j.memsci.2005.12.063

Tale, V.P., Maki, J.S., Struble, C. a., Zitomer, D.H., 2011. Methanogen community structure-activity relationship and bioaugmentation of overloaded anaerobic digesters. Water Res. 45, 5249–5256. doi:10.1016/j.watres.2011.07.035

van de Graaf, A.A., de Bruijn, P., Robertson, L.A., Jetten, M.S.M., Kuenen, J.G., 1996. Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. Microbiology 142, 2187–2196. doi:10.1099/13500872-142-8-2187

van den Berg, L., 1977. Effect of temperature on growth and activity of a methanogenic culture utilising acetate. Can. J. Microbiol. 23, 898–902.

van den Berg, L., Lenz, C.P., 1979. Comparison between up and downflow anaerobic fixed film reactors of varying surface to volume ratios for the treatment of bean blanching waste, in: 34th Purdue Industrial Waste Conference. pp. 39–325.

van der Gast, C.J., Jefferson, B., Reid, E., Robinson, T., Bailey, M.J., Judd, S.J., Thompson, I.P., 2006. Bacterial diversity is determined by volume in membrane bioreactors. Environ. Microbiol. 8, 1048–55. doi:10.1111/j.1462-2920.2006.00996.x

van Haandel, A., van der Lubbe, J.G.M., 2012. Handbook of Biological Wastewater Treatment: Design and Optimisation of Activated Sludge Systems. IWA Publishing.

van Lier, J.B., Rebac, S., Lens, P., van Bijnen, F., Elferink, S., Stams, A.J.M., Lettinga, G., 1997. Anaerobic treatment of partly acidified wastewater in a two-stage expanded granular sludge bed (EGSB) system at 8oC. Water Sci. Technol. 36, 317–324.

Vanwonterghem, I., Jensen, P.D., Ho, D.P., Batstone, D.J., Tyson, G.W., 2014. Linking microbial community structure, interactions and function in anaerobic digesters using new molecular techniques. Curr. Opin. Biotechnol. 27, 55–64. doi:10.1016/j.copbio.2013.11.004

von Sperling, M., 2007. Wastewater characteristics, treatment and disposal, Volume 1. IWA Publishing, New York.

Wen, C., Huang, X., Qian, Y., 1999. Domestic wastewater treatment using an anaerobic bioreactor coupled with membrane filtration. Process Biochem. 35, 335–340. doi:10.1016/S0032-9592(99)00076-X

Williams, A.T., Zitomer, D.H., Mayer, B.K., 2015. Ion exchange-precipitation for nutrient recovery from dilute wastewater. Environ. Sci. Water Res. Technol. 1, 832-838. doi:10.1039/C5EW00142K

Yoon, S.H., Kang, I.J., Lee, C.H., 1999. Fouling of inorganic membrane and flux enhancement in membrane-coupled anaerobic bioreactor. Sep. Sci. Technol. 34, 709–724. doi:10.1080/01496399908951140

Young, J.C., McCarty, P.L., 1969. The Anaerobic Filter for Waste Treatment. J. (Water Pollut. Control Fed. 41, R160–R173.

Zhang, J., Padmasiri, S.I., Fitch, M., Norddahl, B., Raskin, L., Morgenroth, E., 2007. Influence of cleaning frequency and membrane history on fouling in an anaerobic membrane bioreactor. Desalination 207, 153–166. doi:10.1016/j.desal.2006.07.009

Zhang, S., Qu, Y., Liu, Y., Yang, F., Zhang, X., Furukawa, K., Yamada, Y., 2005. Experimental study of domestic sewage treatment with a metal membrane bioreactor. Desalination 177, 83–93. doi:10.1016/j.desal.2004.10.034

Zitomer, D.H., Bachman, T.C., Vogel, D.S., 2005. Thermophilic anaerobic digester with ultrafilter for solids stabilization. Water Sci. Technol. 52, 525–30.

3 REDUCED ENERGY DEMAND FOR MUNICIPAL WASTEWATER RECOVERY USING AN ANAEROBIC FLOATING FILTER MEMBRANE BIOREACTOR

"I only feel angry when I see waste. When I see people throwing away things we could use."

Mother Teresa
A Gift for God, 1975

This chapter has been submitted to the journal *Environmental Science: Water Research & Technology* as:

Seib, M.D., Berg, K.J., Zitomer, D.H., 2015. Reduced energy demand for municipal wastewater recovery using an anaerobic floating filter membrane bioreactor. Environ. Sci.: Water Res. Technol. Submitted.

3.1 INTRODUCTION

Sustainable scenarios for municipal wastewater management often involve replacing aerobic systems with anaerobic biotechnology (Verstraete & Vlaeminck 2011; McCarty et al. 2011). Wastewater management scenarios for cities of the future emphasize water, energy, and nutrient (nitrogen, phosphorous, and potassium) recovery (van Lier 2008; Mo & Zhang 2013) with reduced biosolids production and energy usage. For this, anaerobic treatment can be superior to aerobic processes (Speece 2008; Verstraete & Vlaeminck 2011; McCarty et al. 2011). Furthermore, wastewater treatment can be decentralized to reuse water locally without the need for extensive conveyance systems. This can be done by constructing water reclamation facilities within self-contained eco-blocks or dense urban areas (Novotny 2011; van Lier & Lettinga 1999). Again, anaerobic systems may offer an advantage by requiring smaller footprint area than aerobic systems.

Although anaerobic systems have benefits**,** challenges must be overcome before they can be widely employed for municipal water recovery in cold climates. For example, anaerobic biotechnology traditionally has been only applied to high-strength wastewater, manure, and biosolids (van Haandel et al. 2006; Switzenbaum 1983). Further, anaerobic processes are traditionally performed at mesophilic or thermophilic temperatures (25-50^{o}C), which are cost prohibitive for municipal wastewater recovery if heating is required (Lettinga et al. 2001; Martin et al. 2011). Anaerobic biotechnology for municipal wastewater recovery must be feasible at low temperatures without reactor heating in order to be more sustainable for widespread application in cold and/or temperate climates (Collins et al. 2006; Smith et al. 2013).

Low temperature operation, however, creates organic removal challenges for anaerobic systems. Low temperature decreases microorganism growth and metabolism rates, potentially

leading to poor organic removal (Switzenbaum 1983), especially at the short hydraulic residence times (HRTs) necessary for low energy and small footprint applications. Also, low strength municipal wastewater does not contain sufficient organic pollutant concentrations to produce enough methane to be practically useful if heating is necessary for effective treatment (Lettinga et al. 2001; Martin et al. 2011; Smith et al. 2012). Lastly, anaerobic processes convert most of the nitrogen and phosphorus to soluble ammonia and phosphate rather than removing them via nitrification/denitrification and biological accumulation as is done in aerobic processes. Therefore, additional nutrient removal steps often will be required after anaerobic treatment to achieve effluent quality sufficient for discharge to receiving waters.

Progress has recently been made to overcome the organic removal challenges faced by anaerobic municipal wastewater recovery. Over the past decade, anaerobic membrane bioreactor (AnMBR) technology has gained much attention. Several reviews have summarized laboratory and pilot scale studies examining AnMBRs for both industrial and municipal applications (Sutton et al. 2004; Liao et al. 2006; Skouteris et al. 2012; Smith et al. 2012; Stuckey 2012; Lin et al. 2013). These studies focused on operational parameters such as HRT, solids retention time (SRT), temperature, membrane flux, transmembrane pressure (TMP), reactor design, and membrane configuration. Recent low temperature (as low as 6^{o}C) AnMBR studies treating municipal wastewater by Ho and Sung (2009), Smith et al. (2013), Smith et al. (2015), and Shin et al. (2014) have all successfully demonstrated low effluent five day biochemical oxygen demand (BOD_5) (< 20 mg/L) and chemical oxygen demand (COD) (<40 mg/L) while employing different bioreactor and membrane configurations.

Existing AnMBR studies reveal two main strategies for bioreactor selection. In the first strategy, complete-mix stirred tank reactors (CSTRs) are used to maintain flocculent biomass (Ho & Sung 2010). AnMBR studies employing CSTRs with submerged membranes have shown

promising results with energy demands competitive to those required for organic removal with conventional activated sludge aeration (Smith et al. 2013; Smith et al. 2015; Martin et al. 2011). However, if operated with high suspended solids concentrations, membrane fouling potential is increased in these systems (Meng et al. 2009). In the second strategy, attached growth technologies such as the upflow anaerobic sludge blanket (UASB) or fluidized bed reactors (FBRs) are used to maintain granules or biofilms and reduce bulk liquid suspended solids, thus reducing bulk liquid foulants seen by the membrane (Shin et al. 2014). Biofilm technologies are often more efficient than flocculent systems because biofilm formation enhances interspecies substrate degradation and mass transfer and may allow for direct electron transfer between individual cells (McCarty & Smith 1986; Morita et al. 2015); making biofilm technologies a promising option (McHugh et al. 2005; Sutton et al. 2004; Rittmann & McCarty 2001; McCarty et al. 2011; Kim et al. 2011).

Advancement of AnMBR technology is dependent upon a reliable bioreactor and design that minimizes energy demands both for bioreactor and membrane operation. While biofilm technologies may demonstrate high substrate conversion rates, there are drawbacks such as difficulty forming granular biomass and retaining biosolids in UASB reactors (O'Flaherty et al. 2006) and high energy requirements for fluidizing recycle flow in FBRs. Additionally, biofilm reactors are often coupled to external membrane configurations (Liao et al. 2006) that have historically required 3.0 to 7.3 kWh/m^3, but have been reported as high as 10 kWh/m^3 (Le-Clech et al. 2006). This energy consumption is well above the 0.3-0.6 kWh/m^3 typically required for activated sludge (Metcalf & Eddy 2003), and is also above the energy that can be gained from the CH_4 produced. However, in recent years new methods of external membrane operation have been developed that drastically reduce energy demands. For example, Kim et al. (2011) operated a two-stage fluidized-bed AnMBR and indicated energy demands for the first-stage

FBR accounted for 52% of total energy demand. In order to minimize energy needed to operate AnMBRs, bioreactor recycle pumping rates should be reduced.

The objectives of this study were to develop an AnMBR using a biological downflow floating media filter (DFF) that required less energy than a FBR to achieve effluent BOD_5 concentrations less than 10 mg/L for municipal wastewater management and to demonstrate the feasibility of implementing anaerobic biotechnology as a viable alternative to activated sludge. Alternative attached growth bioreactor configurations have been developed in the past, including the anaerobic packed bed (APB) or anaerobic filter (AF) (Speece 1983; Switzenbaum 1983), which require significantly lower recycle pumping rates than an FBR. However, these configurations have historically not been widely adopted (van Lier 2008). While membrane incorporation has been shown to improve organic removal in other bioreactor configurations, no reports were found describing membranes coupled to a DFF for low strength municipal wastewater recovery.

3.2 MATERIALS AND METHODS

3.2.1 ANMBR CONFIGURATIONS

Two different AnMBR configurations, having different biofilm carrier materials, recycle flows and membrane types, were employed (Figure 3.1). The first AnMBR configuration was a DFF utilizing buoyant media coupled to an external polymeric cross-flow membrane. The DFF bioreactor contained 165 g of buoyant media (Aqwise, Herzliya, Israel) and was operated with a downflow recycle velocity of 11 m/h. The DFF polymeric tubular membrane consisted of two 750 mm long, 12.5 mm diameter polyvinylidene fluoride (PVDF) tubes (surface area = 0.059 m^2) with nominal molecular weight cutoff of 100 kDa (~0.018 µm nominal pore size) encased in a

stainless steel housing (FP100, PCI Membranes, Fareham, UK). The second was a FBR using granular activated carbon (GAC) media coupled to an external ceramic cross-flow membrane. The FBR bioreactor contained 300 g of 12 x 30 mesh GAC (TIGG 5DC 1230, TIGG Corp, Oakdale, PA) fluidized at an upflow velocity of 30 m/h. The FBR ceramic tubular membrane was a single, 100 cm long, 16 mm diameter aluminum oxide tube (surface area = 0.05 m^2) with a 0.05 µm nominal pore size encased in a stainless steel housing (Type 1/16, atech innovations, Gladbeck, Germany).

Each bioreactor consisted of an 80 cm tall, 6.35 cm diameter clear polyvinylchloride tube with a working volume of 2.3 L. Each external membrane system consisted of an equalization tank, pulse dampener, and membrane unit with combined working volume of 1 L (Figure 3.1). A recycle line was used to transfer retentate from the membrane equalization tanks back to the bioreactors. All membranes were mounted vertically and TMP was recorded at the top and bottom of each module using gauges (NOSHOK Inc., Berea, OH). Peristaltic pumps (Masterflex, Vernon Hills, IL) were used for bioreactor recycle, fluid transfer, and membrane cross-flow. Recycle head losses were determined using a digital manometer (EXTECH Instruments, Nashua, NH).

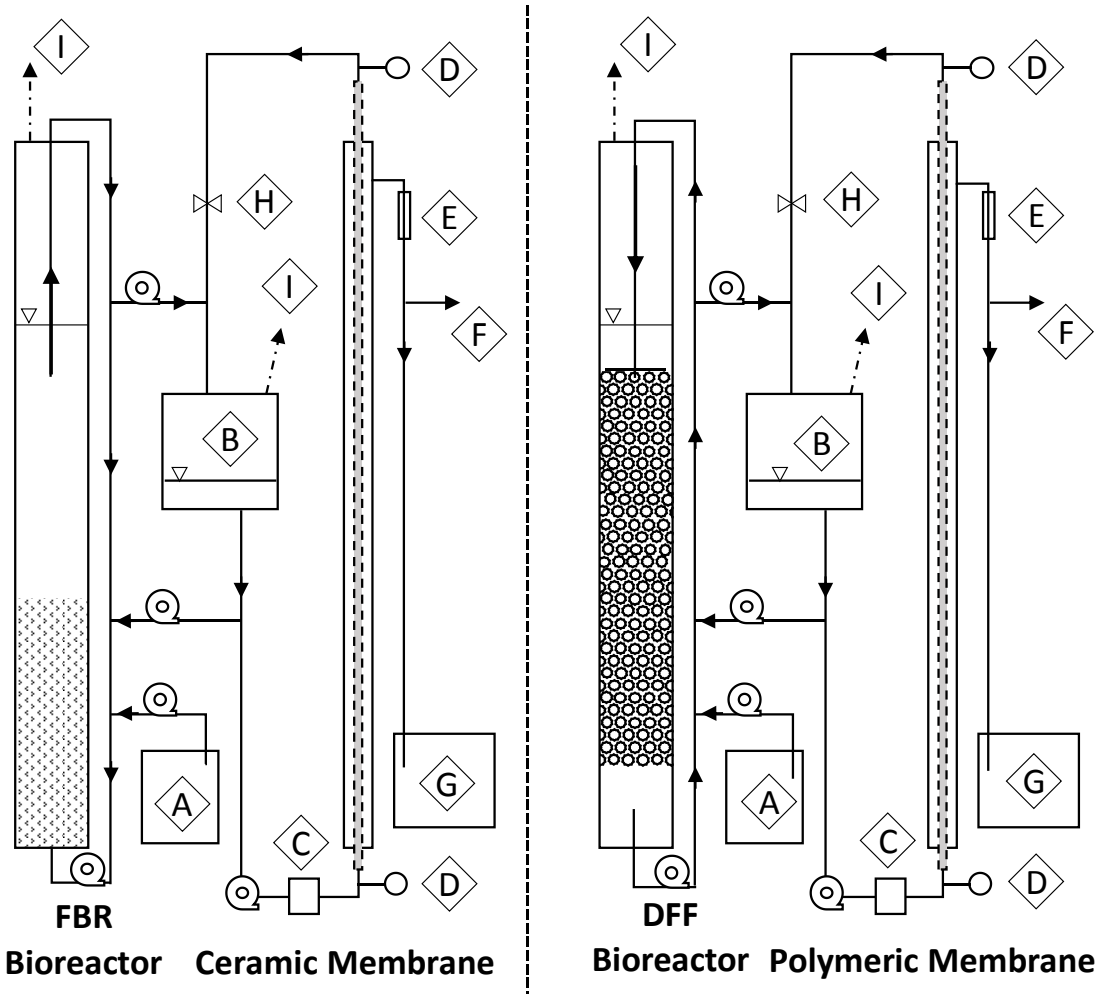

Figure 3.1 Schematic of FBR with ceramic and DFF with polymeric membrane. A. Feed tank, B. Equalization tank, C. Pulse dampener, D. Pressure gauge, E. Flow meter, F. Excess permeate flow return, G. Permeate tank, H, Pressure control valve, I. Biogas collection

3.2.2 BIOREACTOR INOCULA AND OPERATION

Each bioreactor was inoculated with 2 g VSS of a biomass mix from five sources including two different mesophilic upflow anaerobic sludge blanket (UASB) reactors treating brewery wastewaters, a mesophilic municipal anaerobic digester treating primary and waste activated sludges, an ambient-temperature industrial anaerobic lagoon treating sugar beet waste, and a laboratory, mesophilic anaerobic propionate enrichment culture previously described by Tale et al. (2011).

Bioreactors were fed a synthetic primary effluent (SPE) wastewater that was modeled after primary effluent at the South Shore Water Reclamation Facility (Oak Creek, WI). SPE was formulated with constituents adapted from the SYNTHES recipe developed by Aiyuk and Verstraete (2004) and an inorganic nutrient media developed by Speece (2008) (Table 3.1). SPE contained the following average constituent concentrations in deionized water: 235 mg/L BOD_5, 480 mg/L total chemical oxygen demand (TCOD), 18 mg/L ammonia nitrogen (NH_3-N), 43 mg/L organic nitrogen (N_{org}) 2.5 mg/L phosphate-phosphorus (PO_4^{-3}-P), 5 mg/L total phosphorus (TP), 120 mg/L total suspended solids (TSS), and 115 mg/L volatile suspended solids (VSS).

Each AnMBR configuration was evaluated at both 10 and 25°C, for a total of four systems (FBR10, FBR25, DFF10, DFF25). During start-up, all AnMBRs were acclimated for 45 days at 25°C with a total system HRT of 18 hr (12.5 h bioreactor, 5.5 h membrane compartment). After day 45, the temperature in FBR10 and DFF10 AnMBRs was reduced to 10°C. The AnMBRs were allowed to acclimate until day 79; during this time no performance data were collected. From day 80 to 145, total system HRT for all AnMBRs was reduced to 9 h. On day 146, HRT for each system was adjusted to the lowest value required to achieve membrane permeate BOD_5 <10 mg/L. During acclimation, the influent flowrate to the AnMBRs was less than the membrane permeate flow rate and a portion of membrane permeate was returned to each membrane equalization tank. Once HRT was adjusted on day 146, the influent flow rate to some AnMBRs was greater than the membrane permeate flow, so any excess bioreactor flow to membrane equalization tanks was directly removed from the system before it passed through the membrane.

Table 3.1 Synthetic primary effluent (SPE) constituents

Constituent	mg/L
Organic	
Non-fat dry milk	133
Soluble potato starch	133
Yeast extract	67
Casein peptone	67
$CH_3COONa \cdot 3H_2O$	75
Cysteine	10
Inorganic	
$NaHCO_3$	510
$MgCl_2 \cdot 6H_2O$	260
$CaCl_2 \cdot 2H_2O$	275
NaCl	140
NH_4Cl	64
$MgSO_4$	36
$FeSO_4 \cdot 7H_2O$	23
KCl	12
KI	10
$MgHPO_4 \cdot 3H_2O$	7
$(NaPO_3)_6$	4
$CoCl_2 \cdot 6H_2O$; $NiCl_2 \cdot 6H_2O$; $ZnCl_2$	1
$MnCl_2 \cdot 4H_2O$; NH_4VO_3; $CuCl_2 \cdot 2H_2O$; $AlCl_3 \cdot 6H_2O$; $NaMoO_4 \cdot 2H_2O$; H_2BO_3; $NaWO_4 \cdot 2H_2O$; Na_2SeO_3	0.5[a]

[a] The concentration of each compound was this value

3.2.3 MEMBRANE OPERATION

The membranes were operated at target fluxes of 5.9 to 7.4 $L/m^2 \cdot h$ by manually controlling TMP. The ceramic and polymeric membranes were operated at cross-flow velocities of 0.27 to 0.30 m/s, respectively. Membranes were considered fouled when the average TMP increased above 0.9 bar. Once a membrane fouled, it was removed and cleaned by spraying the inside of the membrane tube with a water jet to remove the fouling cake layer then chemically

cleaned by soaking in a high pH bath for 60 minutes and then an acidic bath for 25 minutes. For the ceramic membrane, the high pH bath consisted of a solution of NaClO (200 ppm free chlorine) adjusted to a pH of 11 using 6N NaOH. For the polymeric membrane, the high pH bath consisted of a solution of NaClO (200 ppm free chlorine) with a pH of 10. The acidic bath for both membranes consisted of distilled water adjusted to a pH of 2 using HNO_3. Solids removed during cleaning were collected and quantified along with liquid wasted from equalization tanks to determine VSS mass wasting rate from each system.

3.2.4 ANALYTICAL PROCEDURES

Influent and effluent BOD_5, TCOD, NH_3-N, N_{org}, PO_4^{-3}-P, TP, TSS, and VSS concentrations were determined by standard methods (APHA et al. 1999). Volatile fatty acid concentrations were determined by gas chromatography with a flame ionization detector (FID) (Agilent 7890A, Santa Clara, CA). Sulfate concentrations were determined using an ion chromatograph (Dionex ICS-1100, Sunnyvale, CA) and packed column (Ionpac AS22, Dionex, Sunnyvale, CA). Biogas methane and permeate dissolved methane content were determined using gas chromatography with a thermal conductivity detector (TCD) (Agilent 7890A, Santa Clara, CA). Biogas was collected in 2 L Tedlar bags and the volume quantified using a 140 mL syringe. Dissolved methane in membrane permeate was quantified using the method of Kim et al. (2011). Briefly, permeate samples were collected in 60 mL serum bottles that were previously dried and weighed. Each serum bottle contained 0.2 mL of 6N NaOH. Serum bottles were filled with approximately 50 mL of permeate and immediately sealed with rubber stoppers. The sealed bottle was then weighed to determine the exact volume of liquid in the bottle. Bottles were then incubated at 35°C and shaken at 200 rpm using an orbital shaker table for one hour. Serum bottle headspace gas was sampled and analyzed for methane content using gas chromatography

and the initial dissolved methane concentration was calculated based on Henry's law and measured headspace methane content.

3.2.5 ENERGY ESTIMATE

An energy estimate was performed to determine the overall energy requirements to treat 40,000 m^3/day municipal wastewater using either aerobic or anaerobic processes, both with primary sedimentation and anaerobic solids digestion. Energy inputs/outputs for different unit processes including BOD_5 removal, nutrient recovery/removal, solids processing, anaerobic solids digestion, and energy generated from methane were determined from literature values. Activated sludge aeration energy required for BOD_5 removal was reported by Speece (1996). Energies required for conventional biological nutrient removal and solids processing as well as produced from methane in anaerobic solids digestion were obtained from previous literature (WEF 2009). AnMBR normalized energy requirements for each bioreactor used in this study were determined using the power equation for pumping (Yoo et al. 2012), $P=(Q\gamma E)/(Q_i\eta)$, where P is power requirement per cubic meter treated (kWh/m^3), Q is recycle flow rate (m^3/s), γ is specific weight of water (kN/m^3), E is headloss (m H_2O), Q_i is influent flow to that portion of the system (m^3/h), and η is pump efficiency (assumed 66%). Recycle headlosses were determined for the FBR and DFF bioreactors using a manometer.

To compare nutrient removal in aerobic and anaerobic systems, ion exchange was assumed for recovery of N and P in the anaerobic system and the energy requirement for ion exchange systems (both N and P) was reported by Howe et al. (2012). Energies for anaerobic biosolids digestion for the AnMBR systems were considered to be 75% of that of activated sludge systems; this assumes a 25% reduction in the overall dry mass of waste biosolids that need to be processed from combining primary sludge with solids removed from AnMBR primary

effluent treatment versus primary sludge combined with activated sludge treatment of primary effluent. Energy needed for dissolved methane stripping/recovery was reported by McCarty et al. (2011). AnMBR energy generation from methane production was estimated based on COD reduction assuming 0.28 m^3 CH_4 per kg COD removed (1 atm, 0°C) and 37 MJ/m^3 CH_4 (Khartchenko et al. 1997). From estimated AnMBR produced methane, electrical energy production was estimated assuming 33% conversion of methane energy to electricity (Kim et al. 2011).

3.3 RESULTS AND DISCUSSION

3.3.1 ANMBR PERFORMANCE AND ORGANIC REMOVAL COMPARISON

All AnMBRs produced high quality effluent based on BOD_5, with average permeate concentration less than 5 mg/L for the FBR25, FBR10, and DFF25 systems and less than 8 mg/L for the DFF10 after day 146 (Figure 3.2). It should be noted that HRT in all systems was adjusted on day 146 to achieve average permeate BOD_5 concentration less than 10 mg/L, resulting in bioreactor HRT values for the FBR25, DFF25, FBR10 and DFF10 systems of 4.2 h, 4.2 h, 5.6 h, and 9.8 h, respectively. Permeate BOD_5 consistently remained low once HRT values were adjusted.

All AnMBRs achieved greater than 95% TCOD removal. Average permeate TCOD concentrations were less than 14 mg/L for DFF25 and DFF10 systems and less than 25 mg/L TCOD for the FBR25 and FBR10 systems after day 145. Permeate TCOD and soluble chemical oxygen demand (SCOD) were similar, as was expected since the membrane nominal pore sizes were smaller than the standard 0.45 μm filter used for SCOD analysis. Influent organics were converted to CH_4, which was detected in biogas and dissolved in membrane permeate (Table 3.2). Average CH_4 production was low because of poor capture due to suspected leaking from

system headspace. Influent SPE sulfate concentration was 35 mg/L SO_4^{-2} and, if reduced to sulfide, would account for removal of 23 mg/L TCOD. Average VSS production ranged from 0.01-0.07 VSS/g COD_r.

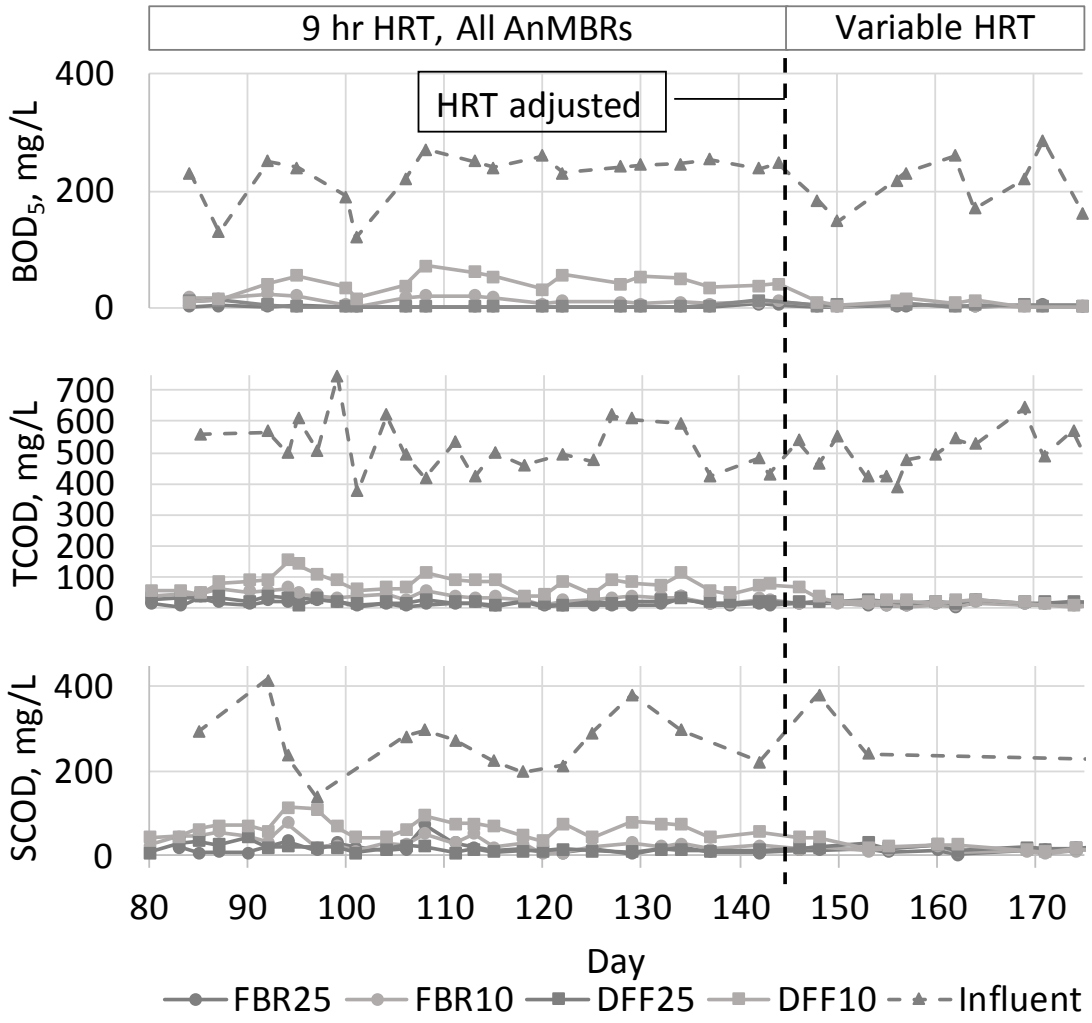

Figure 3.2 AnMBR organic concentrations.
Influent data is SPE concentration fed to each AnMBR. Data shown for FBR25, DFF25, FBR10, DFF10 are membrane permeate concentrations. Bioreactor HRT after day 145 for the FBR25, DFF25, FBR10, DFF10 systems were adjusted to 4.2h, 4.2h, 5.6h, 9.8h, respectively.

Table 3.2 AnMBR CH_4 yield after day 146 (mL CH_4/g COD_r)

	FBR25	FBR10	DFF25	DFF10
Gaseous	119	45	109	37
Dissolved	37	30	36	28
Total	156	75	145	65

Average influent phosphorus to each system was 40% phosphate, whereas effluent from each system was approximately 100% phosphate, indicating essentially all phosphorus leaving each AnMBR had been fully converted to phosphate. Approximately 0.9 and 1.2 mg/L of influent total phosphorus to the 25°C and 10°C systems, respectively, was apparently incorporated into biomass. Average influent nitrogen to each system was 40% NH_3-N, whereas effluent from each AnMBR was 85% NH_3-N. Approximately 6.5 mg/L of influent total nitrogen was apparently incorporated into biomass.

AnMBRs were able to achieve the same organic removal as conventional activated sludge technology under similar hydraulic loading conditions. Both the FBR25 and DFF25 systems achieved the same organic removal efficiency while operating at a 4.2 h bioreactor HRT, indicating little difference in BOD_5 removal based on the type of fixed-film media or membrane material selected under the same temperature and hydraulic conditions. Results from all four AnMBRs in this study were comparable to results found in other recent AnMBR studies describing different configurations (Table 3.3). Permeate BOD_5 concentrations observed were within typical values reported for conventional activated sludge treatment with biological nutrient removal (5-20 mg/L BOD_5) (Metcalf & Eddy 2003). Additionally, AnMBR solids production rates (0.01-0.07 g VSS/g COD_r) were much lower than typical solids yields of 0.4-0.7 g VSS/g BOD_r for aerobic activated sludge (Metcalf & Eddy 2003). Therefore, energy for solids processing is expected to be lower for anaerobic versus activated sludge systems.

Table 3.3 Effluent comparison with other recent AnMBR studies

	This Study		Ho and Sung 2009	Smith et al. 2013	Shin et al. 2014
Bioreactor	FBR	DFF	Complete mix	Complete mix	FBR
Membrane	external tubular, ceramic	external tubular, polymeric	external tubular, polymeric	internal flat sheet, polymeric	internal hollow fiber, polymeric
Waste	synthetic primary effluent	synthetic primary effluent	synthetic primary effluent	synthetic wastewater	real wastewater
Scale	bench	bench	bench	bench	pilot
HRT (h)	6-8	6-14	6-12	16	4.5-6.8
Temp (^{o}C)	10-25	10-25	25	15	8-30
Inf. COD (mg/L)	500	500	500	440	198-362
Eff. COD (mg/L)	<14	<25	<40	36	<25
Eff. BOD_5 (mg/L)	<4	<8	-	18	<10

The decrease in BOD_5 and TCOD permeate concentration seen in the DFF10 AnMBR when HRT was increased on day 146 (Figure 3.2) demonstrated that permeate from this system contained readily biodegradable BOD_5 when operated at a 9 h total system HRT. The required HRT increase for DFF10 AnMBR was consistent with expectations of reduced biomass activity at lower temperature. The relatively longer HRT necessary to achieve permeate BOD_5 less than 10 mg/L from DFF10 is likely due to a lower biomass concentration on the DFF media compared to FBR media and/or due to substrate diffusion limitations with thicker biofilm layers expected on the DFF media (Rittmann & Manem 1992; Mitchell & Gu 2010).

3.3.2 ENERGY REQUIREMENTS

Flow normalized recycle energy requirements for the DFF bioreactor were 60-75% lower compared to the FBR bioreactor (Figure 3.3). Membrane recycle energy requirements ranged between 1.9 and 2.2 kWh/m^3 for the ceramic systems and 3.3 to 3.8 kWh/m^3 for the polymeric membranes, depending on temperature.

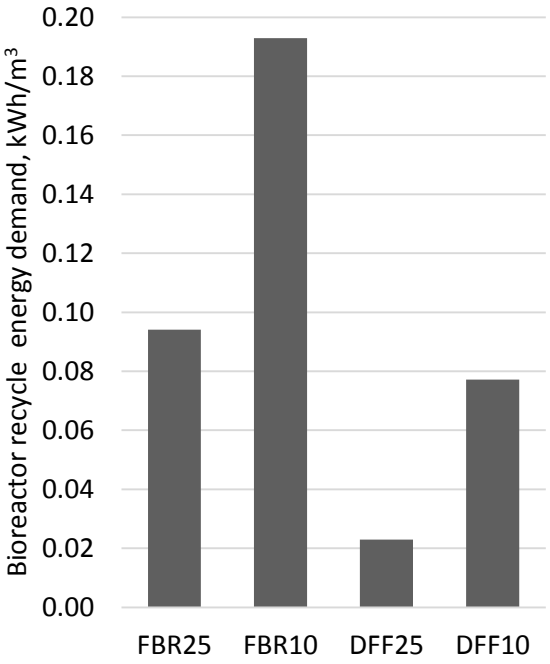

Figure 3.3 AnMBR flow normalized bioreactor recycle energy requirements to achieve permeate <10 mg/L BOD_5.

Anaerobic biotechnology can offer significant energy savings compared to activated sludge for BOD_5 removal by eliminating the need for aeration and offsetting internal energy needs by producing methane that can be used as fuel. However, these savings may be diminished by the pumping demands or membrane biogas sparging required for various membrane bioreactor configurations. Bioreactor configurations such as the FBR require recycle pumping at rates much higher than influent flow to fluidize the biocarrier. Since recycle pumping is fixed relative to hydraulic loading, it is imperative to minimize HRT, not simply to keep bioreactor volume to a minimum, but also to minimize the amount of energy needed per unit of flow treated. For example, the energy requirements for FBR10 and DFF10 bioreactors were higher relative to the FBR25 and DFF25 bioreactors due to the formers' increased HRT and increased headloss from the viscosity decrease due to lower temperature. Therefore, special attention should be given to minimize headlosses from piping and unnecessary pumping in

order to optimize hydraulic efficiency. The DFF bioreactors in this study required between 60 and 75% less energy than the FBRs due to significantly lower recycle pumping requirements. The DFF systems were also able to achieve the same organic removal as the FBR systems, which demonstrates fixed-film bioreactor technology does not necessarily require high recycle rates to produce low effluent BOD_5.

3.3.3 RECOVERY NEEDS FOR CONVERTED SUBSTRATES

Nutrient removal remains a challenge when using AnMBRs. Aerobic processes can successfully remove nitrogen and accumulate phosphorus in wasted biosolids. Anaerobic biotechnology, on the other hand, converts nitrogen to soluble ammonia and phosphorus to soluble phosphate. Both of these products typically must be recovered or removed in order to prevent environmental degradation in the form of eutrophication (Sala & Mujeriego 2001; WEF 2010).

Most of the N and P entering the AnMBRs was converted to ammonia and phosphate in membrane permeate. In order for AnMBRs to become more widely applicable, nutrients must be removed or recovered before they enter receiving waters. Since the AnMBR permeate in this study was virtually free of organic carbon and oxygen, conventional aerobic biological nutrient removal processes after AnMBR treatment may not be suitable. Partial nitritation/nitrification coupled with Anammox (van de Graaf et al. 1996; Stuckey 2012) has been suggested as an autotrophic biological process to remove nitrogen with an energy demand of 1.2 kWh/kg N removed (Batstone et al. 2015), but process control is challenging for mainstream applications, whereas it is more easily applied to digested sludge filtrate with high ammonia concentration at mesophilic temperatures (Smith et al. 2012).

In contrast, physical/chemical processes such as ion exchange (Aiyuk et al. 2006) or struvite precipitation (Mo & Zhang 2013) may be more sustainable than biological methods. Ion exchange may be appropriate because AnMBR permeate contains no suspended solids that can clog ion exchange beds and most of the N and P exiting AnMBRs is in the form of ammonia and phosphate that can be captured using ion exchange resins. Struvite precipitation, on the other hand, requires the addition of magnesium and can only remove a portion of the nitrogen since the maximum extent of struvite formation from municipal wastewater is typically phosphate limited when excess magnesium is added. Nutrient recovery, concentration, and precipitation using ion exchange may be particularly attractive since concentrated nutrients in ion exchange regeneration brine could be utilized in agricultural applications to offset new fertilizer production (Rittmann et al. 2011; Williams et al. 2015).

Dissolved methane lost in AnMBR permeate poses a concern as a greenhouse gas, especially at lower temperature operation when methane solubility is higher (Hatamoto et al. 2010; Lin et al. 2013). Dissolved methane lost in membrane permeate can also result in lost renewable energy available from biogas. Air stripping has been proposed to recover dissolved methane from AnMBR permeate (McCarty et al. 2011), with the off-gas blended with primary sludge anaerobic digester biogas for energy production in internal combustion engines. Air stripping would also help aerate AnMBR permeate to increase dissolved oxygen concentration prior to discharge. This also may be achieved simply by cascading the effluent or with a small aeration basin, but special attention should be given to greenhouse gas collection as well as potential concerns with sulfurous gasses (von Sperling 2007; van Haandel & van der Lubbe 2012) and odors (Switzenbaum 1995).

3.3.4 ANMBR ENERGY AND FEASIBILITY

While energy reduction in bioreactor operation is important, it is clear that the high energy demand for traditional external cross-flow membrane operation is not economical compared to the 0.3-0.6 kWh/m^3 typically required for activated sludge (Metcalf & Eddy 2003). The cross-flow tubular membranes used in this study were operated at cross-flow velocities significantly lower than traditionally used velocities of 2 to 3 m/s (Liao et al. 2006), but still the energy demand was 2 to 3 kWh/m^3. It should be noted, however, that the AnMBRs were not optimized to minimize head losses and membranes were operated at relatively low fluxes. Estimates conducted by Le-Clech et al. (2006) on previous AnMBR studies showed that a cross-flow membrane operated at low cross-flow velocity and flux of 30 L/m^2·h was expected to require 0.23 kWh/m^3. This demonstrates that hydraulic optimization and proper membrane selection can significantly reduce membrane energy requirements. The membrane energy estimate of Le-Clech et al. (2006) along with DFF energy results from this study result in a total AnMBR system energy demand of approximately 0.25-0.31 kWh/m^3. This significant result shows that AnMBRs can be energy competitive with the activated sludge process for BOD_5 removal, even without considering the renewable energy gains made from utilizing produced methane.

Overall, AnMBR treatment coupled with ion exchange for nutrient recovery and air stripping for dissolved methane recovery is expected to require 30-50% less energy than current aerobic treatment with biological nutrient removal (Table 3.4). The wide range in energy reduction for AnMBRs is due to the large variability of required HRT values and head losses observed in this study. Previous estimates that municipal wastewater anaerobic treatment can result in an energy positive process (Speece 1996; McCarty et al. 2011) are challenging to achieve based on requirements for recycle flow, nutrient removal and/or dissolved methane removal. Nutrient removal and dissolved methane processes are expected to account for one

third of the total energy demand for municipal water recovery by anaerobic treatment. Energy potential from AnMBR biogas production may be enough to offset energy demands for ion exchange nutrient recovery and effluent dissolved methane recovery, but is not estimated herein to satisfy all energy demands. More research is required to optimize systems and reduce total energy requirements for AnMBR systems.

Table 3.4 Comparison of energy demands for aerobic and anaerobic treatment of 40,000 m^3/day municipal wastewater

Treatment Process	Aerobic treatment with nitrification	Anaerobic treatment with ion exchange
	kWh/d	kWh/d
Aeration (diffused air)[1]	12,000	-
Biological nitrification[2]	3,400	-
AnMBR[3]	-	10,100-12,300
Ion exchange nutrient removal[4]	-	4,800
Anaerobic digestion**	1,700	1,300
Belt filter press[2]	500	350
Dissolved methane recovery[5]	-	2,000
Energy recovered from AnMBR biogas	-	(8,900)
Energy from primary digester biogas[2]	(3,500)	(2,600)
Total	14,100	7,050-10,100
kWh/m^3 treated	0.35	0.18 - 0.25

[1]From Speece 1996, [2]From WEF 2009, [3]From this study and Le-Clech et al. 2006, [4]From Howe et al. 2012, [5]From McCarty et al. 2011

3.4 FUTURE WORK

DFF AnMBRs achieved the same organic removal as conventional activated sludge technology and were estimated to require between 30 and 50% less energy than currently required for activated sludge. Future work should focus on hydraulic optimization to reduce pumping and headlosses and on optimal membrane selection to maximize hydraulic loading

while minimizing energy demands. Additionally, low energy processes for dissolved methane and nutrient removal should be identified for the anaerobic permeate from an AnMBR.

3.5 CONCLUSIONS

Bench scale AnMBRs utilizing different fixed-film media were operated to treat synthetic primary effluent municipal wastewater at 10 and 25°C. Effluent BOD_5 less than 8 mg/L was observed for all AnMBR systems, even at 10°C, indicating the AnMBRs are able to achieve high organic removal rates greater than 95% while treating low-strength municipal wastewater. The DFF bioreactor in this study required 60-75% less energy for recycle pumping than the FBR configuration, demonstrating that low energy alternatives to high recycle fixed-film anaerobic systems are possible. Additionally, a DFF AnMBR coupled with additional steps to remove nutrients and dissolved methane was estimated to require 30-50% less energy than currently required for activated sludge. Further investigation is needed to understand hydraulic loading limitations, optimal selection of cross-flow membranes, and strategies to minimize headlosses to reduce energy demands. Additionally, dissolved methane and nutrient removal requires additional study in order to identify low energy processes well-suited for the low carbon, anaerobic permeate from an AnMBR.

3.6 ACKNOWLEDGEMENTS

The authors wish to thank Xylem, Inc. for assisting with project development and for providing materials, funding, and technical support throughout this study. This article was also developed under STAR Fellowship Assistance Agreement no. FP-91766901-0 awarded by the U.S. Environmental Protection Agency (EPA). It has not been formally reviewed by EPA. The

views expressed in this article are solely those of the authors and EPA does not endorse any products or commercial services mentioned in this article.

3.7 REFERENCES

Aiyuk, S., Forrez, I., Lieven, D.K., van Haandel, A., Verstraete, W., 2006. Anaerobic and complementary treatment of domestic sewage in regions with hot climates - A review. Bioresour. Technol. 97, 2225–2241. doi:10.1016/j.biortech.2005.05.015

Aiyuk, S., Verstraete, W., 2004. Sedimentological evolution in an UASB treating SYNTHES, a new representative synthetic sewage, at low loading rates. Bioresour. Technol. 93, 269–278. doi:10.1016/j.biortech.2003.11.006

APHA, AWWA, WEF, 1999. Standard Methods for the Examination of Water and Wastewater, 20th ed, Standard Methods for the Examination of Water and Wastewater. American Water Works Association.

Batstone, D.J., Hülsen, T., Mehta, C.M., Keller, J., 2015. Platforms for energy and nutrient recovery from domestic wastewater: A review. Chemosphere 140, 2–11. doi:10.1016/j.chemosphere.2014.10.021

Collins, G., McHugh, S., Connaughton, S., Enright, A.-M., Kearney, A., Scully, C., Mahony, T., Madden, P., O'Flaherty, V., 2006. New low-temperature applications of anaerobic wastewater treatment. J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng. 41, 881–95. doi:10.1080/10934520600614504

Hatamoto, M., Yamamoto, H., Kindaichi, T., Ozaki, N., Ohashi, A., 2010. Biological oxidation of dissolved methane in effluents from anaerobic reactors using a down-flow hanging sponge reactor. Water Res. 44, 1409–18. doi:10.1016/j.watres.2009.11.021

Ho, J., Sung, S., 2010. Methanogenic activities in anaerobic membrane bioreactors (AnMBR) treating synthetic municipal wastewater. Bioresour. Technol. 101, 2191–6. doi:10.1016/j.biortech.2009.11.042

Ho, J., Sung, S., 2009. Anaerobic membrane bioreactor treatment of synthetic municipal wastewater at ambient temperature. Water Environ. Res. 81, 922–928. doi:10.2175/106143009X407339

Howe, K.J., Hand, D.W., Crittenden, J.C., Trussell, R.R., Tchobanoglous, G., 2012. Principles of Water Treatment. John Wiley & Sons, Inc., Hoboken, NJ.

Khartchenko, N., Khartchenko, N.V., Kharchenko, V.M., 1997. Advanced Energy Systems. Taylor & Francis, Washington DC.

Kim, J., Kim, K., Ye, H., Lee, E., Shin, C., McCarty, P.L., Bae, J., 2011. Anaerobic fluidized bed membrane bioreactor for wastewater treatment. Environ. Sci. Technol. 45, 576–581. doi:10.1021/es1027103

Le-Clech, P., Chen, V., Fane, T.A.G., 2006. Fouling in membrane bioreactors used in wastewater treatment. J. Memb. Sci. 284, 17–53. doi:10.1016/j.memsci.2006.08.019

Lettinga, G., Rebac, S., Zeeman, G., 2001. Challenge of psychrophilic anaerobic wastewater treatment. Trends Biotechnol. 19, 363–70. doi:10.1016/S0167-7799(01)01701-2

Liao, B.Q., Kraemer, J.T., Bagley, D.M., 2006. Anaerobic membrane bioreactors: Applications and research directions. Crit. Rev. Environ. Sci. Technol. 36, 489–530. doi:10.1080/10643380600678146

Lin, H., Peng, W., Zhang, M., Chen, J., Hong, H., Zhang, Y., 2013. A review on anaerobic membrane bioreactors: Applications, membrane fouling and future perspectives. Desalination 314, 169–188. doi:10.1016/j.desal.2013.01.019

Martin, I., Pidou, M., Soares, a., Judd, S., Jefferson, B., 2011. Modelling the energy demands of aerobic and anaerobic membrane bioreactors for wastewater treatment. Environ. Technol. 32, 921–932. doi:10.1080/09593330.2011.565806

McCarty, P.L., Bae, J., Kim, J., 2011. Domestic wastewater treatment as a net energy producer-can this be achieved? Environ. Sci. Technol. 45, 7100–7106. doi:10.1021/es2014264

McCarty, P.L., Smith, D.P., 1986. Anaerobic wastewater treatment. Environ. Sci. Technol. 20, 1200–1206. doi:10.1021/es00154a002

McHugh, S., Collins, G., Mahony, T., O'Flaherty, V., 2005. Biofilm reactor technology for low temperature anaerobic waste treatment: microbiology and process characteristics. Water Sci. Technol. 52, 107–113.

Meng, F., Chae, S.-R., Drews, A., Kraume, M., Shin, H.-S., Yang, F., 2009. Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material. Water Res. 43, 1489–512. doi:10.1016/j.watres.2008.12.044

Metcalf & Eddy, 2003. Wastewater Engineering, Treatment and Reuse, 4th ed.

Mitchell, R., Gu, J.-D. (Eds.), 2010. Environmental Microbiology, 2nd ed. Wiley-Blackwell, Hoboken, NJ.

Mo, W., Zhang, Q., 2013. Energy-nutrients-water nexus: Integrated resource recovery in municipal wastewater treatment plants. J. Environ. Manage. 127, 255–267. doi:10.1016/j.jenvman.2013.05.007

Morita, M., Malvankar, N.S., Franks, A.E., Summers, Z.M., Giloteaux, L., Rotaru, A.E., Rotaru, C., Lovleya, D.R., 2015. Potential for direct interspecies electron transfer in methanogenic

wastewater digester aggregates. MBio 2. doi:10.1128/ mBio.00159-11

Novotny, V., 2011. Water and energy link in the cities of the future - Achieving net zero carbon and pollution emissions footprint. Water Sci. Technol. 63, 184–190. doi:10.2166/wst.2011.031

O'Flaherty, V., Collins, G., Mahony, T., 2006. The microbiology and biochemistry of anaerobic bioreactors with relevance to domestic sewage treatment. Rev. Environ. Sci. Bio/Technology 5, 39–55. doi:10.1007/s11157-005-5478-8

Rittmann, B.E., Manem, J., 1992. Development and experimental evaluation of a steady-state, multispecies biofilm model. Biotechnol. Bioeng. 39, 914–922. doi:10.1002/bit.260390906

Rittmann, B.E., Mayer, B., Westerhoff, P., Edwards, M., 2011. Capturing the lost phosphorus. Chemosphere 84, 846–853. doi:10.1016/j.chemosphere.2011.02.001

Rittmann, B.E., McCarty, P.L., 2001. Environmental Biotechnology: Principles and Applications. McGraw-Hill.

Sala, L., Mujeriego, R., 2001. Cultural eutrophication control through water reuse. Water Sci. Technol. 43, 109–116.

Shin, C., McCarty, P.L., Kim, J., Bae, J., 2014. Pilot-scale temperate-climate treatment of domestic wastewater with a staged anaerobic fluidized membrane bioreactor (SAF-MBR). Bioresour. Technol. 159, 95–103. doi:10.1016/j.biortech.2014.02.060

Skouteris, G., Hermosilla, D., López, P., Negro, C., Blanco, Á., 2012. Anaerobic membrane bioreactors for wastewater treatment: A review. Chem. Eng. J. 198-199, 138–148. doi:10.1016/j.cej.2012.05.070

Smith, A.L., Skerlos, S.J., Raskin, L., 2015. Anaerobic membrane bioreactor treatment of domestic wastewater at psychrophilic temperatures ranging from 15 °C to 3 °C. Environ. Sci. Water Res. Technol. 1, 56–64. doi:10.1039/C4EW00070F

Smith, A.L., Skerlos, S.J., Raskin, L., 2013. Psychrophilic anaerobic membrane bioreactor treatment of domestic wastewater. Water Res. 47, 1655–1665. doi:10.1016/j.watres.2012.12.028

Smith, A.L., Stadler, L.B., Love, N.G., Skerlos, S.J., Raskin, L., 2012. Perspectives on anaerobic membrane bioreactor treatment of domestic wastewater: A critical review. Bioresour. Technol. 122, 149–159. doi:10.1016/j.biortech.2012.04.055

Speece, R., 2008. Anaerobic Biotechnology and Odor/Corrosion Control for Municipalities and Industries. Archae Press, Nashville, TN.

Speece, R., 1996. Anaerobic Biotechnology for Industrial Wastewaters. Archae Press, Nashville, TN.

Speece, R., 1983. Anaerobic biotechnology for industrial wastewater. Environ. Sci. Technol. 17, 416A–427A.

Stuckey, D.C., 2012. Recent developments in anaerobic membrane reactors. Bioresour. Technol. 122, 137–148. doi:10.1016/j.biortech.2012.05.138

Sutton, P.M., Berube, P., Hall, E.R., 2004. Membrane bioreactors for anaerobic treatment of wastewaters. Water Environment Research Foundation, Alexandria, VA.

Switzenbaum, M.S., 1995. Obtacles in the implementation of anaerobic treatment technology. Bioresour. Technol. 53, 255–262.

Switzenbaum, M.S., 1983. Anaerobic fixed film wastewater treatment. Enzyme Microb. Technol. doi:10.1016/0141-0229(83)90072-8

Tale, V.P., Maki, J.S., Struble, C. a., Zitomer, D.H., 2011. Methanogen community structure-activity relationship and bioaugmentation of overloaded anaerobic digesters. Water Res. 45, 5249–5256. doi:10.1016/j.watres.2011.07.035

van de Graaf, A.A., de Bruijn, P., Robertson, L.A., Jetten, M.S.M., Kuenen, J.G., 1996. Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. Microbiology 142, 2187–2196. doi:10.1099/13500872-142-8-2187

van Haandel, A., Kato, M.T., Cavalcanti, P.F.F., Florencio, L., 2006. Anaerobic reactor design concepts for the treatment of domestic wastewater. Rev. Environ. Sci. Bio/Technology 5, 21–38. doi:10.1007/s11157-005-4888-y

van Haandel, A., van der Lubbe, J.G.M., 2012. Handbook of Biological Wastewater Treatment: Design and Optimisation of Activated Sludge Systems. IWA Publishing.

van Lier, J.B., 2008. High-rate anaerobic wastewater treatment: Diversifying from end-of-the-pipe treatment to resource-oriented conversion techniques. Water Sci. Technol. 57, 1137–1148. doi:10.2166/wst.2008.040

van Lier, J.B., Lettinga, G., 1999. Appropriate technologies for effective management of industrial and domestic waste waters - the decentralised approach. Water Sci. Technol. 40, 171–183.

Verstraete, W., Vlaeminck, S., 2011. ZeroWasteWater: short-cycling of wastewater resources for sustainable cities of the future. Int. J. Sustain. Dev. World Ecol. 18, 253–264. doi:10.1080/13504509.2011.570804

von Sperling, M., 2007. Wastewater characteristics, treatment and disposal, Volume 1. IWA Publishing, New York.

WEF, 2010. Nutrient Removal, WEF Manual of Practice No. 34. WEF Press, Alexandria, VA.

WEF, 2009. Energy Conservation in Water and Wastewater Treatment Facilities, WEF Manual of

Practice No. 32. WEF Press, Alexandria, VA.

Williams, A.T., Zitomer, D.H., Mayer, B.K., 2015. Ion exchange-precipitation for nutrient recovery from dilute wastewater. Environ. Sci. Water Res. Technol. 17–19. doi:10.1039/C5EW00142K

Yoo, R., Kim, J., McCarty, P.L., Bae, J., 2012. Anaerobic treatment of municipal wastewater with a staged anaerobic fluidized. Bioresour. Technol. 120, 133–139. doi:10.1016/j.biortech.2012.06.028

4 LOW ENERGY ANAEROBIC MEMBRANE BIOREACTOR FOR MUNICIPAL WASTEWATER TREATMENT

"A good scientist is a person with original ideas. A good engineer is a person who makes a design that works with as few original ideas as possible. There are no prima donnas in engineering."

Freeman Dyson
Disturbing the Universe, 1979

This chapter has been submitted to *Journal of Membrane Science* as:

Seib, M.D., Berg, K.J., Zitomer, D.H., 2015. Low Energy Anaerobic Membrane Bioreactor for Municipal Wastewater Treatment. J. Memb. Sci. Submitted.

4.1 INTRODUCTION

New scenarios for municipal wastewater recovery focus on replacing aerobic processes such as activated sludge with anaerobic biotechnology (Verstraete & Vlaeminck 2011; McCarty et al. 2011). Recently, anaerobic membrane bioreactors (AnMBRs) have become a focal point because of distinct advantages membrane separation offers for biomass retention and low effluent chemical oxygen demand (COD) concentrations, especially at low temperature (Jeison 2007; Smith et al. 2012; Stuckey 2012). Anaerobic biotechnology also offers advantages including reduced biosolids production, reduced energy requirements due to aeration elimination, and methane production for energy generation (van Lier 2008; Mo & Zhang 2013; McCarty et al. 2011; Verstraete & Vlaeminck 2011). Additionally, AnMBRs can provide footprint savings due to higher organic loading rate and greater reactor depth compared to standard activated sludge. AnMBR permeate is free of suspended solids and lends itself to post-treatment such as ion exchange for nutrient (nitrogen, phosphorous and potassium) concentration and recovery (Williams et al. 2015). Previous studies have shown AnMBRs are capable of producing effluent with very low five day biochemical oxygen demand (BOD_5) concentration, even at temperatures less than 10°C (Ho & Sung 2009; Smith et al. 2013; Shin et al. 2014). However, novel configurations that reduce energy demands would be beneficial.

In order for the AnMBR energy requirement to be less than that of activated sludge, the energy demand for membrane operation and maintenance must be below the typical activated sludge demand of between 0.3 and 0.6 kWh/m^3 (Metcalf & Eddy 2003). However, existing membrane operational techniques that help decrease membrane fouling, such as gas sparging or high crossflow velocity (CFV), are more energy intensive than aeration for activated sludge. For submerged AnMBR configurations using biogas sparging, Liao et al. (2006) reported energy

demands of 0.25-1.0 kWh/m^3, whereas estimates from other studies range from 0.69-3.41 kWh/m^3 (Martin et al. 2011).

External crossflow membrane configurations typically require much more energy than submerged membranes due to high CFV required to maintain flux. Liao et al. (2006) reported external crossflow energy demands of 3-7.3 kWh/m^3 and Le-Clech et al. (2006) indicated demands as high as 10 kWh/m^3. However, lower CFV external examples were found with estimated CFV energy demands ranging from 0.23-0.48 kWh/m^3 (Martin et al. 2011). Submerged membrane energy demands can also be higher than activated sludge due to the biogas sparging required to prevent membrane fouling. Actual energy demand is highly dependent on membrane selection and operating strategy, as indicated by the wide ranges reported for each configuration.

In the past, several fouling mitigation strategies have been evaluated, but only two reports have been found that describe the addition of fluidized granular activated carbon (GAC) as a method to reduce membrane fouling and eliminate the energy demand of gas sparging to maintain operation for wastewater treatment (Kim et al. 2011; Shin et al. 2014). These important reports were limited to a submerged configuration and employed only one membrane material/configuration. Other strategies to improve membrane efficiency and reduce membrane energy demands have centered on methods to minimize membrane fouling through membrane surface modification (Hilal et al. 2005; Stuckey 2012), use of adsorbents such as activated carbon (Hu & Stuckey 2007; Akram & Stuckey 2008; Kim et al. 2011), physical scouring mechanisms via fluidization of plastic media (Krause et al. 2010; Siembida et al. 2010), as well as backflushing and relaxation (Liao et al. 2006; Berube & Hall 2006; Meng et al. 2009)

In this study we evaluated the impact of greatly reducing CFV in polymeric and ceramic external crossflow membranes treating synthetic and actual municipal wastewater in order to

reduce energy demands below typical values required for conventional activated sludge. In addition, fluidized GAC was used successfully as a fouling control strategy for both polymeric and ceramic external crossflow membranes.

4.2 EXPERIMENTAL

4.2.1 ANMBR CONFIGURATIONS

Two different lab-scale AnMBR configurations having different biofilm and membrane types were employed, as previously described (Seib et al. 2015a). Briefly, the first configuration consisted of a downflow floating media filter (DFF) bioreactor (2.3 L working volume) coupled to a polymeric tubular membrane module (1 L working volume) (Figure 4.1). The polymeric module contained two, 750 mm long, 12.5 mm diameter polyvinylidene fluoride (PVDF) membranes (total surface area = 0.059 m^2) with nominal molecular weight cutoff of 100 kDa (~0.018 µm nominal pore size) (FP100, PCI Membranes, Fareham, UK). The second configuration was a fluidized bed (FBR) bioreactor (2.3 L working volume) coupled to a ceramic tubular membrane module (1 L working volume) (Figure 4.1). The ceramic module was a 100 cm long, 16 mm diameter aluminum oxide tube (surface area = 0.05 m^2) with a 0.05 µm nominal pore size (Type 1/16, Atech Innovations, Gladbeck, Germany). Membranes were mounted vertically and transmembrane pressure (TMP) was monitored at the top and bottom of modules using gauges (NOSHOK Inc., Berea, OH). All fluid transfer was done with peristaltic pumps (Masterflex, Vernon Hills, IL). A digital manometer was used to determine headloss (EXTECH Instruments, Nashua, NH).

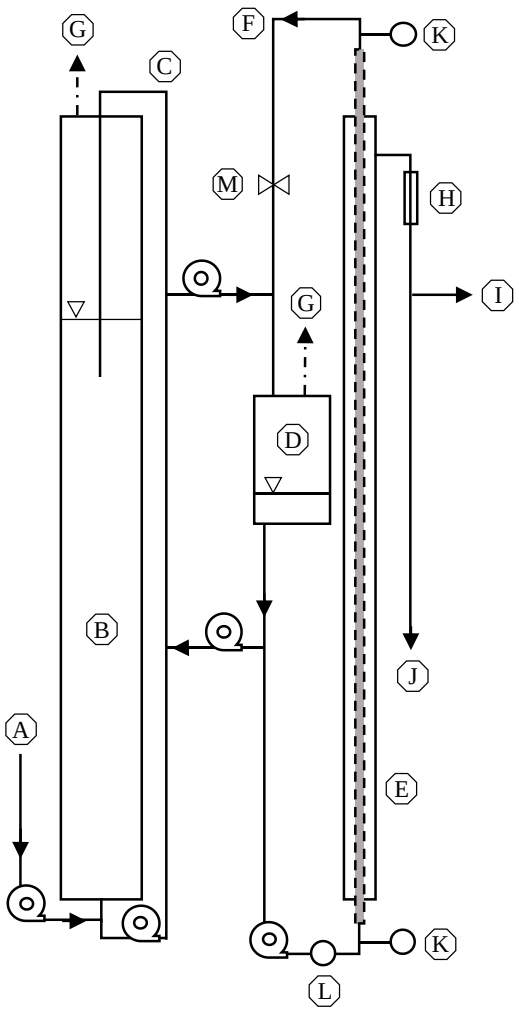

Figure 4.1 Schematic of individual AnMBR setup.
A. Influent wastewater, B. Bioreactor (FBR or DFF), C. Bioreactor recycle line, D. Equalization tank, E. Membrane module (ceramic or polymeric), F. Membrane recycle line, G. Biogas collection, H. Permeate flow meter, I. Excess permeate return to equalization tank, J. Final permeate, K. Pressure meter, L. Pulse dampener, M. Pressure control

4.2.2 BIOREACTOR OPERATION

Both the DFF and FBR configurations were evaluated at 10 and 25°C, for a total of four systems (FBR25, FBR10, DFF25, DFF10). Each bioreactor was inoculated with methanogenic biomass and fed synthetic primary effluent wastewater (SPE) modeled after primary effluent at the South Shore Water Reclamation Facility (SSWRF) (Oak Creek, WI) for the first 320 days of

operation as previously described (Seib et al. 2015a). The SPE characteristics were as follows: 235 mg/L BOD_5, 480 mg/L total chemical oxygen demand (TCOD), 18 mg/L ammonia nitrogen (NH_3-N), 43 mg/L organic nitrogen (N_{org}) 2.5 mg/L phosphate-phosphorus (PO_4^{-3}-P), 5 mg/L total phosphorus (TP), 120 mg/L total suspended solids (TSS), and 115 mg/L volatile suspended solids (VSS). After day 320, all systems were fed real primary effluent (PE) from SSWRF that was collected weekly and stored at 4°C. From day 80 to 145, the total system hydraulic residence time (HRT) in all AnMBRs was 9 hr. After day 145, the total system HRT was adjusted to the minimum needed to achieve BOD_5 <10 mg/L in permeate from each AnMBR (Seib et al. 2015a).

4.2.3 MEMBRANE OPERATIONAL PARAMETERS

Membranes were operated at fluxes of 5.9 to 7.4 L/m^2·h by varying TMP. Each membrane was operated in three distinct crossflow modes: high crossflow (HXF), low crossflow (LXF), and low crossflow with GAC fluidized within the membrane (LXF+GAC). During HXF mode, CFV for the ceramic and polymeric membranes was 0.30 and 0.27 m/s, respectively. During LXF+GAC mode, 50% of each membrane tube volume was filled with 12 x 30 mesh GAC (TIGG 5DC 1230, TIGG Corp, Oakdale, PA) and the CFV was set as the flow required to achieve 100% fluidization of GAC within a membrane tube (GAC fluidized along entire membrane surface). LXF and LXF+GAC CFV values were approximately 90% lower than HXF velocities. For both of the ceramic membranes, LXF and LXF+GAC CFV values were 0.024 m/s. For the 25°C polymeric membranes, CFV was 0.018 m/s during both LXF and LXF+GAC modes. For the 10°C polymeric membrane, CFV was 0.020 m/s during both LXF and LXF+GAC modes. Membrane energy requirements were determined using measured headlosses as well as recycle and permeate flow rates assuming a pump efficiency of 66% (Yoo et al. 2012).

Membranes were periodically cleaned when the membrane TMP increased to greater than 0.5 bar. First the fouling cake layer on the inside of the membrane was removed with a water jet. Membranes were then immersed in a high pH bath for one hour followed by a low pH bath for 25 minutes. The high pH bath for the ceramic membranes was a solution of NaClO (200 ppm free chlorine) adjusted to a pH of 11 using 6N NaOH. The high pH bath for the polymeric membranes was a solution of NaClO (200 ppm free chlorine) with a pH of 10. The same low pH bath consisting of distilled water adjusted to a pH of 2 using HNO_3 was used for both the ceramic and polymeric membranes. During LXF+GAC mode, GAC was removed from each membrane before cleaning and then re-inserted after cleaning. The GAC was not cleaned/regenerated during membrane cleaning.

4.2.4 ANALYTICAL PROCEDURES

Influent and effluent BOD_5, COD, NH_3-N, N_{org}, PO_4^{-3}-P, TP, TSS, and VSS concentrations were determined by standard methods (APHA et al. 1999). Methane masses in biogas and dissolved in permeate were determined using gas chromatography with a thermal conductivity detector (TCD) (Agilent 7890A, Santa Clara, CA). Biogas was collected in 2 L Tedlar bags and produced biogas volume was measured using a 140 mL syringe. Dissolved methane in permeate was quantified using the method of Kim et al. (2011).

4.3 RESULTS

4.3.1 MEMBRANE FOULING IMPACT OF GAC

During operation with PE, fouling rates increased for both membranes relative to SPE operation (Figures 4.2 and 4.3). However, addition of GAC during LXF+GAC mode reduced the fouling rate compared to LXF mode, especially during PE operation. Both membranes exhibited similar fouling rates for all modes during SPE operation with the exception of the polymeric membranes during LXF mode.

When treating PE at low CFV, the ceramic and polymeric membrane run-times were 55% and 120% longer, respectively, when GAC was present (Figure 4.4). In addition, run-times between polymeric membrane cleanings for HXF and LXF+GAC modes were not statistically different ($p<0.05$) when treating SPE. Both HXF and LXF+GAC conditions exhibited longer run-times than LXF mode for the polymeric membranes. Run-times between ceramic membrane cleanings were not different among all three CFV modes employed while treating SPE ($p<0.05$).

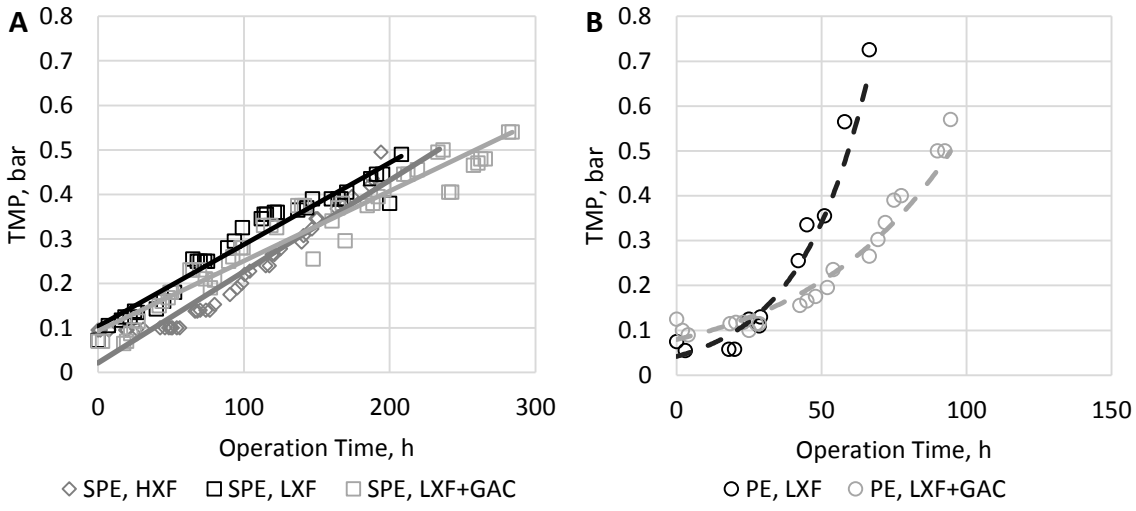

Figure 4.2 Typical transmembrane pressure (TMP) change over time between cleanings for ceramic membranes treating A.) synthetic primary effluent (SPE) and B.) primary effluent (PE) during high crossflow velocity (HXF), low crossflow velocity (LXF), and low crossflow velocity with granular activated carbon (LXF+GAC) modes. Data shown are typical of each mode. Lines fit to data are approximate and do not represent specific trends.

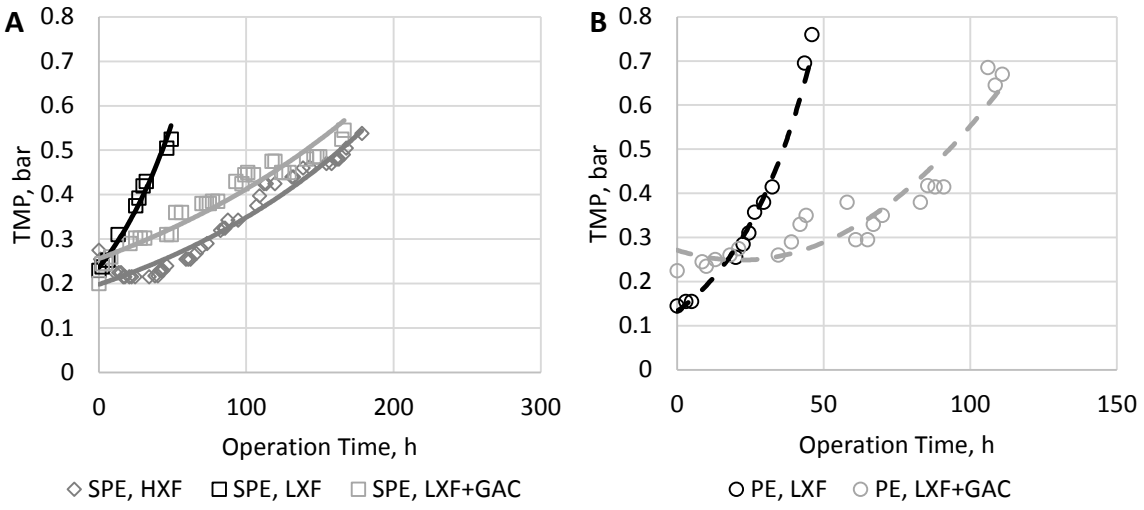

Figure 4.3 Typical transmembrane pressure (TMP) change over time between cleanings for polymeric membranes treating A.) synthetic primary effluent (SPE) and B.) primary effluent (PE) during high crossflow velocity (HXF), low crossflow velocity (LXF), and low crossflow velocity with granular activated carbon (LXF+GAC) modes. Data shown is typical of each mode. Lines fit to data are approximate and do not represent specific trends.

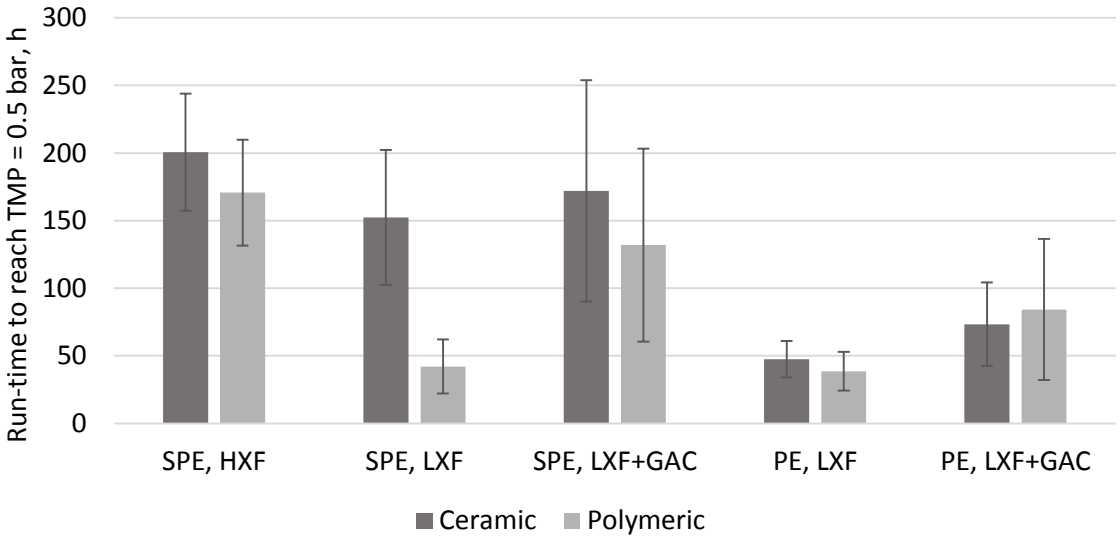

Figure 4.4 Comparison of membrane run-time between cleanings treating synthetic primary effluent (SPE) and primary effluent (PE) during high crossflow velocity (HXF) (n=6), low crossflow velocity (LXF) (n=4 to 20), and low crossflow velocity with granular activated carbon (LXF+GAC) (n=6 to10). Error bars represent ±1 standard deviation.

4.3.2 ENERGY REQUIREMENTS

Energy consumption during HXF mode was at least 30 times greater than during LXF and LXF+GAC modes (Figure 4.5). During HXF mode, membrane crossflow rates were 1,200 to 1,500% higher than during LXF and LXF+GAC, respectively. While CFV values for LXF and LXF+GAC modes were identical, LXF+GAC mode required more energy than LXF mode due to additional headloss from GAC fluidization.

Headloss measured in each system (inclusive of membranes and hoses/connections on either side of membranes) varied throughout the study based on CFV value, temperature, and presence of GAC. During HXF mode, headlosses ranged from 0.76 to 1.32 m H_2O, whereas headlosses for LXF and LXF+GAC modes ranged from 0.01 to 0.146 and 0.12 to 0.23 m H_2O, respectively. When headloss was determined across the polymeric membranes separate from hoses and connections, results were 25% of system headloss, indicating the majority of system headloss was from tubing and connections.

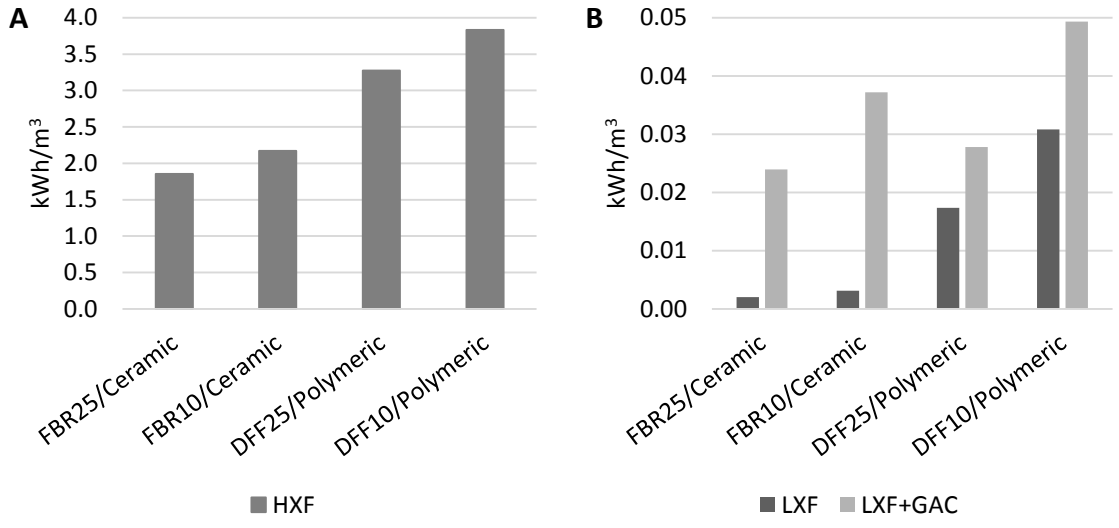

Figure 4.5 Membrane crossflow energy demand during A.) HXF mode and B.) LXF and LXF+GAC modes.

4.3.3 ORGANIC REMOVAL

AnMBR permeates averaged <9±7 mg/L BOD_5 while treating SPE, and ≤10±9 mg/L BOD_5 while treating real PE (Figure 4.6). BOD_5 reduction in AnMBR permeate was 96% for the 25°C systems and 94% for the 10°C systems while treating real PE. Average TCOD in AnMBR permeate was <26±15 mg/L during SPE operation and <46±10 mg/L during PE operation and did not vary with the addition or removal of GAC. Permeate SCOD concentrations were the same as TCOD and this was expected given the pore size of each membrane was less than 0.45 µm.

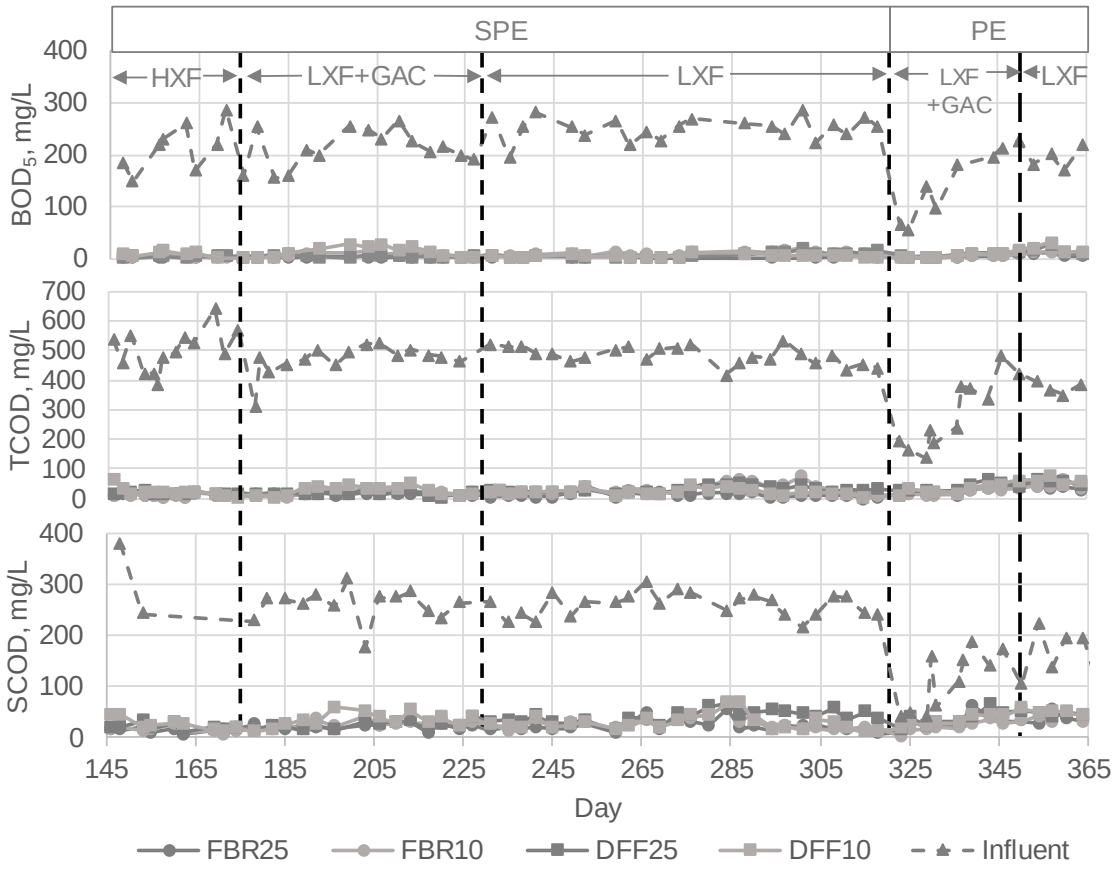

Figure 4.6 Organic removal during operation with SPE and PE under different membrane CFV conditions. Bioreactor HRT for the FBR25, DFF25, FBR10, and DFF10 systems were 4.2 h, 4.2 h, 5.6 h, and 9.8 h, respectively.

4.4 DISCUSSION

4.4.1 IMPACT OF GAC

For tubular crossflow membrane configurations, the primary method of flux maintenance has been to induce hydraulic shear across the membrane surface by operating at CFV values ranging from 2 to 5 m/s (Liao et al. 2006). Several studies have employed much lower CFVs ranging from 0.1 to 0.4 m/s (Baek & Pagilla 2006; Ho et al. 2007; Ho & Sung 2009; An et al. 2009). One study used an estimated CFV of 0.0008 m/s (Baek & Pagilla 2006) although high solids deposition rates were observed, making such a low CFV impractical. Aside from CFV, other typical fouling control strategies reported for AnMBRs using tubular membranes have included backflushing (Ho et al. 2007; Ho & Sung 2009; An et al. 2009; Torres et al. 2011), relaxation (An et al. 2009; Wijekoon et al. 2011), brushing (Ho & Sung 2009; Torres et al. 2011), and chemical cleaning with NaClO (Ho & Sung 2009; Herrera-Robledo et al. 2010; Salazar-Peláez et al. 2011; Calderón et al. 2011; Torres et al. 2011). In instances where chemical cleaning was used, frequency of cleaning ranged from every 6 h to monthly (Baek & Pagilla 2006; Zhang et al. 2007; Ho & Sung 2009; An et al. 2009).

The combination of GAC and low CFV in LXF+GAC mode resulted in polymeric membrane run-time between cleanings similar to run-time at high CFV without GAC; this allowed for recycle pumping rates that were more than one hundred times less than those traditionally used (Figure 4.4). During operation with SPE, similar run-times were observed for the polymeric membranes in HXF and LXF+GAC mode, whereas run-time decreased significantly during LXF mode without GAC. Differences in run-times for each mode were not observed for the ceramic membranes during SPE operation. However, when using PE, both membranes

showed increased average run-times for LXF+GAC compared to LXF. Average LXF+GAC mode run-times using CFV of <0.025 m/s during operation with SPE were 172 h and 132 h for the ceramic and polymeric membranes, respectively, compared to ~96-144 h run-times for similar systems treating SPE using significantly higher CFV values of 0.1 to 0.2 m/s (Ho et al. 2007; Ho & Sung 2009). This indicates similar if not longer run-times between cleanings can be achieved by adding GAC while using only 10% of the CFV pumping energy. Average LXF+GAC mode run-times were reduced to 73h and 84 h for the ceramic and polymeric membranes, respectively, when treating PE compared to SPE. These run-times fall within regular interval for chemical maintenance cleaning of 3 to 7 d (Le-Clech et al. 2006) that are typically associated with much higher CFV of 2 to 5 m/s. It should be noted that membrane cleanings were not performed in-situ and the best method for membrane chemical cleaning with GAC contained in the membranes requires further evaluation. Also, additional membrane operation strategies such as backflushing and relaxation were not evaluated. These strategies should be investigated since they may decrease chemical usage and costs while increasing membrane run-time between cleanings.

The impact of GAC as a means to maintain flux while reducing CFV is more significant for the polymeric membranes compared to the ceramic (Figure 4.4). This may be due to factors including membrane material, hydrophobicity, and tube diameter. In general, fouling from cake layer deposition is more significant in polymeric membranes than ceramic (inorganic) membranes (Sutton et al. 2004). Additionally, cake layer formation occurs more easily on hydrophobic membrane material, such as the PVDF polymeric membrane used in the study, compared to hydrophilic membrane material, such as the ceramic membrane (Meng et al. 2009). Lastly, increasing membrane tube diameter has been shown to increase membrane

permeability while CFV is constant (An et al. 2009). These factors may explain the more significant impact of GAC on polymeric compared to ceramic membranes.

4.4.2 REDUCED ENERGY DEMANDS

Reducing CFV from HXF to LXF modes in each membrane decreased energy consumption by 99% (Figure 4.5). GAC addition in LXF+GAC mode increased energy consumption compared to LXF mode due to increased headloss from GAC fluidization, but still achieved 98-99% energy reduction compared to HXF mode. Results from LXF+GAC mode resulted in CFV energy requirements ranging from 0.03 to 0.05 kWh/m^3, which is orders of magnitude less than previously reported requirements of 3 to 7.3 kWh/m^3 for external crossflow systems using CFVs of 3 to 5 m/s (Liao et al. 2006) and ten times less than the 0.21-0.44 kWh/m^3 energy requirements estimated by Martin et al. (2011) for several low energy external crossflow AnMBRs.

The low membrane energy requirements determined in this study, along with previously determined DFF bioreactor energy demands of 0.02 to 0.08 kWh/m^3 (Seib et al. 2015a) and TMP energy demand of 0.017 kWH/m^3 result in a total DFF AnMBR energy demand of 0.07 to 0.15 kWh/m^3. Previous low energy AnMBR examples have resulted in energy demands of 0.23 kWh/m^3 for a sidestream AnMBR (Martin et al. 2011) and 0.227 kWh/m^3 for a two-stage AnMBR using fluidized GAC in conjunction with hollow fiber membranes (Shin et al. 2014). Results for the DFF AnMBR in this study represent an energy savings of >30% compared to these examples and at least >50% energy savings compared to activated sludge energy demand of 0.3-0.6 kWh/m^3 (Metcalf & Eddy 2003). However, activated sludge can remove nutrients in addition to BOD_5 and does not require dissolved methane removal that is needed for AnMBR effluent. These additional steps for nutrient and dissolved methane removal require additional energy.

Dissolved methane removal is expected to require 0.05 kWh/m^3 (McCarty et al. 2011) and ion exchange as a nutrient removal technology is expected to require 0.12 kWh/m^3 for nitrogen and phosphorus capture (Howe et al. 2012), resulting in a complete DFF AnMBR system energy demand of 0.24 to 0.32 kWh/m^3. Compared to activated sludge, this AnMBR system represents up to a 60% energy savings without taking into account energy production from produced methane. Assuming a theoretical energy yield of 0.22 kWh/m^3 from produced methane in the AnMBR (Seib et al. 2015a), system energy demand is estimated to be 0 to 0.08 kWh/m^3. This results in energy savings of 70 to 100% compared to activated sludge and indicates that although difficult to achieve, AnMBRs may be capable of net neutral energy demand. When energy demand for solids handling and energy production from primary and AnMBR solids digestion are considered (Seib et al. 2015a), all the low energy AnMBR scenarios in this study result in energy demands less than activated sludge, with the DFF25 systems resulting in net positive energy production (Figure 4.7).

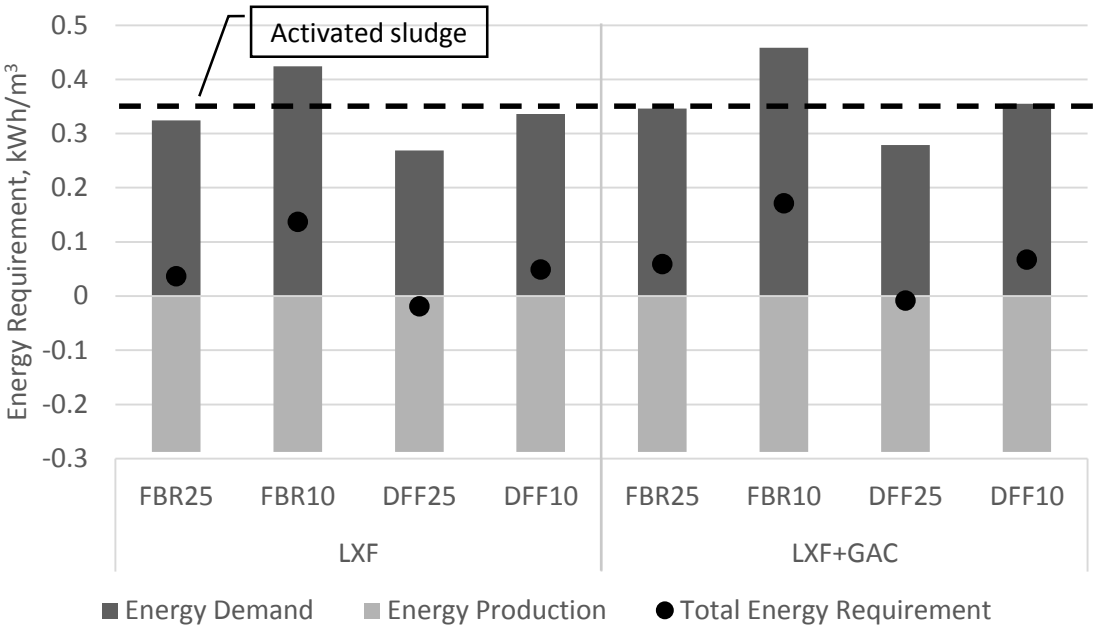

Figure 4.7 Overall AnMBR process energy balance. Energy demand includes energies for bioreactor recycle, membrane recycle, membrane TMP, ion exchange nutrient removal, dissolved methane recovery, anaerobic digestion, and belt filter press (from Seib et al. 2015a). Energy production includes energies from AnMBR and digester biogas (from Seib et al. 2015a). Activated sludge energy was taken from Seib et al. (2015a).

A relatively low fraction of the total operating energy was required to maintain TMP compared to the energy needed for traditional fouling control methods (gas sparging or high CFV). At a TMP of 0.5 bar, energy to maintain TMP was 0.017 kWh/m^3 for all AnMBRs. Similar estimated permeate energy requirements ranging from 0.02-0.04 kWh/m^3 were reported for external crossflow AnMBRs using TMP values ranging from 0.4-0.9 bar (Martin et al. 2011). Therefore, efforts to reduce energy demands should focus on minimizing energy for fouling control.

Piping headloss plays a significant role in energy demand and future design should avoid unnecessary headloss. Shin et al. (2014) recently reported that slight adjustments to pipe sizing would have reduced their AnMBR energy demands from 0.227 kWh/m^3 to 0.133 kWh/m^3. Likewise, results from this study indicated that the majority of headloss measured in each

membrane system resulted from minor losses in conduit connections that could have been reduced or avoided. Further energy demand reduction can be expected if piping configurations are designed to minimize head losses. Future work should include identification of process design elements where hydraulic optimization can be best implemented to reduce headloss.

4.4.3 ORGANIC REMOVAL

All AnMBRs successfully achieved permeate BOD_5 <10 mg/L when treating SPE and PE. HRT was adjusted in each AnMBR to meet this threshold. The FBR25 and DFF25 systems both required a 4.2 h bioreactor HRT (6 h total system HRT), whereas bioreactor HRT values of 5.6 and 9.8 h were required for FBR10 and DFF10, respectively (total system HRT values of 8 h and 14 h, respectively). The longer HRT required for DFF10 was likely due to a lower biomass concentration on the DFF media and/or substrate diffusion limitations with thicker biofilm layers on DFF compared to FBR media (Rittmann & Manem 1992; Mitchell & Gu 2010). Permeate COD characteristics remained similar during periods with and without GAC in the membrane units, indicating that the presence of GAC had no observable impact on system COD removal (Figure 4.6).

Low temperature treatment of municipal wastewater using anaerobic biotechnology was considered to be very difficult in the past because of limitations with biomass activity, especially at low temperature (Lettinga et al. 2001). The low permeate BOD_5 and TCOD concentrations produced from the AnMBRs in this study demonstrate that membranes are a beneficial process component for anaerobic biotechnology treating dilute municipal and other dilute wastewater at low temperature. This is confirmed by the successful results of other low temperature AnMBR studies (Ho & Sung 2009; Smith et al. 2013; Shin et al. 2014), that along

with this study described BOD_5 effluent concentrations consistent with effluent for conventional aerobic treatment (5 to 20 mg/L BOD_5) (Metcalf & Eddy 2003).

4.5 CONCLUSIONS

AnMBRs operated using external tubular membranes with fluidized GAC and CFV of 0.018-0.024 m/s resulted in energy demands of 0.05-0.13 kWh/m^3. Use of fluidized GAC resulted in 55 to120% longer membrane run-time between chemical cleaning and reduced CFV energy demands by 98 to 99% compared to traditional tubular membrane operational strategy using higher CFV of 3 to 5 m/s. The FBR and DFF AnMBRs in this study achieved permeate BOD_5 concentrations ≤10 mg/L, even at 10^{o}C. Energy demands for AnMBRs using external tubular membranes in this study represent up to 60% energy savings compared to activated sludge without considering energy production from produced methane. When factoring in theoretical energy production, AnMBRs are estimated to require 70 to 100% less energy compared to activated sludge, indicating net neutral energy demand may be feasible for BOD_5 and nutrient removal from municipal wastewater. Additional work in system hydraulic optimization, membrane operational strategies, and membrane cleaning techniques would be beneficial to further reduce AnMBR energy demands and prolong membrane run-time between cleanings.

4.6 ACKNOWLEDGEMENTS

The authors wish to thank Xylem, Inc. for assisting with project development and for providing materials, funding, and technical support throughout this study. This article was also developed under STAR Fellowship Assistance Agreement no. FP-91766901-0 awarded by the U.S. Environmental Protection Agency (EPA). It has not been formally reviewed by EPA. The

views expressed in this article are solely those of the authors and EPA does not endorse any products or commercial services mentioned in this article.

4.7 REFERENCES

Akram, A., Stuckey, D.C., 2008. Flux and performance improvement in a submerged anaerobic membrane bioreactor (SAMBR) using powdered activated carbon (PAC). Process Biochem. 43, 93–102. doi:10.1016/j.procbio.2007.10.020

An, Y.Y., Yang, F.L., Bucciali, B., Wong, F.S., 2009. Municipal wastewater treatment using a UASB coupled with cross-flow membrane filtration. J. Environ. Eng. 135, 86–91. doi:10.1061/(ASCE)0733-9372(2009)135:2(86)

APHA, AWWA, WEF, 1999. Standard Methods for the Examination of Water and Wastewater, 20th ed, Standard Methods for the Examination of Water and Wastewater. American Water Works Association.

Baek, S.H., Pagilla, K.R., 2006. Aerobic and anaerobic membrane bioreactors for municipal wastewater treatment. Water Environ. Res. 78, 133–140. doi:10.2175/106143005X89599

Berube, P., Hall, E., 2006. Parameters governing permeate flux in an anaerobic membrane bioreactor treating low-strength municipal wastewaters: A literature review. Water Environ. Res. 78, 887. doi:10.2175/106143005X72858

Calderón, K., Rodelas, B., Cabirol, N., González-López, J., Noyola, A., 2011. Analysis of microbial communities developed on the fouling layers of a membrane-coupled anaerobic bioreactor applied to wastewater treatment. Bioresour. Technol. 102, 4618–4627. doi:10.1016/j.biortech.2011.01.007

Herrera-Robledo, M., Morgan-Sagastume, J.M., Noyola, A., 2010. Biofouling and pollutant removal during long-term operation of an anaerobic membrane bioreactor treating municipal wastewater. Biofouling 26, 23–30. doi:10.1080/08927010903243923

Hilal, N., Ogunbiyi, O.O., Miles, N.J., Nigmatullin, R., 2005. Methods employed for control of fouling in MF and UF membranes: A comprehensive review. Sep. Sci. Technol. doi:10.1081/SS-200068409

Ho, J., Sung, S., 2009. Anaerobic membrane bioreactor treatment of synthetic municipal wastewater at ambient temperature. Water Environ. Res. 81, 922–928. doi:10.2175/106143009X407339

Ho, J.H., Khanal, S.K., Sung, S., 2007. Anaerobic membrane bioreactor for treatment of synthetic municipal wastewater at ambient temperature. Water Sci. Technol. 55, 79.

doi:10.2166/wst.2007.130

Howe, K.J., Hand, D.W., Crittenden, J.C., Trussell, R.R., Tchobanoglous, G., 2012. Principles of Water Treatment. John Wiley & Sons, Inc., Hoboken, NJ.

Hu, A.Y., Stuckey, D.C., 2007. Activated carbon addition to a submerged anaerobic membrane bioreactor: effect on performance, transmembrane pressure, and flux. J. Environ. Eng. 133, 73. doi:10.1061/(ASCE)0733-9372(2007)133:1(73)

Jeison, D., 2007. Anaerobic membrane bioreactors for wastewater treatment - feasibility and potential applications. Wageningen University.

Kim, J., Kim, K., Ye, H., Lee, E., Shin, C., McCarty, P.L., Bae, J., 2011. Anaerobic fluidized bed membrane bioreactor for wastewater treatment. Environ. Sci. Technol. 45, 576–581. doi:10.1021/es1027103

Krause, S., Zimmermann, B., Meyer-Blumenroth, U., Lamparter, W., Siembida, B., Cornel, P., 2010. Enhanced membrane bioreactor process without chemical cleaning. Water Sci. Technol. 61, 2575–80. doi:10.2166/wst.2010.183

Le-Clech, P., Chen, V., Fane, T.A.G., 2006. Fouling in membrane bioreactors used in wastewater treatment. J. Memb. Sci. 284, 17–53. doi:10.1016/j.memsci.2006.08.019

Lettinga, G., Rebac, S., Zeeman, G., 2001. Challenge of psychrophilic anaerobic wastewater treatment. Trends Biotechnol. 19, 363–70. doi:10.1016/S0167-7799(01)01701-2

Liao, B.Q., Kraemer, J.T., Bagley, D.M., 2006. Anaerobic membrane bioreactors: Applications and research directions. Crit. Rev. Environ. Sci. Technol. 36, 489–530. doi:10.1080/10643380600678146

Martin, I., Pidou, M., Soares, a., Judd, S., Jefferson, B., 2011. Modelling the energy demands of aerobic and anaerobic membrane bioreactors for wastewater treatment. Environ. Technol. 32, 921–932. doi:10.1080/09593330.2011.565806

McCarty, P.L., Bae, J., Kim, J., 2011. Domestic wastewater treatment as a net energy producer-can this be achieved? Environ. Sci. Technol. 45, 7100–7106. doi:10.1021/es2014264

Meng, F., Chae, S.-R., Drews, A., Kraume, M., Shin, H.-S., Yang, F., 2009. Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material. Water Res. 43, 1489–512. doi:10.1016/j.watres.2008.12.044

Metcalf & Eddy, 2003. Wastewater Engineering, Treatment and Reuse, 4th ed.

Mitchell, R., Gu, J.-D. (Eds.), 2010. Environmental Microbiology, 2nd ed. Wiley-Blackwell, Hoboken, NJ.

Mo, W., Zhang, Q., 2013. Energy-nutrients-water nexus: Integrated resource recovery in municipal wastewater treatment plants. J. Environ. Manage. 127, 255–267.

doi:10.1016/j.jenvman.2013.05.007

Rittmann, B.E., Manem, J., 1992. Development and experimental evaluation of a steady-state, multispecies biofilm model. Biotechnol. Bioeng. 39, 914–922. doi:10.1002/bit.260390906

Salazar-Peláez, M.L., Morgan-Sagastume, J.M., Noyola, A., 2011. Influence of hydraulic retention time on fouling in a UASB coupled with an external ultrafiltration membrane treating synthetic municipal wastewater. Desalination 277, 164–170. doi:10.1016/j.desal.2011.04.021

Seib, M.D., Berg, K.J., Zitomer, D.H., 2015. Reduced energy demand for municipal wastewater recovery using an anaerobic floating filter membrane bioreactor. Environ. Sci. Water Res. Technol. Submitted.

Shin, C., McCarty, P.L., Kim, J., Bae, J., 2014. Pilot-scale temperate-climate treatment of domestic wastewater with a staged anaerobic fluidized membrane bioreactor (SAF-MBR). Bioresour. Technol. 159, 95–103. doi:10.1016/j.biortech.2014.02.060

Siembida, B., Cornel, P., Krause, S., Zimmermann, B., 2010. Effect of mechanical cleaning with granular material on the permeability of submerged membranes in the MBR process. Water Res. 44, 4037–4046. doi:10.1016/j.watres.2010.05.016

Smith, A.L., Skerlos, S.J., Raskin, L., 2013. Psychrophilic anaerobic membrane bioreactor treatment of domestic wastewater. Water Res. 47, 1655–1665. doi:10.1016/j.watres.2012.12.028

Smith, A.L., Stadler, L.B., Love, N.G., Skerlos, S.J., Raskin, L., 2012. Perspectives on anaerobic membrane bioreactor treatment of domestic wastewater: A critical review. Bioresour. Technol. 122, 149–159. doi:10.1016/j.biortech.2012.04.055

Stuckey, D.C., 2012. Recent developments in anaerobic membrane reactors. Bioresour. Technol. 122, 137–148. doi:10.1016/j.biortech.2012.05.138

Sutton, P.M., Berube, P., Hall, E.R., 2004. Membrane bioreactors for anaerobic treatment of wastewaters. Water Environment Research Foundation, Alexandria, VA.

Torres, a., Hemmelmann, a., Vergara, C., Jeison, D., 2011. Application of two-phase slug-flow regime to control flux reduction on anaerobic membrane bioreactors treating wastewaters with high suspended solids concentration. Sep. Purif. Technol. 79, 20–25. doi:10.1016/j.seppur.2011.03.006

van Lier, J.B., 2008. High-rate anaerobic wastewater treatment: Diversifying from end-of-the-pipe treatment to resource-oriented conversion techniques. Water Sci. Technol. 57, 1137–1148. doi:10.2166/wst.2008.040

Verstraete, W., Vlaeminck, S., 2011. ZeroWasteWater: short-cycling of wastewater resources for sustainable cities of the future. Int. J. Sustain. Dev. World Ecol. 18, 253–264.

doi:10.1080/13504509.2011.570804

Wijekoon, K.C., Visvanathan, C., Abeynayaka, A., 2011. Effect of organic loading rate on VFA production, organic matter removal and microbial activity of a two-stage thermophilic anaerobic membrane bioreactor. Bioresour. Technol. 102, 5353–5360. doi:10.1016/j.biortech.2010.12.081

Williams, A.T., Zitomer, D.H., Mayer, B.K., 2015. Ion exchange-precipitation for nutrient recovery from dilute wastewater. Environ. Sci. Water Res. Technol. 1, 832-838. doi:10.1039/C5EW00142K

Yoo, R., Kim, J., McCarty, P.L., Bae, J., 2012. Anaerobic treatment of municipal wastewater with a staged anaerobic fluidized. Bioresour. Technol. 120, 133–139. doi:10.1016/j.biortech.2012.06.028

Zhang, J., Padmasiri, S.I., Fitch, M., Norddahl, B., Raskin, L., Morgenroth, E., 2007. Influence of cleaning frequency and membrane history on fouling in an anaerobic membrane bioreactor. Desalination 207, 153–166. doi:10.1016/j.desal.2006.07.009

5 WASTEWATER MICROBIOTA AND TEMPERATURE INFLUENCE MICROBIAL COMMUNITY IN ANAEROBIC MEMBRANE BIOREACTORS

"Lots of people talk to animals," said Pooh.
"Not that many listen though."
"That's the problem."

Benjamin Hoff
The Tao of Pooh, 1982

This chapter has been submitted to the journal *Water Research* as:

Seib, M.D., Berg, K.J., Zitomer, D.H., 2015. Wastewater microbiota and temperature influence microbial community in anaerobic membrane bioreactors. Water Res. Submitted.

5.1 INTRODUCTION

Sustainable municipal wastewater recovery scenarios have highlighted anaerobic biotechnology (Verstraete & Vlaeminck 2011), with special attention being given to the anaerobic membrane bioreactor (AnMBR) (McCarty et al. 2011). AnMBR configurations have successfully achieved effluent with <40 mg/L chemical oxygen demand (COD) from dilute or municipal wastewaters at temperatures as low as 6^{o}C (Shin et al. 2014; Smith et al. 2015; Seib et al. 2015b). These results indicate that historical anaerobic biotechnology challenges including poor operation at low temperature with low strength wastewater, and low effluent organic concentration (Switzenbaum 1995; Lettinga et al. 2001) can be overcome.

While AnMBR technology shows great promise, remaining challenges require further investigation including high energy requirements for membrane operation (Seib et al. 2015b) and post treatment for nutrient and dissolved methane removal (McCarty et al. 2011), as well as lack of fundamental understanding of microbial communities responsible for system function (Smith et al. 2015). Microbial community composition is of particular interest since anaerobic bioprocesses historically have been operated as “black boxes” without accounting for the relationship between microbiology and process function (McKeown et al. 2012).

Increased knowledge of key microbial players is important to understand the potential and limitations of microbially driven processes such as hydrolysis, fermentation, and methanogenesis (McKeown et al. 2012; Vanwonterghem et al. 2014). Links between microbial community composition and function could be used to match inoculum biomass to specific operating conditions including temperature or waste type (McKeown et al. 2012). This information could also be used to warn of impending process upset by identifying adverse shifts

in the microbial community before function significantly deteriorates (Collins, McHugh, et al. 2006).

While the importance of microorganisms in biological systems is recognized (O'Flaherty et al. 2006), the body of knowledge describing microbial consortia in anaerobic wastewater reclamation systems is underdeveloped. To date, the majority of studies have focused on microbial communities in anaerobic digesters reclaiming high strength waste. Less attention has been given to microbial community composition in anaerobic systems reclaiming dilute wastes such as municipal wastewater. However, previous studies have shown that: microbial communities in otherwise similar conditions will vary due to selective pressures such as temperature, substrate, and bioreactor configuration (O'Reilly et al. 2009; Bialek et al. 2011; Bialek et al. 2012), bacterial communities are more even and diverse than archaeal communities in anaerobic systems (Rivière et al. 2009), and hydrogenotrophic methanogenesis becomes the dominant methanogenic pathway at psychrophilic temperatures (Siggins et al. 2011; McKeown et al. 2009; O'Reilly et al. 2009).

While several examples of low/ambient temperature AnMBRs have been previously described, only two studies have investigated the microbial community composition within the bioreactor (Smith et al. 2013; Smith et al. 2015). Both studies evaluated completely mixed submerged AnMBRs with gas sparging treating synthetic wastewater, and concluded that biofilm formation on membranes was important to achieve high organic removal. Possible benefits of biofilms such as faster interspecies hydrogen transfer and enhanced syntrophism have already been described (McCarty & Smith 1986; Lettinga et al. 2001). The results of Smith et al. (2015) coupled with existing understanding of the benefits of biofilms highlights the need for further investigation of biofilm microbial consortia in AnMBRs and suggest that reactors

relying on biofilm technology such as the fluidized bed reactor (FBR) or downflow floating filter reactor (DFF) may offer advantages over flocculant biomass.

The impact of continuous inoculation of anaerobic bioreactors by wastewater microbiota also merits investigation. Municipal wastewater is microbially complex (McLellan et al. 2011) and temporal effects of wastewater microbiota on engineered process microbial community composition have been observed in the aerobic activated sludge process (Lee et al. 2015). Regarding anaerobic systems, no studies have been found which considered the effect of wastewater continuous inoculation on bioreactor anaerobic microbial community.

The objective of this study was to assess AnMBR configurations using different biofilm technologies while treating synthetic and real municipal primary effluent wastewater at low and moderate temperatures. Lab-scale reactors were operated to evaluate treatment performance and bioreactor microbial community composition at common wastewater temperatures (10 and 25^{o}C). To our knowledge no study currently exists that examines the microbial community structure within AnMBRs utilizing biofilm technology while treating dilute primary effluent municipal wastewater.

5.2 METHODS

5.2.1 ANMBR CONFIGURATIONS

Two different AnMBR configurations utilizing different biofilm technologies and membrane types were used as previously described (Seib et al. 2015a). The first configuration was a downflow floating filter (DFF) bioreactor (2.3 L working volume) combined with a polymeric tubular membrane (1 L working volume). The DFF bioreactor contained buoyant plastic media to support biofilm formation (Aqwise, Herzliya, Israel). The polymeric membrane

(polyvinylidene fluoride) had a nominal molecular weight cutoff of 100 kDa (~0.018 µm nominal pore size) (FP100, PCI Membranes, Fareham, UK). The second configuration was a fluidized bed reactor (FBR) (2.3L working volume) combined with a ceramic membrane (1L working volume). The FBR contained 12 x 30 mesh granular activated carbon (GAC) (TIGG 5DC 1230, TIGG Corp, Oakdale, PA). The ceramic membrane was composed of aluminum oxide with a 0.05 µm nominal pore size (Type 1/16, Atech Innovations, Gladbeck, Germany).

5.2.2 BIOREACTOR INOCULATION AND OPERATIONAL PARAMETERS

Each AnMBR configuration was duplicated and individual reactors were operated at different temperatures (10 and 25°C), yielding a total of four systems (FBR10, FBR25, DFF10, DFF25). All AnMBRs were seeded with 2 g VSS/L of a mix of methanogenic biomass from five different sources as previously described (Seib et al. 2015a). For the first 320 days, all AnMBRs were fed synthetic primary effluent wastewater (SPE) as previously described (Seib et al. 2015a). Briefly, the SPE contained 235 mg/L five-day biochemical oxygen demand (BOD_5), 480 mg/L total chemical oxygen demand (TCOD), 18 mg/L ammonia nitrogen (NH_3-N), 43 mg/L organic nitrogen (N_{org}) 2.5 mg/L phosphate-phosphorus (PO_4^{-3}-P), 5 mg/L total phosphorus (TP), 120 mg/L total suspended solids (TSS), and 115 mg/L volatile suspended solids (VSS). After day 320, the feed to all AnMBRs was changed to real primary effluent wastewater (PE). PE was collected weekly from a local water reclamation facility and stored at 4°C before use. From day 80 to 145, total system hydraulic residence time (HRT) in all AnMBRs was 9 h. On day 146, HRT was adjusted to the minimum time necessary to achieve <10 mg/L BOD_5 in AnMBR permeate. Membrane operation was conducted as previously described (Seib et al. 2015a) with membrane flux ranging from 5.9 to 7.4 $L/m^2 \cdot h$ and chemical cleaning performed using NaClO and HNO_3 when transmembrane pressure increased above 0.5 bar.

5.2.3 ANALYTICAL PROCEDURES

Influent and permeate BOD_5, COD, NH_3, N_{org}, PO_4^{-3}, TP, TSS, and VSS concentrations were determined using standard methods (APHA et al. 1999). Volatile fatty acid (VFA) concentrations were determined by gas chromatography with a flame ionization detector (FID) (Agilent 7890A, Santa Clara, CA). Methane content in biogas was determined using gas chromatography with a thermal conductivity detector (TCD) (Agilent 7890A, Santa Clara, CA).

5.2.4 DNA EXTRACTION

Biomass (~0.5 g) from each reactor was removed from the biocarrier and placed in 2 mL centrifuge tubes. Lysis buffer (120 mM phosphate buffer, pH 8.0, 5% sodium dodecylsulfate) was added to each sample and cells were lysed by performing three freeze-thaw cycles (-75^{o}C for 60 min, 35^{o}C for 60 min) followed by a 90 min incubation at 70^{o}C. DNA was extracted using a FastDNA Spin Kit (MP Biomedicals) according to the manufacturer's instructions, and then stored at -20°C until use (up to 30 days).

5.2.5 DNA SEQUENCING

PCR amplification using universal primers for the V4 variable region of 16s rRNA gene (515F and 806R) (Caporaso et al., 2011) was performed using the HotStarTaq Plus Master Mix Kit (Qiagen, USA). This primer has been described as "nearly universal to Archaea and Bacteria" (Walters et al., 2011). PCR consisted of the following steps: 94°C for 3 min followed by 28 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, followed by a final elongation step at 72°C for 5 min. Ampure XP beads (Beckman Coulter, USA) were used to purify PCR products. Purified PCR products were used to prepare a DNA library using the Illumina TruSeq DNA library preparation

protocol. Sequencing was performed by a commercial laboratory (MR DNA, Shallowater, TX, USA) using an Illumina MiSeq v3 300 base pair sequencing platform (Illumina, San Diego, CA, USA) following manufacturer guidelines. Barcodes and primers were removed from Q25 filtered sequences and processed as previously described (Dowd et al. 2008; Eren et al. 2011; Swanson et al. 2011). Briefly, data (average 85,000 reads per sample) were refined by removing sequences <200 bp, sequences with ambiguous base calls, and sequences with homopolymers >6 bp. Denoised sequences were clustered into operational taxonomic units (OTUs) having 97% similarity. Singleton sequences and chimeras were removed. BLASTn was used to taxonomically classify OTUs (average 74,000 reads per sample) against a curated database derived from GreenGenes, RDPII, and NCBI (DeSantis et al. 2006; NCBI 2015; CME 2015).

5.2.6 MICROBIAL COMMUNITY ANALYSIS

Inter-AnMBR comparisons of richness (S), Shannon-Weaver diversity (H), and evenness (E) indices were performed using Illumina sequence results. Richness was calculated as the number of unique OTUs identified at the genus level from Illumina sequencing. Shannon-Weaver diversity index was determined as follows: $H = -\sum p_i \log(p_i)$, where p_i is the relative abundance of genus i of the n genera detected in a sample (i = 1 to n) (Briones et al. 2007). Evenness was calculated as follows: $E = H/\ln(S)$ (Falk et al. 2009). Sequencing results were also used to calculate Pearson's correlation coefficients comparing AnMBR microbial community structures to the microbial community structure of the influent PE.

Ordination techniques including non-metric multi-dimensional scaling (NMDS) and principal component analysis (PCA) were used to compare AnMBR microbial communities. Using Ilumina sequencing data, NMDS using a Bray-Curtis similarity distance matrix was performed in R (version 3.2.0 (20015-04-16)) using the VEGAN and MASS packages. NMDS is considered well-

suited for environmental data because it does not assume a linear distribution (as in PCA) and is unaffected by null values between samples (Ramette 2007). PCA was also performed using R.

5.2.7 METHANOGENIC ACTIVITY

Specific methanogenic activity (SMA) assays were performed using acetate and H_2/CO_2 while AnMBRs were fed SPE (day 300) and PE (day 355) at 10^{o}C. Biocarrier was removed from each AnMBR, placed in a serum bottle with basal nutrient medium (Speece 2008) and agitated in an anaerobic glove box to remove biomass from the biocarrier. Biocarrier was then removed and biomass was placed in 160 mL serum bottles, sparged with O_2-free gas (7:3 v/v N_2/CO_2), sealed with butyl rubber stoppers, and allowed to endogenously produce biogas for two days at 10^{o}C. Produced biogas was then removed and substrate (either acetate or H_2/CO_2) was added. SMA using acetate was performed for 40 days as described by Bocher et al. (2015) using biomass concentration of 1.5 to 1.8 g VS/L and 10 g/L calcium acetate at 10^{o}C. For SMA using H_2/CO_2, biomass concentration was 0.2 g VS/L and serum bottle headspace was charged with 100 mL of a 4:1 mixture of H_2/CO_2 gas that had been previously cooled to 10^{o}C. Decrease in headspace pressure was monitored for 40 days using a glass syringe with wetted glass barrel to stoichiometrically determine hydrogenotrophic methane production.

5.3 RESULTS AND DISCUSSION

5.3.1 ORGANIC REMOVAL

Organic removal in all four AnMBRs was >94% while treating both SPE and PE, with average permeate $BOD_5 \leq 10$ mg/L in all systems. Each AnMBR required a specific bioreactor HRT to achieve low permeate BOD_5, with FBR25 and DFF25 both operated at 4.2 h and FBR10 and

DFF10 operated at 5.6 and 9.8 h, respectively. These values correspond to total system HRTs of 6, 6, 8, and 14 h for the FBR25, DFF25, FBR10, and DFF10 systems, respectively, considering membrane system volumes. Average permeate TCOD was ≤25 mg/L in all AnMBRs while treating SPE and ≤45 mg/L while treating PE. The increased average effluent TCOD was likely due to a combination of higher amount of recalcitrant COD in the PE along with insufficient time for all AnMBRs to acclimate to the PE substrate. Total VFA (as acetic acid) concentrations were also low, with average bioreactor concentration <40 mg/L in all AnMBRs during SPE operation and <15 mg/L during PE operation.

5.3.2 MICROBIAL DIVERSITY AND COMMUNITY STRUCTURE

Microbial community structure and diversity are considered important factors to achieve process stability in engineered biosystems (Briones & Raskin 2003; Falk et al. 2009). Highly diverse communities which contain many unique members within different trophic groups (i.e., fermenting bacteria, syntrophic bacteria, methanogens, etc.) are functionally redundant which is important to maintain system functionality in the event of environmental stress (i.e., pH change, substrate change, toxicity, etc.) (Fernandez et al. 2000; Briones & Raskin 2003; Vanwonterghem et al. 2014). Traditional characterizations of community diversity have included richness, evenness, and Shannon-Weaver index, which are broad measures indicating the number of unique members along with general distribution of members within the community (Stirling & Wilsey 2001). Communities with higher richness and Shannon-Weaver index values are more diverse (Stirling & Wilsey 2001). A high evenness score indicates unique community members are evenly distributed, which is beneficial for functional redundancy (Fernandez et al. 2000; Wittebolle et al. 2009).

Diversity indices derived from sequencing analysis of the V4 region of 16S rRNA gene of biofilm biomass from each bioreactor indicate community bias to only a few dominant OTUs in each AnMBR (Table 5.1). Analysis revealed greater richness, evenness, and Shannon-Weaver diversity values in the bacterial community of each AnMBR compared to the archaeal community, which is consistent with findings of previous anaerobic studies (Rivière et al. 2009; Regueiro et al. 2012). All systems contained a similar number of bacterial and archaeal OTUs, with the exception of FBR10, which had fewer bacterial OTUs. Shannon indices were similar among all AnMBRs with an average index for all systems of 1.62±0.08 for Bacteria and 0.56±0.08 for Archaea. These are lower than values of 1.92 to 3.91 previously reported in mesophilic anaerobic studies treating wastes including swine wastewater, domestic sewage sludge, and synthetic sulfate-rich wastewater (Briones et al. 2007; Roy et al. 2009; Xu et al. 2010). Evenness scores were higher for bacterial communities compared to archaeal communities. Evenness scores were also similar among AnMBRs, with average scores of 0.27±0.01 for Bacteria and 0.20±0.03 for Archaea. Evenness scores found in previous mesophilic digestion studies ranged from 0.73-0.91, indicating more even distribution of OTUs detected in those studies (Briones et al. 2007; Roy et al. 2009; Xu et al. 2010). Diversity index scores in this study were similar between systems run at 10^{o}C and 25^{o}C, but were lower than values previously reported for mesophilic systems.

Table 5.1: Diversity indices for Bacteria and Archaea communities during SPE operation.

		FBR25	DFF25	FBR10	DFF10
Bacteria	Richness	384 ± 30	406 ± 10	330 ± 8	403 ± 18
	Evenness	0.28 ± 0.01	0.27 ± 0.01	0.27 ±0.01	0.28 ± 0.01
	Shannon Index	1.64 ± 0.08	1.64 ± 0.05	1.52 ± 0.05	1.66 ± 0.09
Archaea	Richness	18 ± 3	18 ± 1	15 ± 1	16 ± 2
	Evenness	0.19 ± 0.02	0.19 ± 0.03	0.17 ±0.01	0.23 ± 0.02
	Shannon Index	0.56 ± 0.06	0.55 ± 0.08	0.47 ± 0.04	0.64 ± 0.03

A small group of 5 of over 700 bacterial OTUs identified, including *Clostridium*, *Bacteroides*, *Cytophaga*, *Geobacter*, and *Trichococcus*, comprised 31 to 43% of the total relative abundance in all reactors while treating SPE. This finding is consistent with analysis previously conducted on mesophilic anaerobic communities that describe the predominant bacterial composition in anaerobic digesters being composed of only a few OTUs (Rivière et al. 2009). This was also observed among Archaea, with only three genera (*Methanosaeta*, *Methanobacterium*, and *Methanospirillum)* accounting for >80% of archaeal relative abundance in all AnMBRs while treating SPE.

Despite each reactor containing similar dominant OTUs, unique microbial fingerprints were observed in each system based on the most abundant bacterial OTUs. A comparison of the 20 most abundant OTUs, which represented >50% of the relative abundance in all systems, showed distinct OTU distributions in all AnMBRs (Figure 5.1). During SPE operation, the bacterial community in each AnMBR possessed a unique dominant OTU. For FBR10 and FBR25, an OTU most similar to *Clostridium* was dominant and accounted for >20% of all bacterial relative abundance. For the DFF reactors, an OTU most similar to *Geobacter* was dominant in DFF25, while DFF10 showed higher abundances of OTUs most similar to *Cytophaga* and *Trichococcus*. All of these genera are contained within the phyla Proteobacteria, Bacteroidetes, and Firmicutes, which have been described as being dominant in mesophilic anaerobic systems (Regueiro et al. 2012; McKeown et al. 2009) and have been shown to account for over 65% of relative abundance in a psychrophilic AnMBR treating synthetic domestic wastewater (Smith et al. 2013).

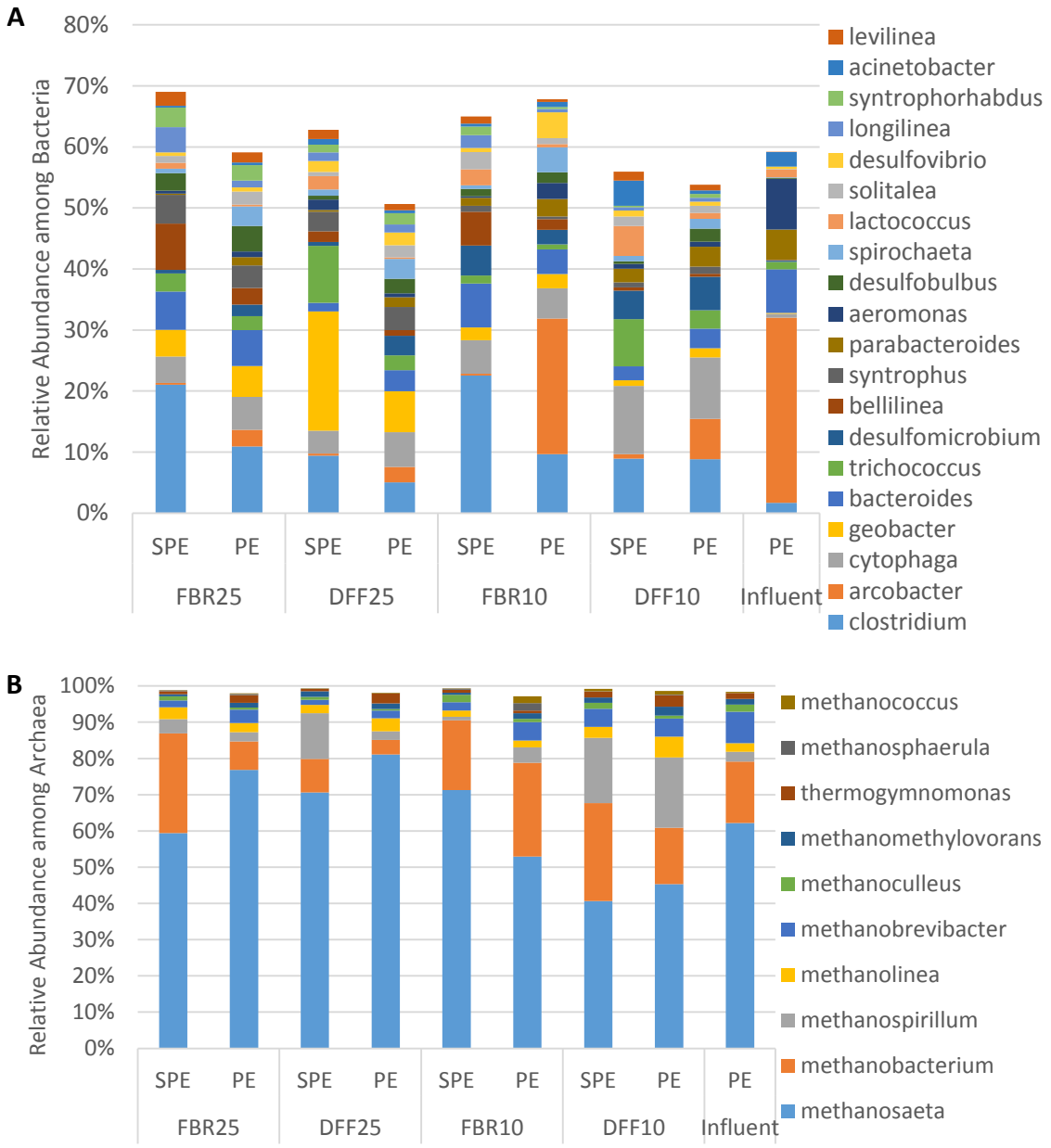

Figure 5.1 Biofilm community structure at the genus level for A.) Bacteria and B.) Archaea during operation with SPE (day 250) and PE (day 355). A.) Relative abundance is shown for the 20 most abundant genera classified in the domain Bacteria and for the 10 most abundant genera classified in the domain Archaea, respectively.

The PE microbiome was significantly different from community structures in the AnMBRs during SPE operation based on Pearson's correlation coefficient and community microbial fingerprint (Figure 5.1). Comparison of Pearson's correlation coefficients revealed

poor correlation between AnMBR bacterial communities during SPE operation and the PE bacterial community (r = 0.08 to 0.16). Microbial fingerprint analysis showed that OTUs most similar to *Arcobacter* represented 30% of the PE bacterial relative abundance, but these OTUs were ≤ 1% of the relative abundance in all the AnMBRs during SPE operation. Other dominant OTUs in the PE included those most similar to *Bacteroides*, *Parabacteroide*s, and *Aeromonas*. These four genera have previously been found to comprise a large portion of the bacterial community in sewage (McLellan et al. 2011; Fisher et al. 2014).

Bacterial communities in the AnMBR systems shifted after reactor feeding with SPE ceased and real PE began to be fed (Figure 5.1). This was likely due to introduction of organisms in the PE into the reactors. Specifically, an OTU most similar to *Arcobacter* appeared in higher relative abundance during PE operation with higher increases in the 10^{o}C bioreactors.

No significant differences among the AnMBR archaeal populations were observed. Only 28 unique archaeal OTUs were identified and over 80% of archaeal relative abundance was accounted for by only three OTUs during both SPE and PE operation; these OTUs were most similar to *Methanosaeta*, *Methanobacterium*, and *Methanospirillum* (Figure 5.1). Unlike the bacterial community composition, the archaeal community during PE and SPE feeding did not change significantly.

Hydrogenotrophic methanogen OTUs made up a larger portion of methanogen relative abundance in the 10^{o}C AnMBRs, which is consistent with previous observations of methanogen population shifts to favor hydrogen utilization under psychrophilic conditions (Lettinga et al. 2001; Siggins et al. 2011). OTUs most similar to hydrogenotrophic methanogens made up 16 to 40% of archaeal relative abundance in the 25^{o}C systems, whereas these OTUs accounted for 27-58% of relative abundance at 10^{o}C. Among methanogens, the OTU most similar to *Methanosaeta* was the most dominant, accounting for at least 40% of archaeal relative

abundance in all systems (Figure 5.1). The facts that bioreactor VFA concentrations remained very low, *Methanosarcina* was virtually absent from all samples, and all AnMBRs were run at temperatures below the mesophilic optimum of 35^{o}C indicates that acetoclastic methanogenesis was achieved primarily by *Methanosaeta* spp. (Bialek et al. 2011).

A decrease in relative abundance of methanogens was seen over time at 10^{o}C in this 365 day study, which suggests that biofilms in all AnMBRs primarily contained psychrotolerant mesophilic methanogens as opposed to developing dominant putatively psychrophilic populations. Methanogens comprised 7 to 12% of total microbial relative abundance in the 25^{o}C AnMBRs, whereas only 2 to 5% methanogens were found at 10^{o}C. Previous psychrophilic anaerobic studies operating up to 300 days have concluded that reactors seeded with mesophilic biomass primarily contained psychrotolerant mesophilic methanogens rather than a population of psychrophilic methanogens (Collins, McHugh, et al. 2006; Smith et al. 2013). However, putatively psychrophilic microbial populations have been found in long term studies (>1200 days), indicating that psychrophilic organisms are present, but require a very long time to establish in significant abundance (Collins, Mahony, et al. 2006; McKeown et al. 2009).

5.3.3 ANMBR MICROBIAL COMPARISONS

Unique microbial communities existed in each AnMBR based on NMDS and PCA analysis despite similar values for gross evenness and diversity index. Cluster analysis using NMDS plots revealed distinct differences among the bacterial communities of AnMBRs during SPE operation (Figure 5.2). The distinct grouping of bacterial profiles from each AnMBR indicate that selective pressures of bioreactor configuration and operational temperature caused differences in the microbial communities of reactors seeded with the same inoculum and fed identical substrate. This observation was also made by Bialek et al. (2011), who found methanogenic community

profiles in different bioreactor configurations clustered using NMDS, and by O'Reilly et al. (2009) and Bialek et al. (2012), who indicated that microbial community profiles are affected by both bioreactor configuration and temperature. However, in contrast to bacterial communities, the archaeal communities in this study did not cluster separately using NMDS (Figure 5.2). Results from the archaeal fingerprints confirm this observation and indicates similar archaeal community structures for all AnMBRs during SPE operation (Figure 5.1).

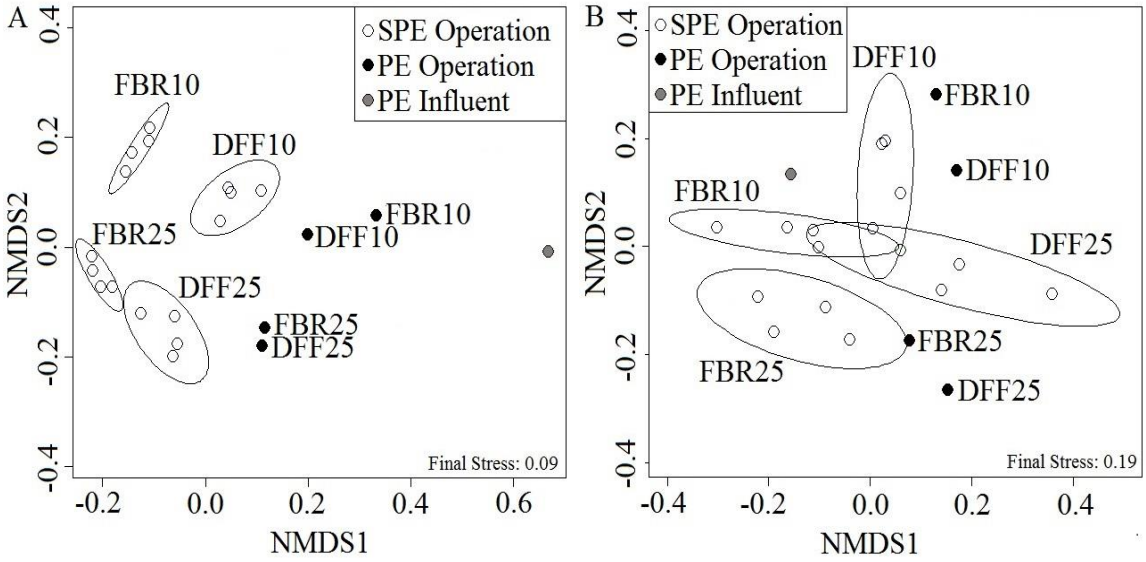

Figure 5.2 Non-metric multidimensional scaling (NMDS) analysis of A.) Bacteria and B.) Archaea 16S rRNA gene sequencing profiles for each AnMBR. Ellipses represent clustering of each AnMBR biomass (95% confidence). During SPE operation samples were taken on day 180, 200, 230, and 250. Samples were taken during PE operation on day 355.

PCA also helps visualize how the most dominant bacterial and archaeal genera are represented among reactors (Figure 5.3). For Bacteria, differences observed among OTUs most similar to the genera *Clostridium*, *Arcobacter*, *Geobacter*, *Trichoccous*, *Acinetobacte*r, and *Cytophaga* in each AnMBR explain 73% of the variance observed within bacterial communities during operation with PE and SPE. Vectors representing specific bacterial OTUs aligned with a

specific AnMBR possessing the highest relative abundance of each OTU, indicating the microbial community differences across AnMBRs was attributed to a specific dominant OTU in each AnMBR. For Archaea, *Methanosaeta*, *Methanobacterium*, and *Methanospirillum* explain 99% of the variance observed among archaeal communities. Unlike results for Bacteria, the vectors representing archaeal OTUs did not align with a specific AnMBR, which reinforces observations made with NMDS and analysis of community fingerprints that unique archaeal community structures did not emerge in each AnMBR.

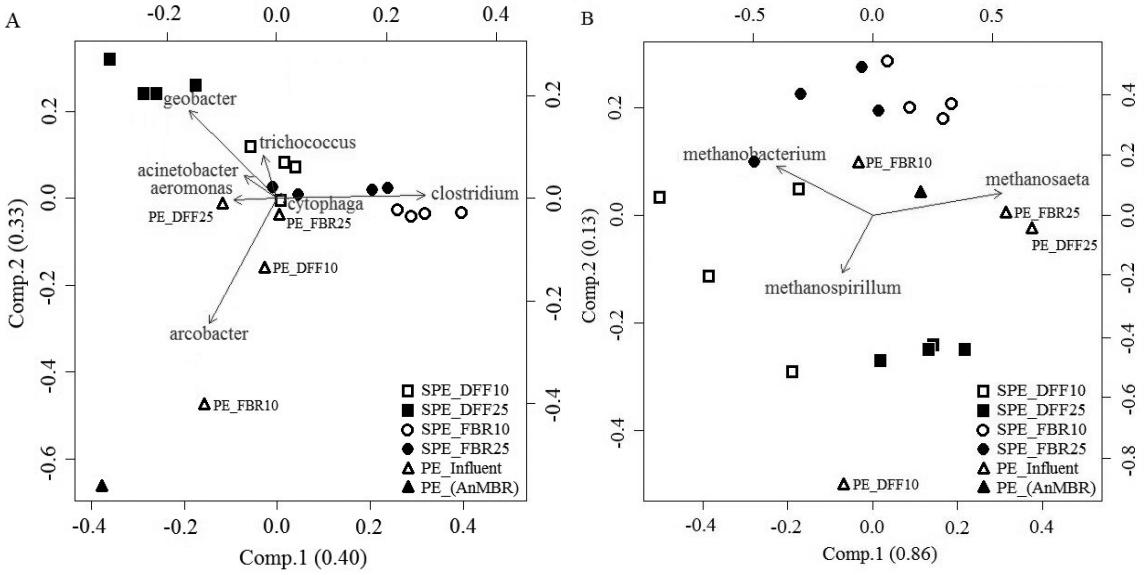

Figure 5.3 Principal components analysis (PCA) analysis of A.) Bacteria and B.) Archaea 16S rRNA gene sequencing profiles for each AnMBR. During SPE operation samples were taken on day 180, 200, 230, and 250. Samples during PE operation were taken on day 355.

5.3.4 IMPACT OF CONTINUOUS INOCULATION

Continuous inoculation by PE caused the community to change in each AnMBR. After wastewater containing a high abundance of *Arcobacter* began to be fed, its relative abundance increased in all bioreactors, ostensibly because reactors were being continuously inoculated

(Figure 5.1). During PE feeding, the bacterial community in all AnMBRs did not cluster with communities analyzed during SPE operation (Figure 5.2). Additionally, *Arcobacter* relative abundance was the primary source of community variance among bioreactor biomass during PE operation, especially for the 10°C AnMBRs (Figure 5.3). Previous work has identified a similar change in microbial community composition within activated sludge systems due to the influent wastewater microbiota (Lee et al. 2015). Influent characteristics are also known to affect microbial community structure (LaPara et al. 2002). The relatively short operation period with PE during this study did not allow time to examine the long term effect of influent continual seeding on AnMBR bioreactor microbial community.

5.3.5 METHANOGENIC ACTIVITY AND SUBSTRATE PREFERENCE

Thermodynamically, hydrogen is a more favorable substrate than acetate at lower temperature (Lettinga et al. 2001). In contrast, acetoclastic methanogenesis has been described by some as the primary methanogenic pathway at low temperatures in natural environments (Metje & Frenzel 2007) and also has been observed in engineered systems at <20°C (Enright et al. 2009). However, hydrogenotrophic methanogenesis has also been observed in low temperature natural (Metje & Frenzel 2007) and engineered environments (Enright et al. 2009; McKeown et al. 2009; Bialek et al. 2011).

Methanogenic activity assays in this study revealed hydrogenotrophic methanogenesis became the primary methanogenic pathway at lower temperature (Figure 5.4). Comparison of SMA at 10°C for biomass from all AnMBRs shows hydrogen utilization was similar among all bioreactors during SPE operation but was higher in the FBR10 and DFF10 biomass compared to FBR25 and DFF25 biomass during PE operation. Additionally, while acetate utilization was observed during SPE operation, acetoclastic methanogenesis was not detected during PE

operation. These results, combined with the higher relative abundance of hydrogenotrophic methanogens at 10°C (Figure 5.1) indicate that hydrogen utilization was the primary pathway for methanogenesis at 10°C and prolonged low temperature operation increased biomass hydrogen utilization rate compared to biomass at 25°C.

The role of *Methanosaeta* detected in each AnMBR is unclear. *Methanosaeta* is commonly found in methanogenic biomass (Harmsen et al. 1996) and is known to be important in forming biofilms in bioreactors such as the upflow anaerobic sludge blanket reactor (Nelson et al. 2012). However, the high relative abundance of *Methanosaeta* in all systems does not correlate to the extremely low or nonexistent methanogenic activity measured with acetate at 10°C. The primary explanation for high *Methanosaeta* detection may stem from the molecular methods used, which relied on sequencing analysis of DNA rather than RNA. DNA-based methods can be biased in that intracellular and extracellular DNA may be included from inactive members within a community (Smith et al. 2015). High detection of *Methanosaeta* coupled with little acetoclastic methanogenic activity suggests that *Methanosaeta* was present but may not have been active. Another possibility is that *Methanosaeta* may have been using a substrate other than acetate. While *Methanosaeta* spp. have been considered to be exclusively acetoclastic since they are not known to use H_2 or formate, a recent study has indicated that *Methanosaeta* may be able to reduce CO_2 to CH_4 via direct interspecies electron transfer in conjunction with *Geobacter* (Rotaru et al. 2014). In this study, *Geobacter* bacterial relative abundance in the 25°C AnMBRs varied from 5 to 20%, whereas they were only 1.0 to 2.3% in the 10°C systems. The presence of *Geobacter* and *Methanosaeta* coupled with low methanogenic acetate utilization suggests that *Methanosaeta* may play a role other than acetate utilizer in anaerobic systems. Further work utilizing RNA or functional gene-based sequencing methods

(such as mcrA) would be useful to characterize the role of *Methanosaeta* in similar fixed-film anaerobic systems.

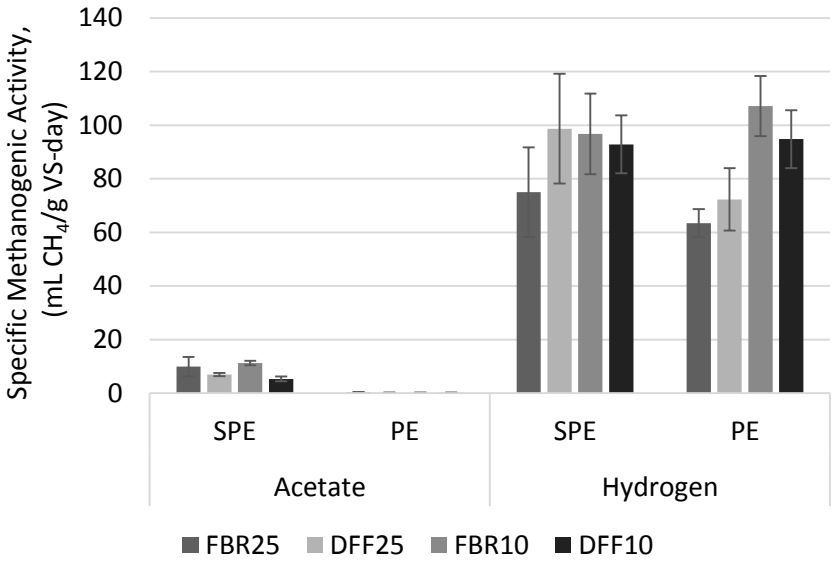

Figure 5.4 Specific methanogenic activity assay (SMA) at 10°C using acetate and H_2/CO_2 (4:1 ratio) for all AnMBRs after treating SPE for 300 days (n=6 for all) and after treating PE for 35 days (n=6 for H_2/CO_2, n=3 to 5 for acetate).

5.4 CONCLUSIONS

Fluidized bed and DFF AnMBRs achieved organic removal >94%, with average permeate BOD_5 remaining ≤10 mg/L in all systems while treating municipal primary effluent wastewater. Bacterial communities in all AnMBRs were dominated by only 5 of over 700 OTUs detected (*Clostridium*, *Bacteroides*, *Cytophaga*, *Geobacter*, and *Trichococcus*) and each of these OTUs had specific significance to a single AnMBR. Unique bioreactor bacterial community structures developed ostensibly due to the combination of operating temperature and bioreactor configuration, but the same observation was not made for Archaea. Relative abundance of hydrogenotrophic methanogens increased at 10°C compared to 25°C. Hydrogenotrophic

methanogenesis emerged as the dominant methanogenic pathway over time at 10°C. Whereas, specific acetoclastic methanogenic activity decreased over time, hydrogenotrophic activity remained similar. The AnMBR microbial community composition changed due to continual seeding with microbiota from the real wastewater fed to the bioreactors. Shifts in microbial community composition were observed after synthetic wastewater feeding ceased and real wastewater was fed. Future work should determine the influence of bioreactor continual seeding by wastewater microbiota and should utilize molecular methods that consider DNA and RNA along with functional genes responsible for particular metabolic functions.

5.5 ACKNOWLEDGEMENTS

The authors thank Xylem, Inc. for assisting with project development and for providing materials, funding, and technical support throughout this study. This article was also developed under STAR Fellowship Assistance Agreement no. FP-91766901-0 awarded by the U.S. Environmental Protection Agency (EPA). It has not been formally reviewed by EPA. The views expressed in this article are solely those of the authors and EPA does not endorse any products or commercial services mentioned in this article.

5.6 REFERENCES

APHA, AWWA, WEF, 1999. Standard Methods for the Examination of Water and Wastewater, 20th ed, Standard Methods for the Examination of Water and Wastewater. American Water Works Association.

Bialek, K., Kim, J., Lee, C., Collins, G., Mahony, T., O'Flaherty, V., 2011. Quantitative and qualitative analyses of methanogenic community development in high-rate anaerobic bioreactors. Water Res. 45, 1298–1308. doi:10.1016/j.watres.2010.10.010

Bialek, K., Kumar, A., Mahony, T., Lens, P.N.L., O'Flaherty, V., 2012. Microbial community structure and dynamics in anaerobic fluidized-bed and granular sludge-bed reactors:

Influence of operational temperature and reactor configuration. Microb. Biotechnol. 5, 738–752. doi:10.1111/j.1751-7915.2012.00364.x

Bocher, B.T.W., Cherukuri, K., Maki, J.S., Johnson, M., Zitomer, D.H., 2015. Relating methanogen community structure and anaerobic digester function. Water Res. 70, 425–435. doi:10.1016/j.watres.2014.12.018

Briones, A., Raskin, L., 2003. Diversity and dynamics of microbial communities in engineered environments and their implications for process stability. Curr. Opin. Biotechnol. 14, 270–276. doi:10.1016/S0958-1669(03)00065-X

Briones, A.M., Daugherty, B.J., Angenent, L.T., Rausch, K.D., Tumbleson, M.E., Raskin, L., 2007. Microbial diversity and dynamics in multi- and single-compartment anaerobic bioreactors processing sulfate-rich waste streams. Environ. Microbiol. 9, 93–106. doi:10.1111/j.1462-2920.2006.01119.x

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. U. S. A. 108, 4516–4522. doi:10.1073/pnas.1000080107/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1000080107

CME, 2015. RDP [WWW Document]. URL http://rdp.cme.msu.edu

Collins, G., Mahony, T., O'Flaherty, V., 2006a. Stability and reproducibility of low-temperature anaerobic biological wastewater treatment. FEMS Microbiol. Ecol. 55, 449–458. doi:10.1111/j.1574-6941.2005.00034.x

Collins, G., McHugh, S., Connaughton, S., Enright, A.-M., Kearney, A., Scully, C., Mahony, T., Madden, P., O'Flaherty, V., 2006b. New low-temperature applications of anaerobic wastewater treatment. J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng. 41, 881–95. doi:10.1080/10934520600614504

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72, 5069–5072. doi:10.1128/AEM.03006-05

Dowd, S.E., Callaway, T.R., Wolcott, R.D., Sun, Y., McKeehan, T., Hagevoort, R.G., Edrington, T.S., 2008. Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). BMC Microbiol. 8, 125. doi:10.1186/1471-2180-8-125

Enright, A.M., McGrath, V., Gill, D., Collins, G., O'Flaherty, V., 2009. Effect of seed sludge and operation conditions on performance and archaeal community structure of low-temperature anaerobic solvent-degrading bioreactors. Syst. Appl. Microbiol. 32, 65–79.

doi:10.1016/j.syapm.2008.10.003

Eren, a. M., Zozaya, M., Taylor, C.M., Dowd, S.E., Martin, D.H., Ferris, M.J., 2011. Exploring the diversity of gardnerella vaginalis in the genitourinary tract microbiota of monogamous couples through subtle nucleotide variation. PLoS One 6. doi:10.1371/journal.pone.0026732

Falk, M.W., Song, K.G., Matiasek, M.G., Wuertz, S., 2009. Microbial community dynamics in replicate membrane bioreactors - Natural reproducible fluctuations. Water Res. 43, 842–852. doi:10.1016/j.watres.2008.11.021

Fernandez, A.S., Hashsham, S. a., Dollhopf, S.L., Raskin, L., Glagoleva, O., Dazzo, F.B., Hickey, R.F., Criddle, C.S., Tiedje, J.M., 2000. Flexible community structure correlates with stable community function in methanogenic bioreactor communities perturbed by glucose. Appl. Environ. Microbiol. 66, 4058–4067. doi:10.1128/AEM.66.9.4058-4067.2000

Fisher, J.C., Levican, A., Figueras, M.J., McLellan, S.L., 2014. Population dynamics and ecology of Arcobacter in sewage. Front. Microbiol. 5, 1–9. doi:10.3389/fmicb.2014.00525

Harb, M., Xiong, Y., Guest, J., Amy, G., Hong, P.-Y., 2015. Differences in microbial communities and performance between suspended and attached growth anaerobic membrane bioreactors treating synthetic municipal wastewater. Environ. Sci. Water Res. Technol. 1, 800–813. doi:10.1039/C5EW00162E

Harmsen, H.J.M., Kengen, H.M.P., Akkermans, A.D.L., Stams, A.J.M., de Vos, W.M., 1996. Detection and localization of syntrophic propionate-oxidizing bacteria in granular sludge by in situ hybridization using 16S rRNA-based oligonucleotide probes. Appl. Environ. Microbiol. 62, 1656–1663.

LaPara, T.M., Nakatsu, C.H., Pantea, L.M., Alleman, J.E., 2002. Stability of the bacterial communities supported by a seven-stage biological process treating pharmaceutical wastewater as revealed by PCR-DGGE. Water Res. 36, 638–646. doi:10.1016/S0043-1354(01)00277-9

Lee, S.-H., Kang, H.-J., Park, H.-D., 2015. Influence of influent wastewater communities on temporal variation of activated sludge communities. Water Res. 73, 132–144. doi:10.1016/j.watres.2015.01.014

Lettinga, G., Rebac, S., Zeeman, G., 2001. Challenge of psychrophilic anaerobic wastewater treatment. Trends Biotechnol. 19, 363–70. doi:10.1016/S0167-7799(01)01701-2

McCarty, P.L., Bae, J., Kim, J., 2011. Domestic wastewater treatment as a net energy producer-can this be achieved? Environ. Sci. Technol. 45, 7100–7106. doi:10.1021/es2014264

McCarty, P.L., Smith, D.P., 1986. Anaerobic wastewater treatment. Environ. Sci. Technol. 20, 1200–1206. doi:10.1021/es00154a002

McKeown, R.M., Hughes, D., Collins, G., Mahony, T., O'Flaherty, V., 2012. Low-temperature anaerobic digestion for wastewater treatment. Curr. Opin. Biotechnol. 23, 444–451. doi:10.1016/j.copbio.2011.11.025

McKeown, R.M., Scully, C., Enright, A.-M., Chinalia, F. a, Lee, C., Mahony, T., Collins, G., O'Flaherty, V., 2009. Psychrophilic methanogenic community development during long-term cultivation of anaerobic granular biofilms. ISME J. 3, 1231–42. doi:10.1038/ismej.2009.67

McLellan, S.L., Huse, S.M., Mueller-Spitz, S.R., Andreihcheva, E.N., Sogin, M.L., 2011. Diversity and population structure of sewage derived microorganisms in wastewater treatment plant influent. Environ. Microbiol. 12, 378–392. doi:10.1111/j.1462-2920.2009.02075.x.Diversity

Metje, M., Frenzel, P., 2007. Methanogenesis and methanogenic pathways in a peat from subarctic permafrost. Environ. Microbiol. 9, 954–964. doi:10.1111/j.1462-2920.2006.01217.x

NCBI, 2015. National Center for Biotechnology Information [WWW Document]. URL http://www.ncbi.nlm.nih.gov/

Nelson, M.C., Morrison, M., Schanbacher, F., Yu, Z., 2012. Shifts in microbial community structure of granular and liquid biomass in response to changes to infeed and digester design in anaerobic digesters receiving food-processing wastes. Bioresour. Technol. 107, 135–143. doi:10.1016/j.biortech.2011.12.070

O'Flaherty, V., Collins, G., Mahony, T., 2006. The microbiology and biochemistry of anaerobic bioreactors with relevance to domestic sewage treatment. Rev. Environ. Sci. Bio/Technology 5, 39–55. doi:10.1007/s11157-005-5478-8

O'Reilly, J., Lee, C., Collins, G., Chinalia, F., Mahony, T., O'Flaherty, V., 2009. Quantitative and qualitative analysis of methanogenic communities in mesophilically and psychrophilically cultivated anaerobic granular biofilims. Water Res. 43, 3365–3374. doi:10.1016/j.watres.2009.03.039

Ramette, A., 2007. Multivariate analyses in microbial ecology. FEMS Microbiol. Ecol. 62, 142–160. doi:10.1111/j.1574-6941.2007.00375.x

Regueiro, L., Veiga, P., Figueroa, M., Alonso-Gutierrez, J., Stams, A.J.M., Lema, J.M., Carballa, M., 2012. Relationship between microbial activity and microbial community structure in six full-scale anaerobic digesters. Microbiol. Res. 167, 581–589. doi:10.1016/j.micres.2012.06.002

Rivière, D., Desvignes, V., Pelletier, E., Chaussonnerie, S., Guermazi, S., Weissenbach, J., Li, T., Camacho, P., Sghir, A., 2009. Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. ISME J. 3, 700–14. doi:10.1038/ismej.2009.2

Rotaru, A.-E., Shrestha, P.M., Liu, F., Shrestha, M., Shrestha, D., Embree, M., Zengler, K., Wardman, C., Nevin, K.P., Lovley, D.R., 2014. A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to Methanosaeta for the reduction of carbon dioxide to methane. Energy Environ. Sci. 7, 408. doi:10.1039/c3ee42189a

Roy, C.S., Talbot, G., Topp, E., Beaulieu, C., Palin, M.F., Massé, D.I., 2009. Bacterial community dynamics in an anaerobic plug-flow type bioreactor treating swine manure. Water Res. 43, 21–32. doi:10.1016/j.watres.2008.09.034

Seib, M.D., Berg, K.J., Zitomer, D.H., 2015a. Low Energy Anaerobic Membrane Bioreactor for Municipal Wastewater Treatment. J. Memb. Sci. Submitted.

Seib, M.D., Berg, K.J., Zitomer, D.H., 2015b. Reduced energy demand for municipal wastewater recovery using an anaerobic floating filter membrane bioreactor. Environ. Sci. Water Res. Technol. Submitted.

Shin, C., McCarty, P.L., Kim, J., Bae, J., 2014. Pilot-scale temperate-climate treatment of domestic wastewater with a staged anaerobic fluidized membrane bioreactor (SAF-MBR). Bioresour. Technol. 159, 95–103. doi:10.1016/j.biortech.2014.02.060

Siggins, A., Enright, A.M., O'Flaherty, V., 2011. Low-temperature (7°C) anaerobic treatment of a trichloroethylene-contaminated wastewater: Microbial community development. Water Res. 45, 4035–4046. doi:10.1016/j.watres.2011.05.013

Smith, A.L., Skerlos, S.J., Raskin, L., 2015. Anaerobic membrane bioreactor treatment of domestic wastewater at psychrophilic temperatures ranging from 15 °C to 3 °C. Environ. Sci. Water Res. Technol. 1, 56–64. doi:10.1039/C4EW00070F

Smith, A.L., Skerlos, S.J., Raskin, L., 2013. Psychrophilic anaerobic membrane bioreactor treatment of domestic wastewater. Water Res. 47, 1655–1665. doi:10.1016/j.watres.2012.12.028

Speece, R., 2008. Anaerobic Biotechnology and Odor/Corrosion Control for Municipalities and Industries. Archae Press, Nashville, TN.

Stirling, G., Wilsey, B., 2001. Empirical Relationships between Species Richness, Evenness, and Proportional Diversity. Am. Nat. 158, 286–299. doi:10.1086/321317

Swanson, K.S., Dowd, S.E., Suchodolski, J.S., Middelbos, I.S., Vester, B.M., Barry, K. a, Nelson, K.E., Torralba, M., Henrissat, B., Coutinho, P.M., Cann, I.K.O., White, B. a, Fahey, G.C., 2011. Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice. ISME J. 5, 639–649. doi:10.1038/ismej.2010.162

Switzenbaum, M.S., 1995. Obtacles in the implementation of anaerobic treatment technology. Bioresour. Technol. 53, 255–262.

Vanwonterghem, I., Jensen, P.D., Ho, D.P., Batstone, D.J., Tyson, G.W., 2014. Linking microbial community structure, interactions and function in anaerobic digesters using new molecular techniques. Curr. Opin. Biotechnol. 27, 55–64. doi:10.1016/j.copbio.2013.11.004

Verstraete, W., Vlaeminck, S., 2011. ZeroWasteWater: short-cycling of wastewater resources for sustainable cities of the future. Int. J. Sustain. Dev. World Ecol. 18, 253–264. doi:10.1080/13504509.2011.570804

Walters, W.A., Caporaso, J.G., Lauber, C.L., Berg-Lyons, D., Fierer, N., Knight, R., 2011. PrimerProspector: de novo design and taxonomic analysis of barcoded polymerase chain reaction primers. Bioinformatics 27, 1159–1161. doi:10.1093/bioinformatics/btr087

Wittebolle, L., Marzorati, M., Clement, L., Balloi, A., Daffonchio, D., Heylen, K., De Vos, P., Verstraete, W., Boon, N., 2009. Initial community evenness favours functionality under selective stress. Nature 458, 623–626. doi:10.1038/nature07840

Xu, K., Liu, H., Chen, J., 2010. Effect of classic methanogenic inhibitors on the quantity and diversity of archaeal community and the reductive homoacetogenic activity during the process of anaerobic sludge digestion. Bioresour. Technol. 101, 2600–2607. doi:10.1016/j.biortech.2009.10.059

6 CONCLUSIONS

"…I leave the evidence I have produced in proof of it to be refuted, if any one can do it; and I leave the ideas that are suggested in the conclusion of the work to rest on the mind of the reader…"

Thomas Paine
The Age of Reason, 1794

The overall goal of this work was to develop an AnMBR that requires less energy than existing technology to increase the sustainability of secondary municipal wastewater treatment. To accomplish this, both conventional fluidized bed reactor and unconventional downflow floating filter bioreactor configurations were operated at low/ambient temperatures of 10^{o}C and 25^{o}C in conjunction with a novel membrane operation strategy. In addition to evaluating process improvements to AnMBR technology, this study also examined the microbial community structure within each system in order to increase understanding of the complex microbial relationships that exist within engineered anaerobic biosystems.

The AnMBRs in this study were able to achieve over 94% organic removal with average, effluent permeate BOD_5 concentrations of 10 mg/L or less while treating either synthetic or real primary effluent municipal wastewater. The consistent effluent organic strength was at or below typical effluent BOD_5 concentrations required for wastewater treatment. Maintaining high organic removal and low effluent organic content was an important outcome for this study; the reduced system energy demands achieved did not come at the expense of reduced organic removal. This work demonstrated that high organic removal could be maintained while reducing system energy demands at hydraulic residence times similar to aerobic activated sludge technology.

The unconventional membrane operation strategies employed in this study resulted in significant energy demand reduction. Use of GAC combined with a low-flowrate pumping strategy resulted in tubular membrane CFV of 0.018 to 0.024 m/s and corresponding energy demands of 0.05 to 0.13 kWh/m^3, which represented an energy savings of at least 98% compared to typical crossflow membrane energy requirements. Additionally, the novel use of fluidized GAC within the tubular membranes resulted in 55 to 120% longer operation time between cleanings compared to operation at similar CFV without GAC. This significant reduction

in membrane operation energy coupled with the low-energy DFF bioreactor configuration results in a secondary treatment process that requires up to 60% less energy than activated sludge while achieving similar organic removal.

This study also provided new insights into the makeup of microbial communities found in AnMBRs. Analysis of 16s rRNA gene profiles from each AnMBR using Illumina sequencing methods detected over 700 unique genera and revealed that of these only 5 genera accounted for a significant portion (31 to 43%) of bacterial relative abundance in each AnMBR during operation with synthetic wastewater. Additionally, the dominant bacteria were found in different relative abundances in each AnMBR, thus revealing different bacterial structures in each bioreactor, ostensibly due to selective pressures including temperature and reactor configuration. Similar analysis did not reveal distinct differences between AnMBR archaeal communities. However, an increase in the relative abundance of hydrogenotrophic methanogens was detected at 10^{o}C compared to 25^{o}C. Methanogenic activity assays also showed consistent hydrogenotrophic activity over time, whereas acetoclastic activity decreased, indicating hydrogenotrophic methanogenesis emerged as the dominant pathway in all AnMBRs. When switching to real wastewater treatment, the microbial communities in each AnMBR shifted, ostensibly due to microbiota in the influent wastewater continuously seeding the AnMBRs. The observed shifts indicated an influence on bioreactor microbial community composition due to continual seeding.

Results from this study are a valuable addition to efforts aimed at altering the conventional wastewater treatment paradigm. Traditional municipal wastewater treatment methods such as activated sludge are designed to mitigate the harmful effects of wastewater by removing organics and nutrients, but require high energy and land costs to do so. Anaerobic biotechnology has been hailed as a sustainable alternative due to expected benefits including

reduced energy demands and ability to capture rather than remove nutrients. The AnMBRs developed for this research demonstrate that anaerobic biotechnology can successfully be used instead of activated sludge for organic removal and can do so with reduced energy demand. However, important aspects that this work did not address include the need to remove/recover nutrients such as nitrogen and phosphorus, along with the need to capture dissolved methane. Low-energy solutions to these challenges need to be developed before anaerobic biotechnology can become a viable alternative to activated sludge. Despite these caveats, this research represents an important milestone for anaerobic biotechnology by demonstrating that a stable, low-temperature, low-energy anaerobic process for organic removal is possible.

APPENDICES

"The idea is to try to give all the information to help others to judge the value of your contribution; not just the information that leads to judgment in one particular direction or another."

Richard P. Feynman
"Surely You're Joking, Mr. Feynman", 1985

APPENDIX A – ANMBR ORGANIC REMOVAL

This Appendix includes all data collected for organic removal during the study that may or may not have been shown in previous chapters.

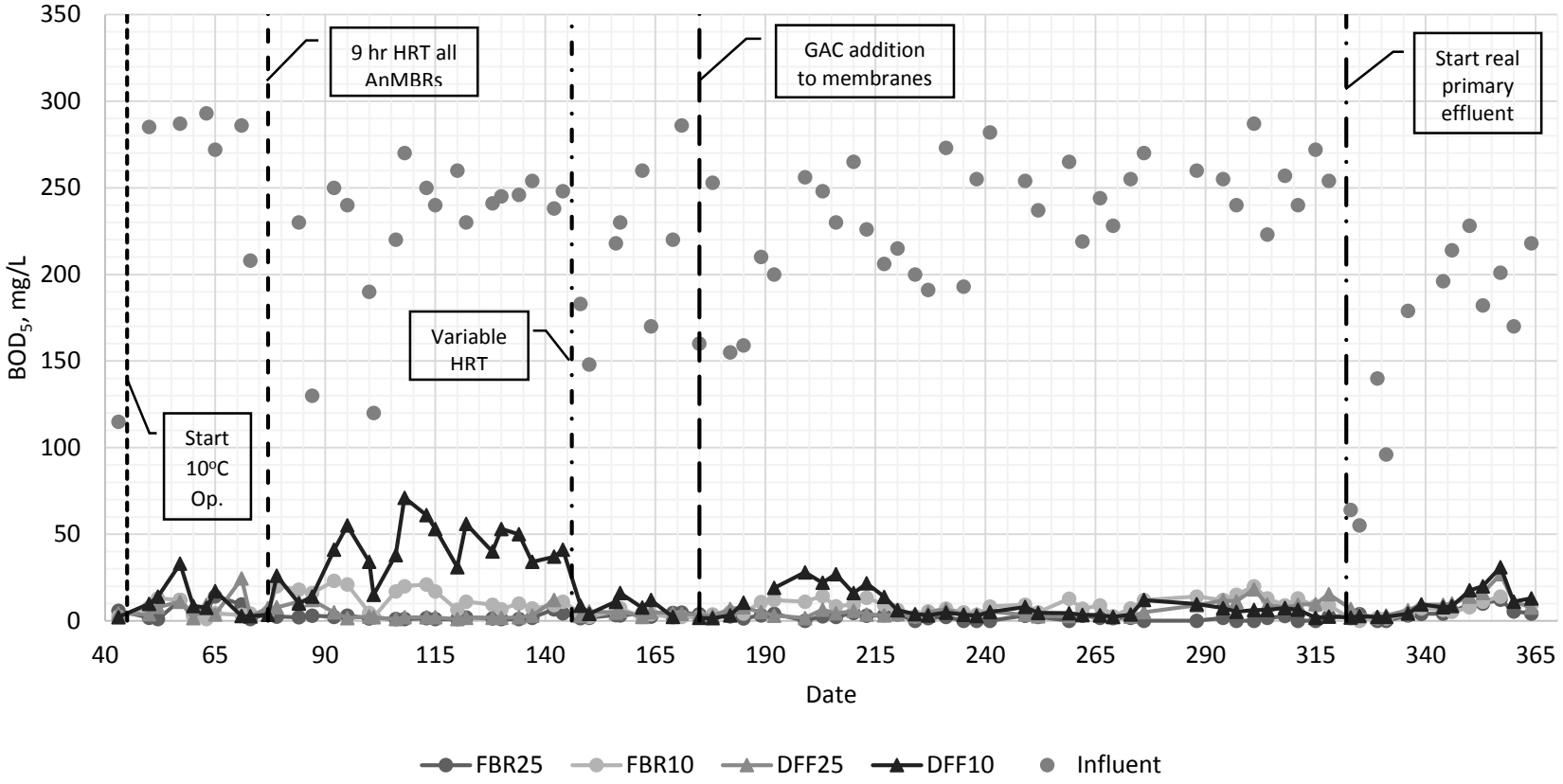

Figure A1 Membrane permeate five day biochemical oxygen demand (BOD_5). FBR25 and FBR10 utilized ceramic membranes. DFF25 and DFF10 utilized polymeric membranes.

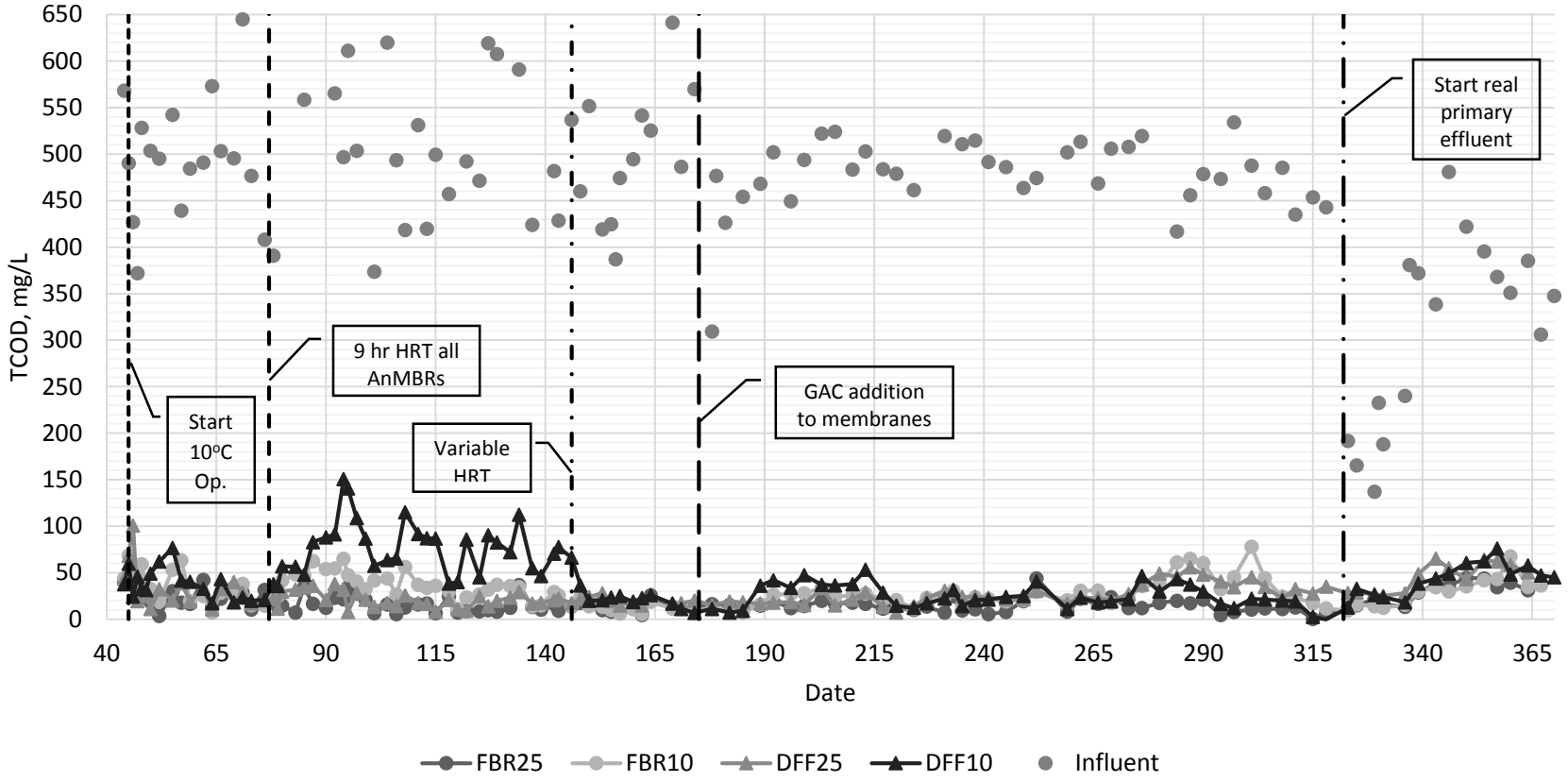

Figure A2 Membrane permeate total chemical oxygen demand (TCOD). FBR25 and FBR10 utilized ceramic membranes. DFF25 and DFF10 utilized polymeric membranes.

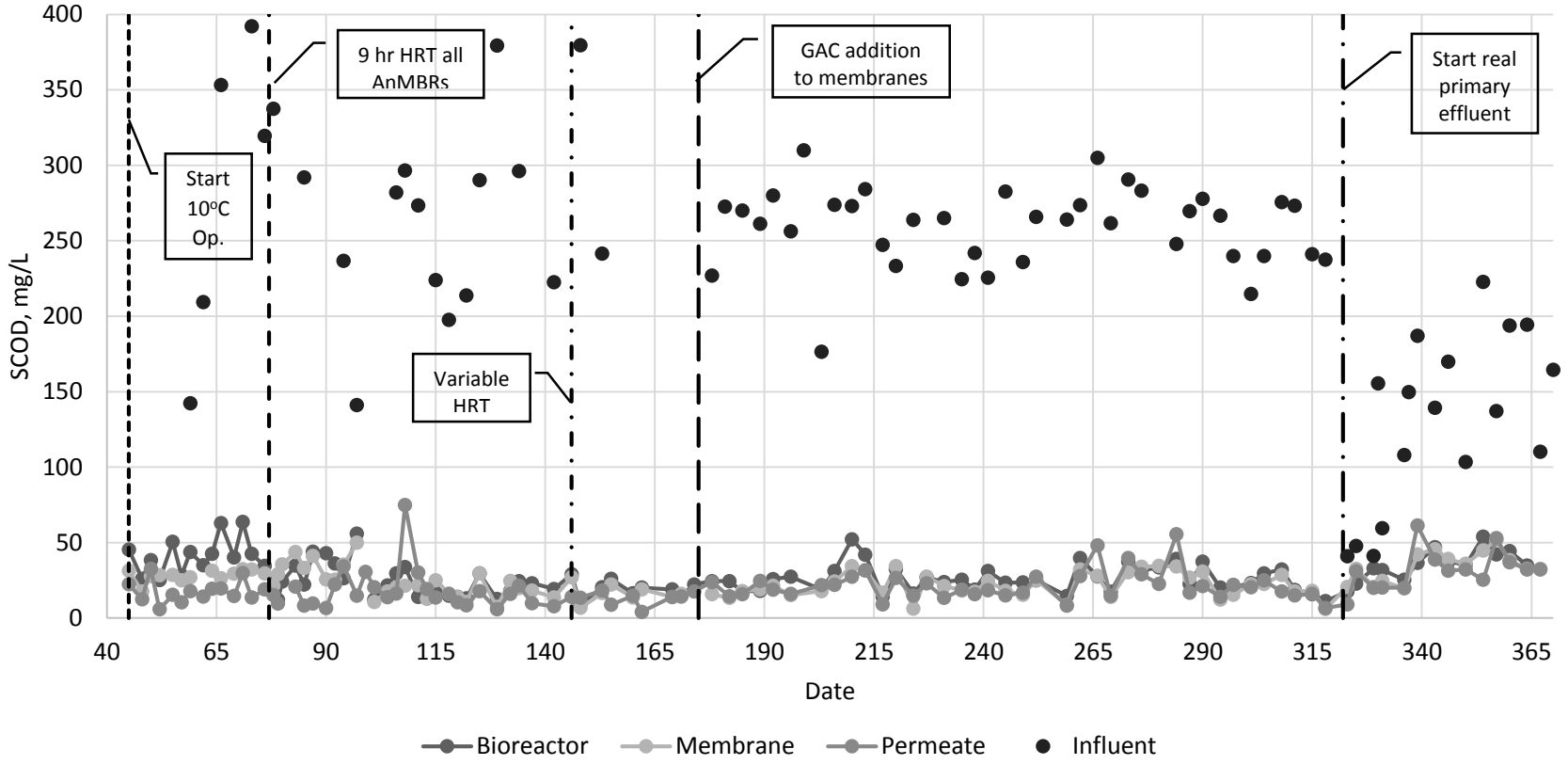

Figure A3 FBR25 soluble chemical oxygen demand (SCOD) in bioreactor, membrane, and permeate.

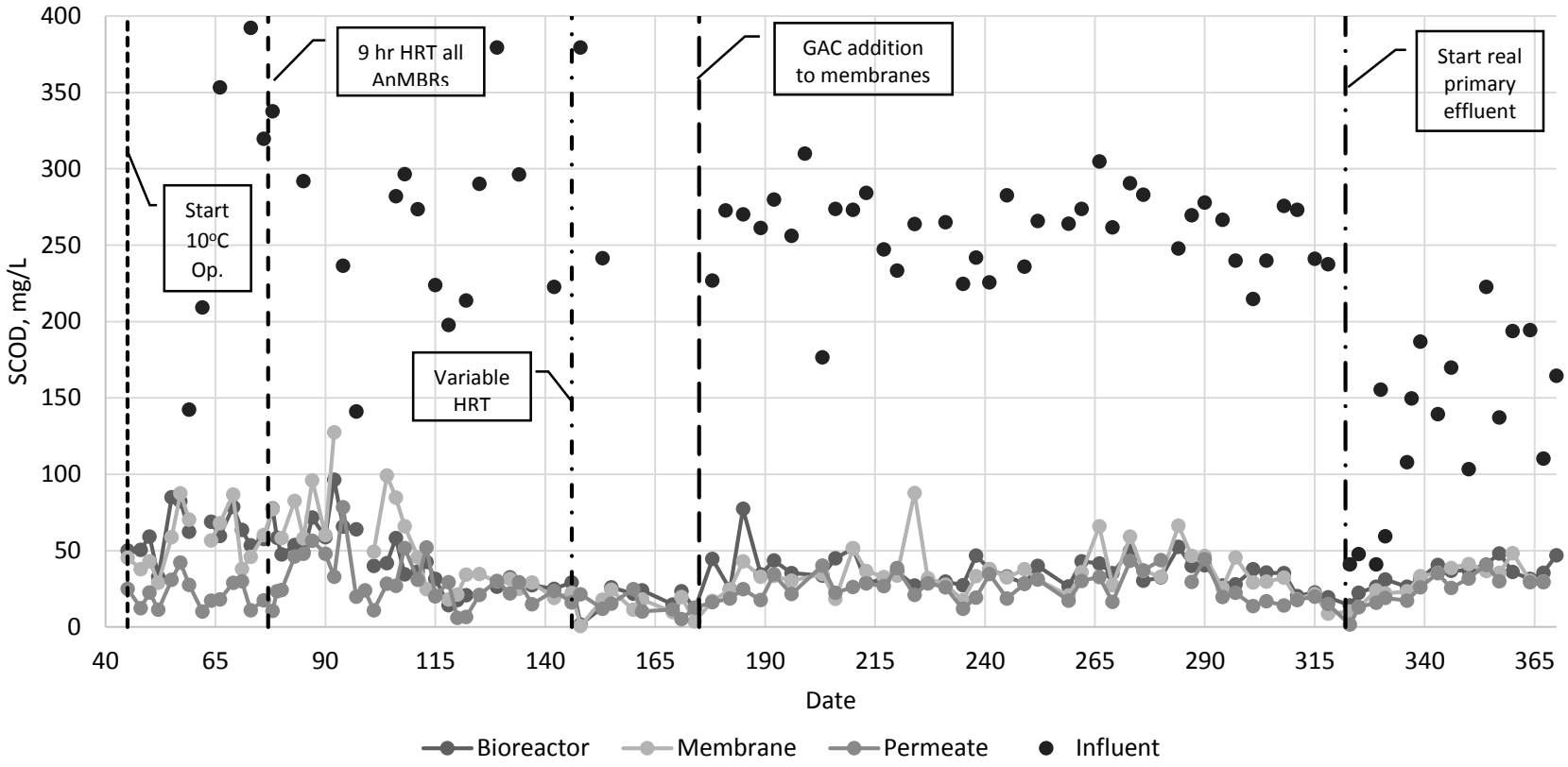

Figure A4 FBR10 soluble chemical oxygen demand (SCOD) in bioreactor, membrane, and permeate.

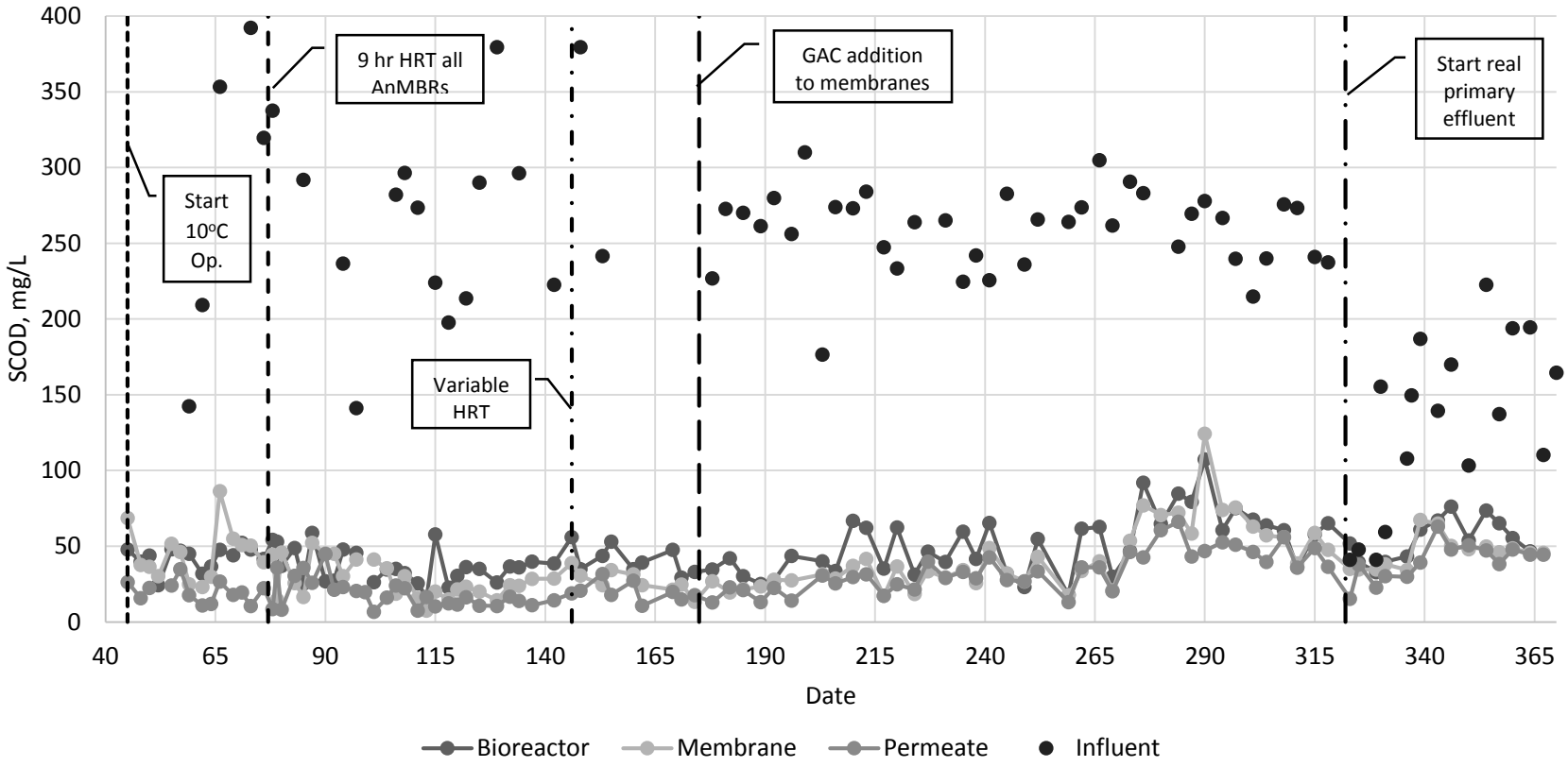

Figure A5 DFF25 soluble chemical oxygen demand (SCOD) in bioreactor, membrane, and permeate.

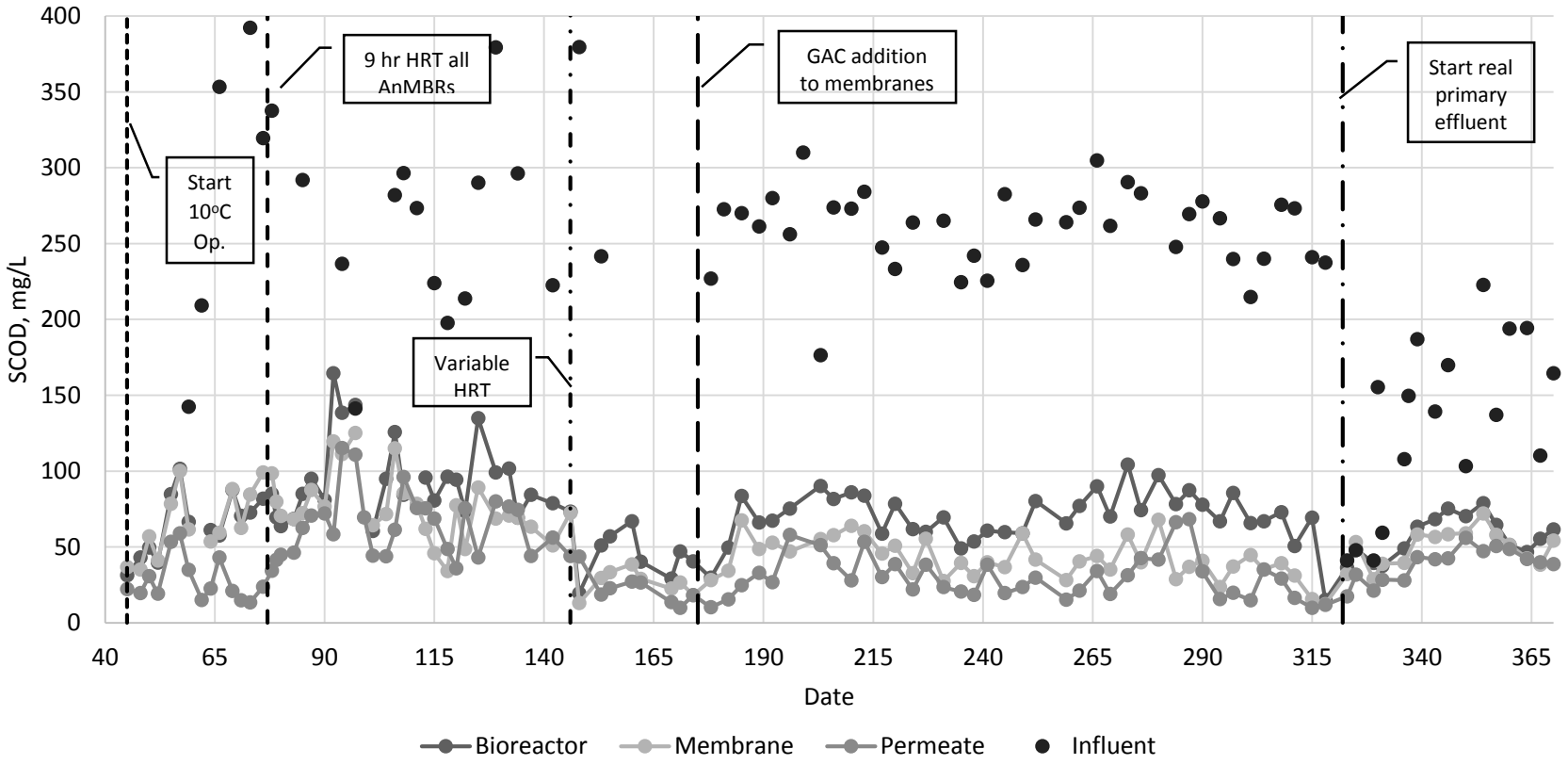

Figure A6 DFF10 soluble chemical oxygen demand (SCOD) in bioreactor, membrane, and permeate.

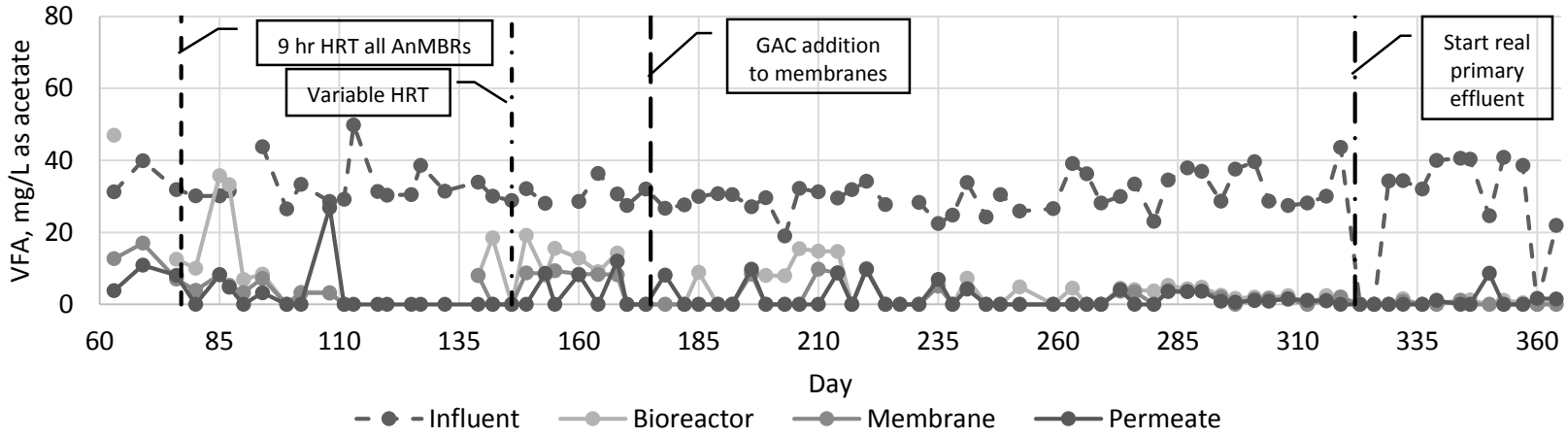

Figure A7 FBR25 influent, bioreactor compartment, membrane compartment, and permeate liquid volatile fatty acid content.

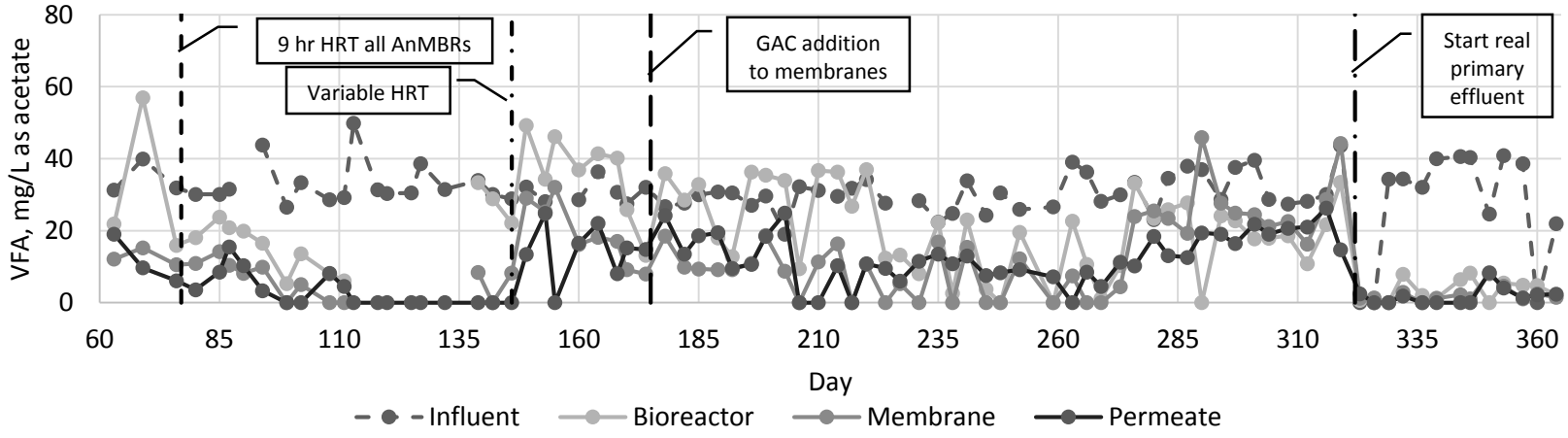

Figure A8 DFF25 influent, bioreactor compartment, membrane compartment, and permeate liquid volatile fatty acid content.

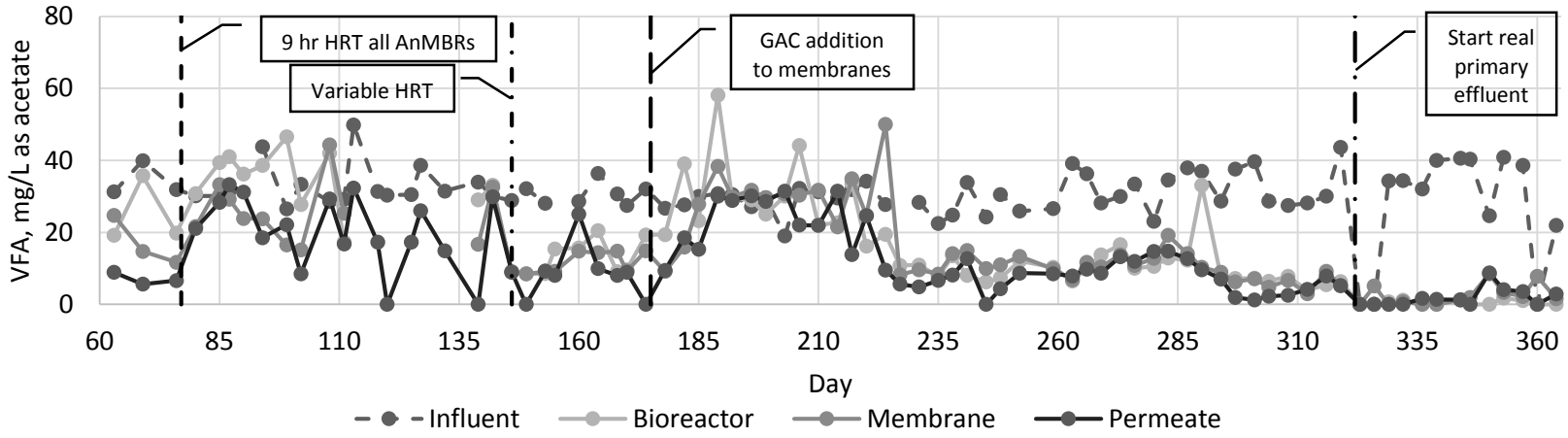

Figure A9 FBR10 influent, bioreactor compartment, membrane compartment, and permeate liquid volatile fatty acid content.

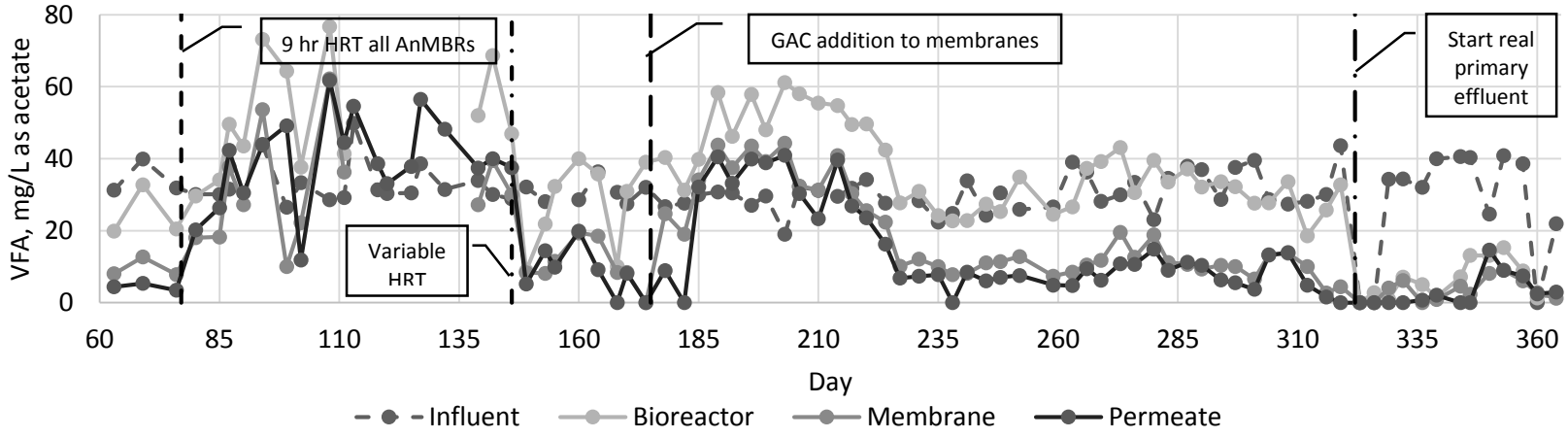

Figure A10 DFF10 influent, bioreactor compartment, membrane compartment, and permeate liquid volatile fatty acid content.

APPENDIX B – SOLIDS AND TURBIDITY

This Appendix includes all data collected for solids and turbidity during the study. This information is provided as a supplement to performance data described in previous chapters.

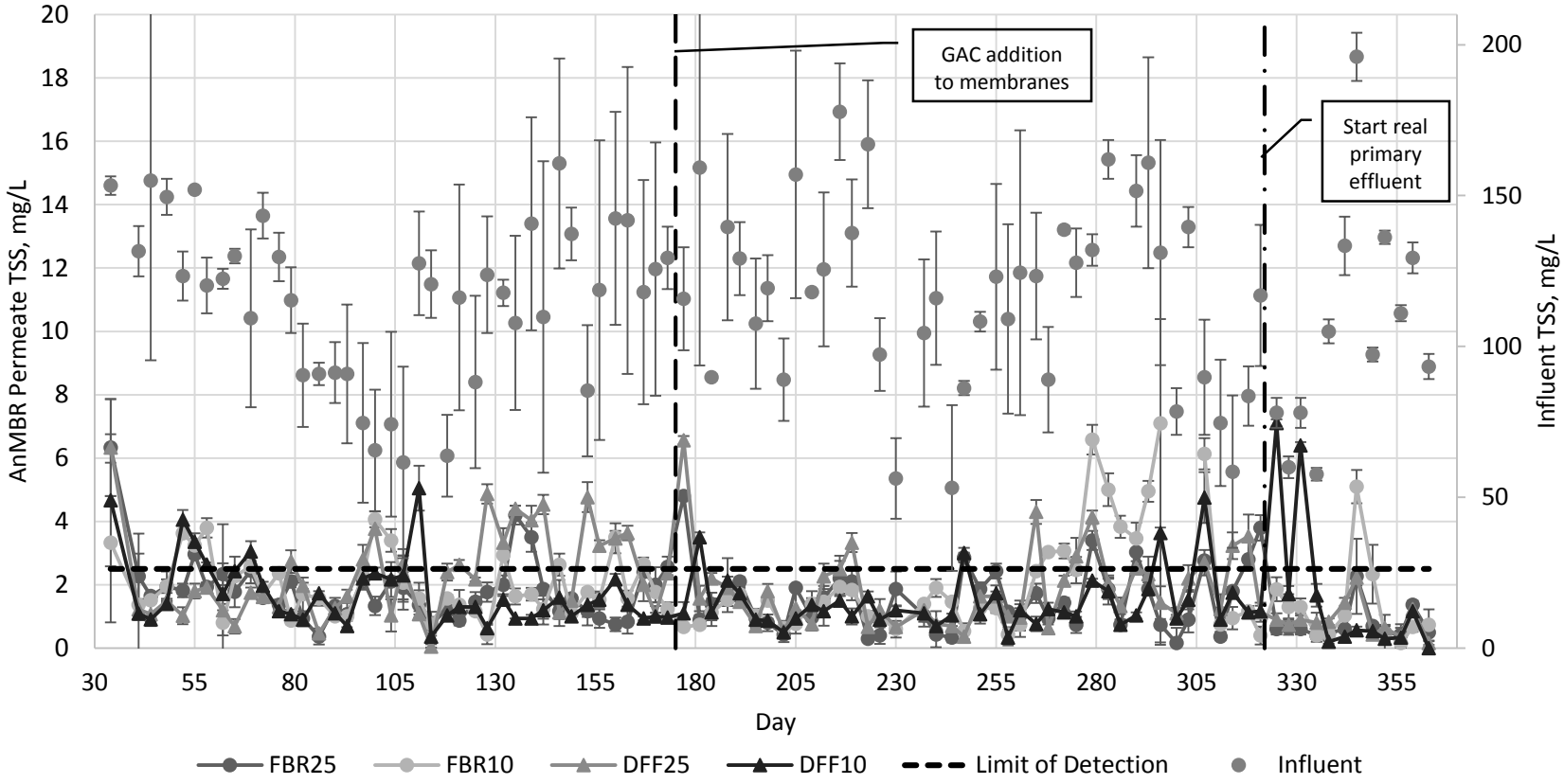

Figure A11 Influent and AnMBR permeate total suspended solids (TSS). Method limit of detection is 2.5 mg/L TSS. Permeate TSS values greater than limit of detection were likely due to biological contamination in permeate collection tanks.

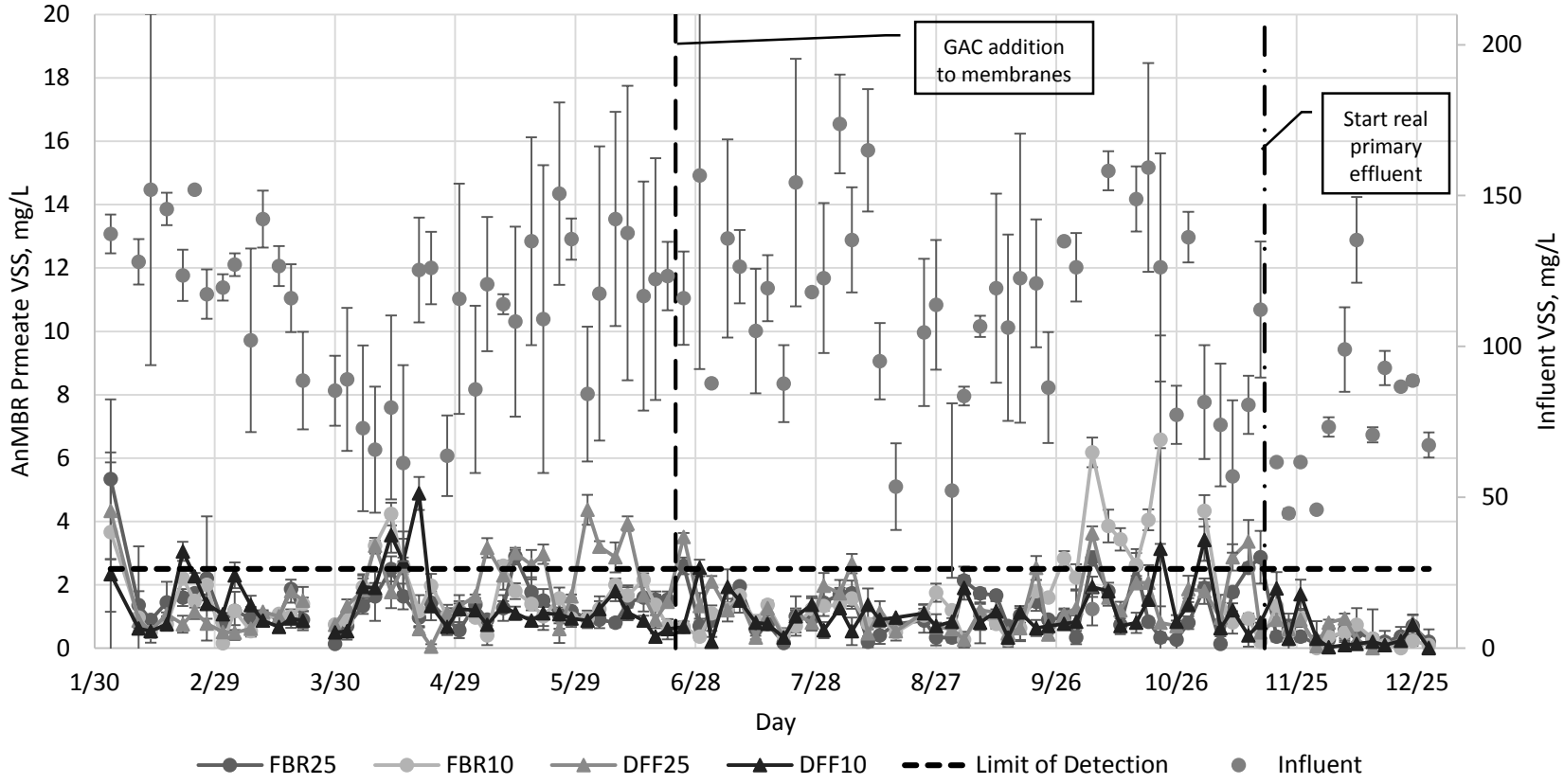

Figure A12 Influent and AnMBR permeate volatile suspended solids (VSS). Method limit of detection is 2.5 mg/L VSS. Permeate VSS values greater than limit of detection were likely due to biological contamination in permeate collection tanks.

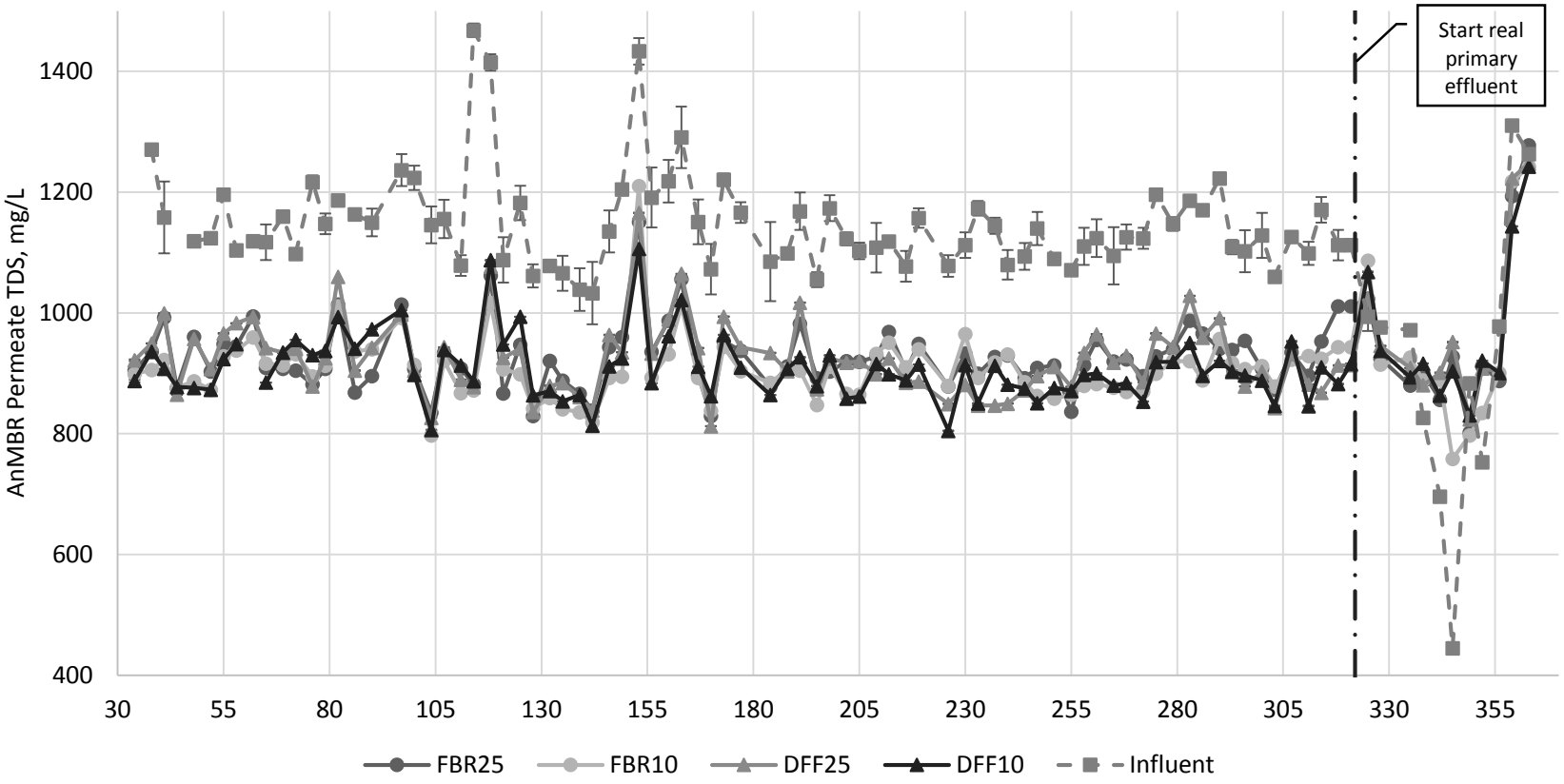

Figure A13 Influent and AnMBR permeate total dissolved solids (TDS).

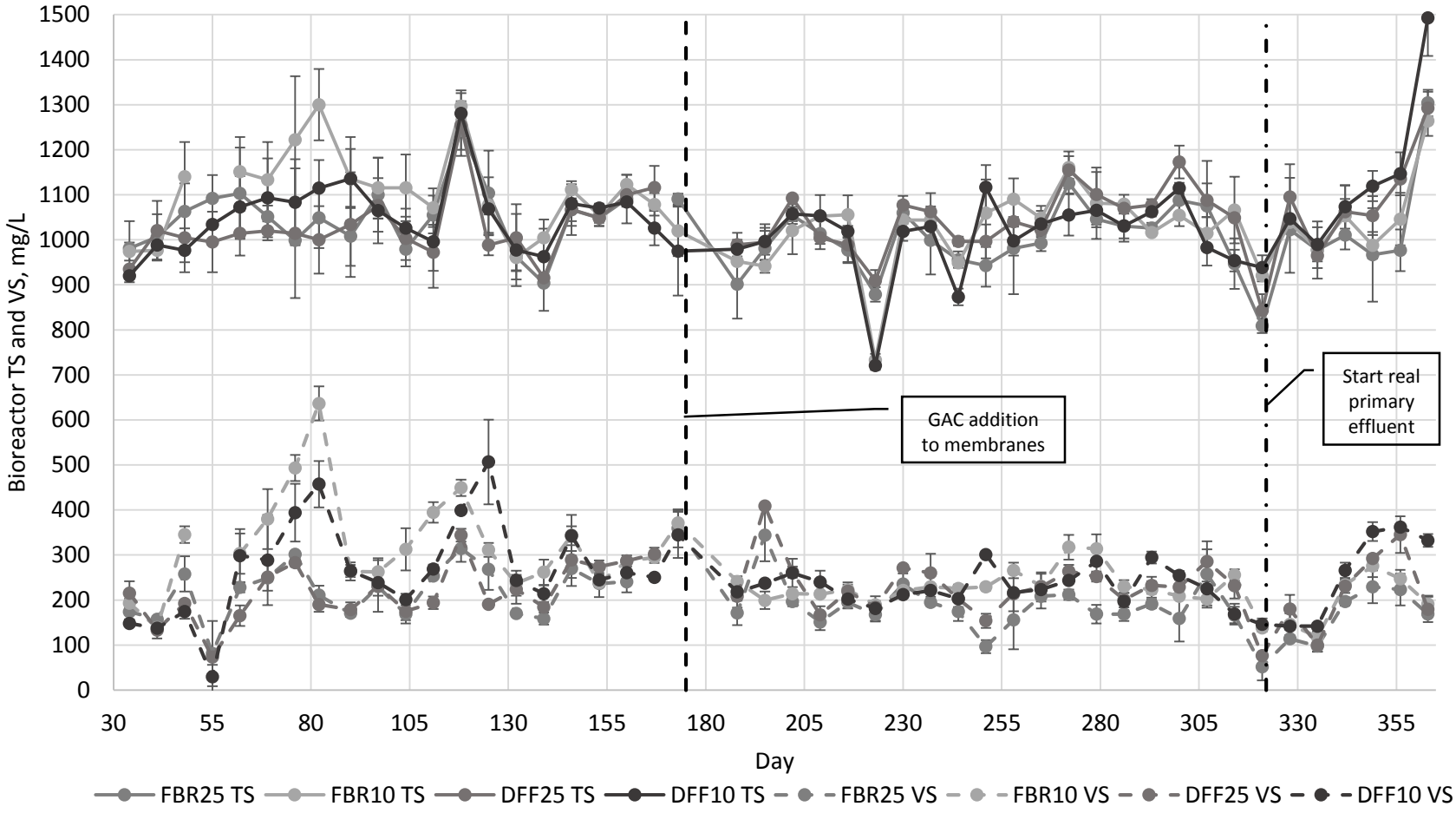

Figure A14 AnMBR bioreactor liquor total solids (TS) and volatile solids (VS).

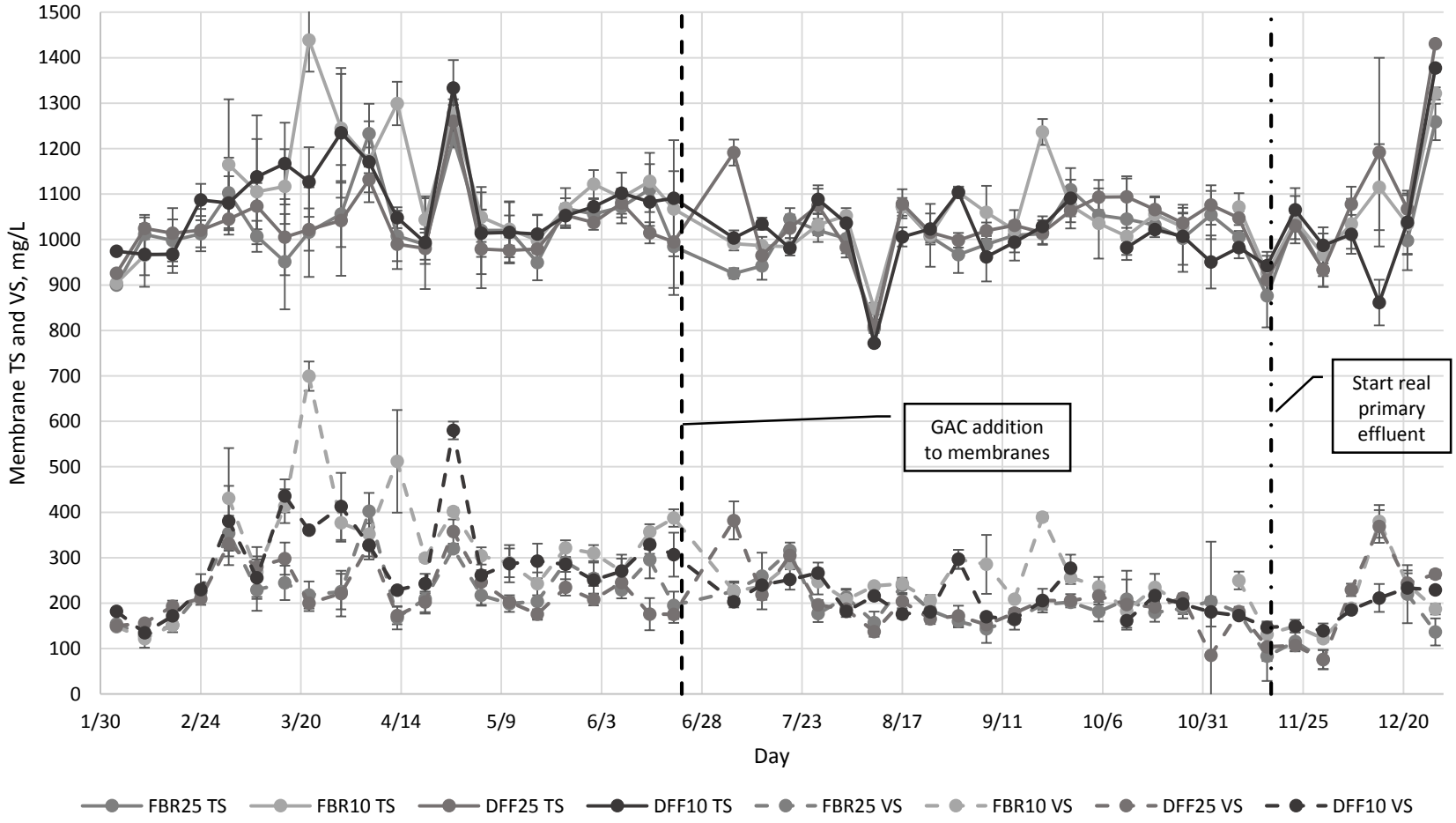

Figure A15 AnMBR membrane liquor total solids (TS) and volatile solids (VS).

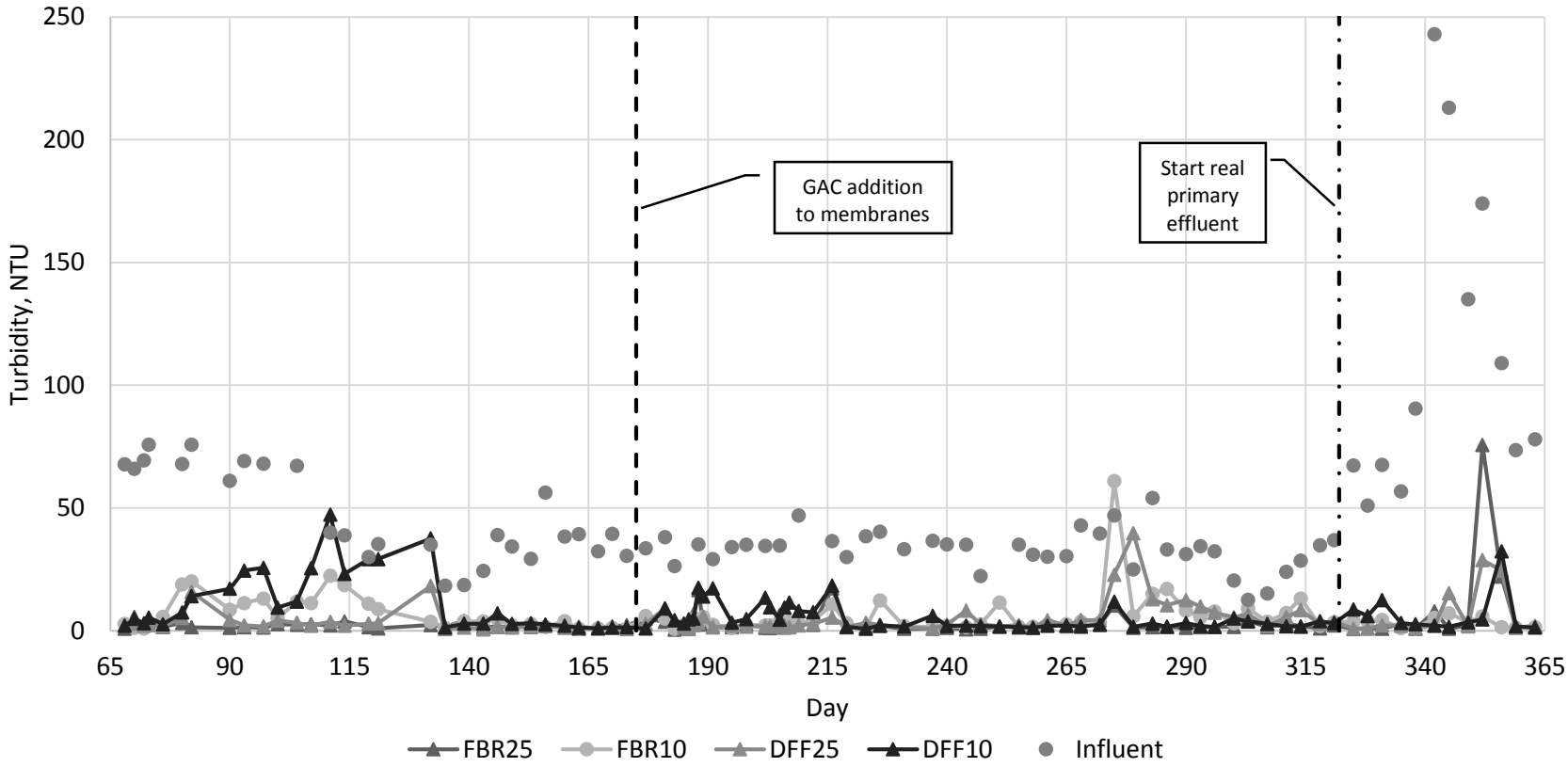

Figure A16 Influent and AnMBR permeate turbidity (NTU).

APPENDIX C – NITROGEN, PHOSPHOROUS, AND SULFATE

This Appendix includes all data collected for nitrogen, phosphorus, and sulfate during the study. This information is provided as a supplement to performance data described in previous chapters.

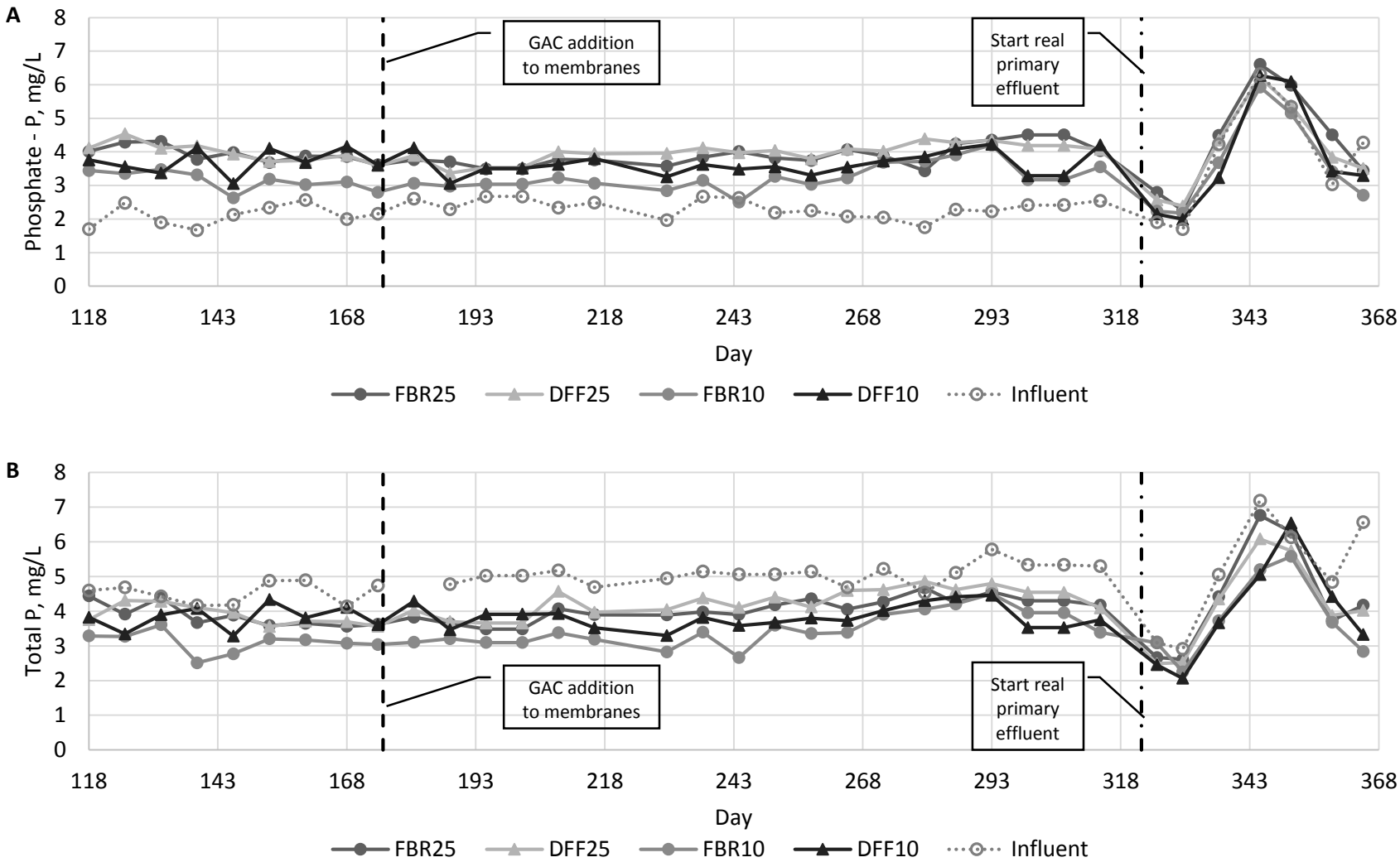

Figure A17 Influent and AnMBR permeate Phosphate-P (A) and Total P (B).

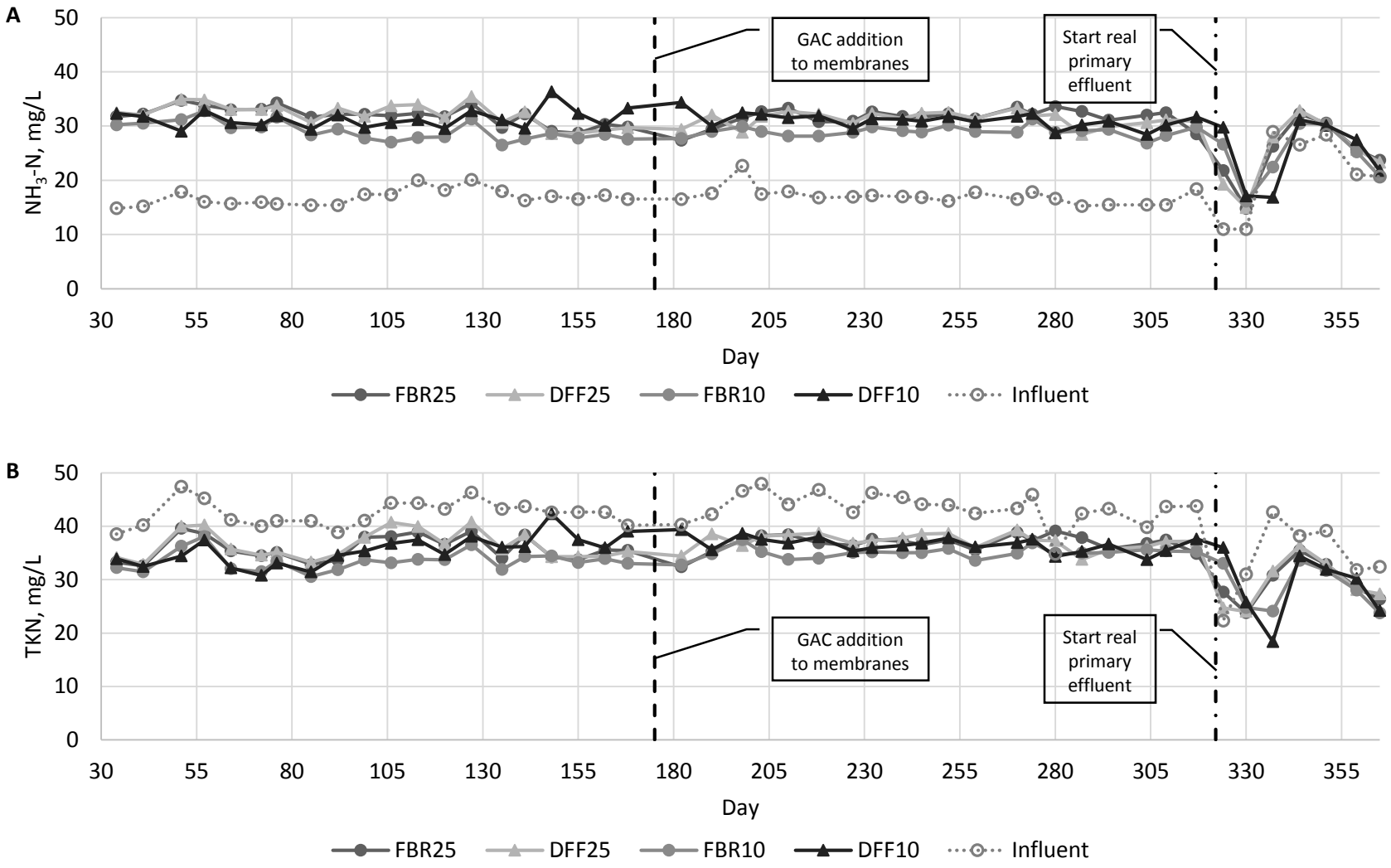

Figure A18 Influent and AnMBR permeate NH_3-N (A) and Total Kjeldahl Nitrogen (TKN) (B).

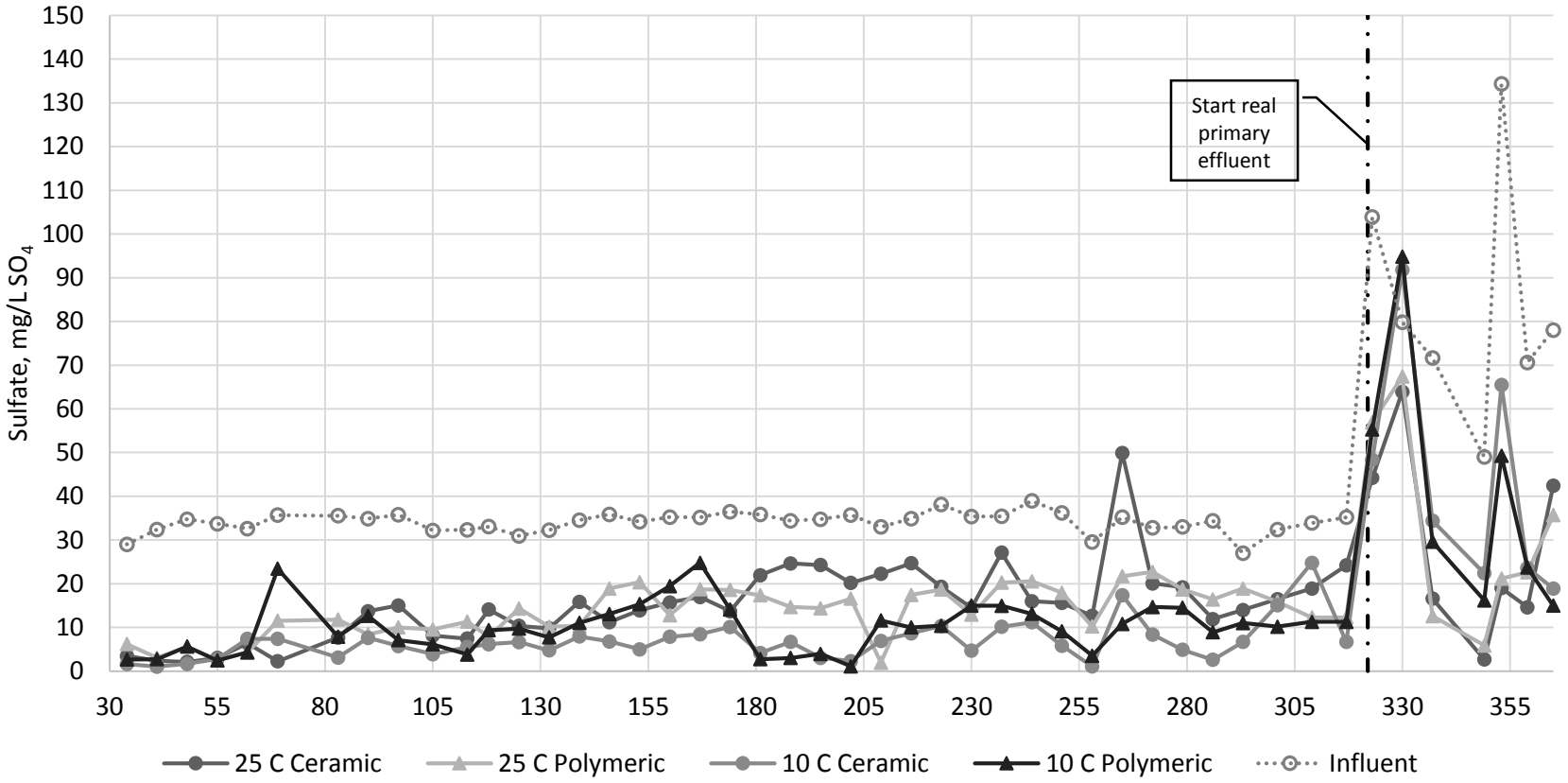

Figure A19 Influent and AnMBR permeate sulfate.

APPENDIX D - METHANE

This Appendix includes all data collected for methane during the study. This information is provided as a supplement to performance data described in previous chapters.

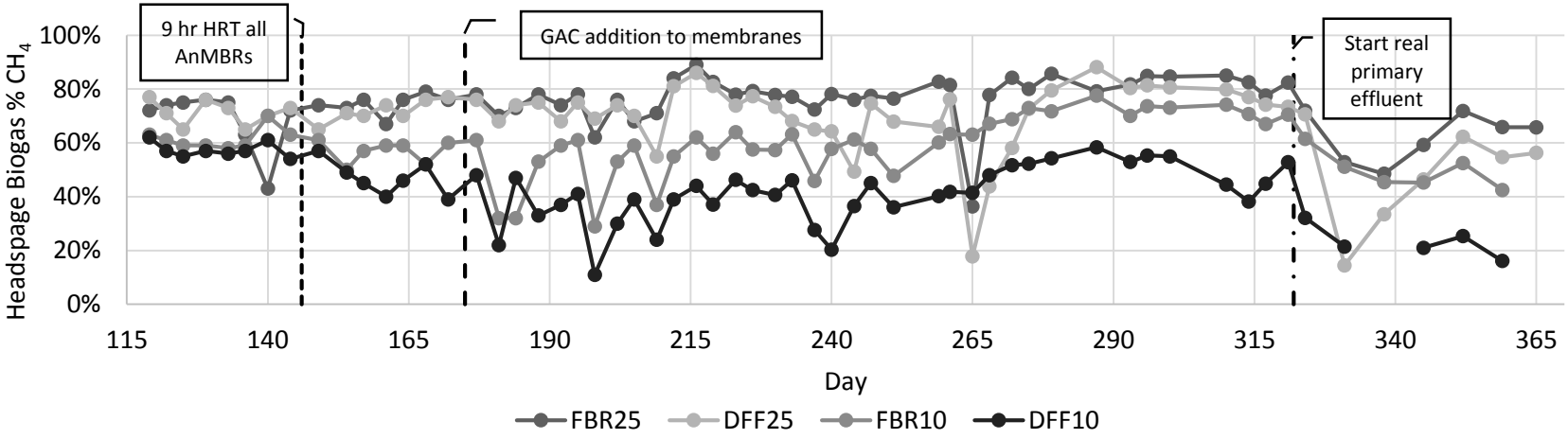

Figure A20 Bioreactor headspace biogas methane content.

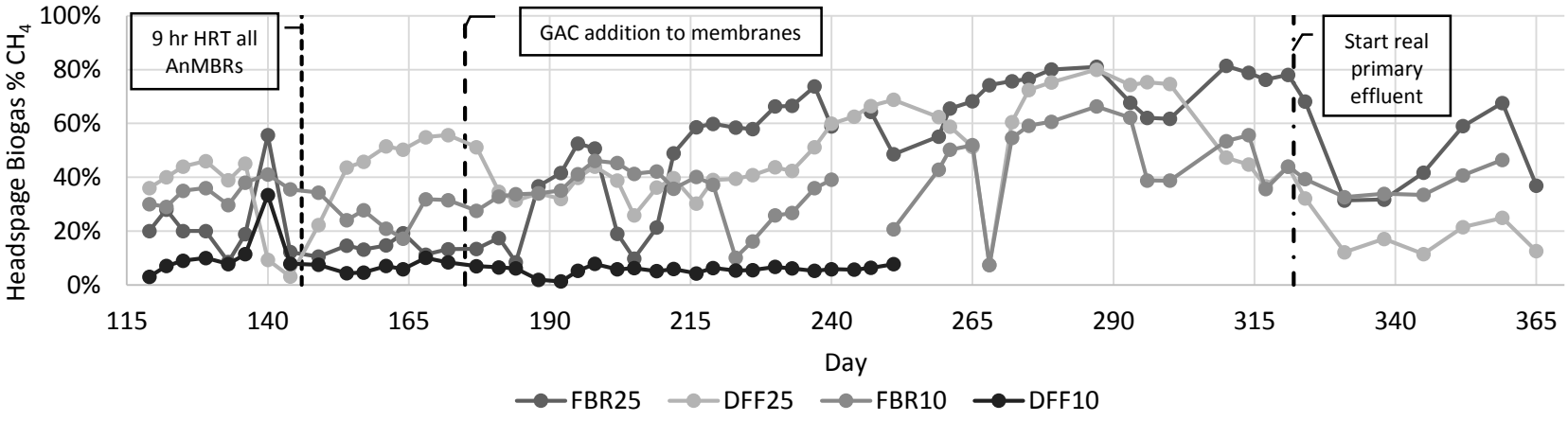

Figure A21 Membrane equalization tank headspace biogas methane content.

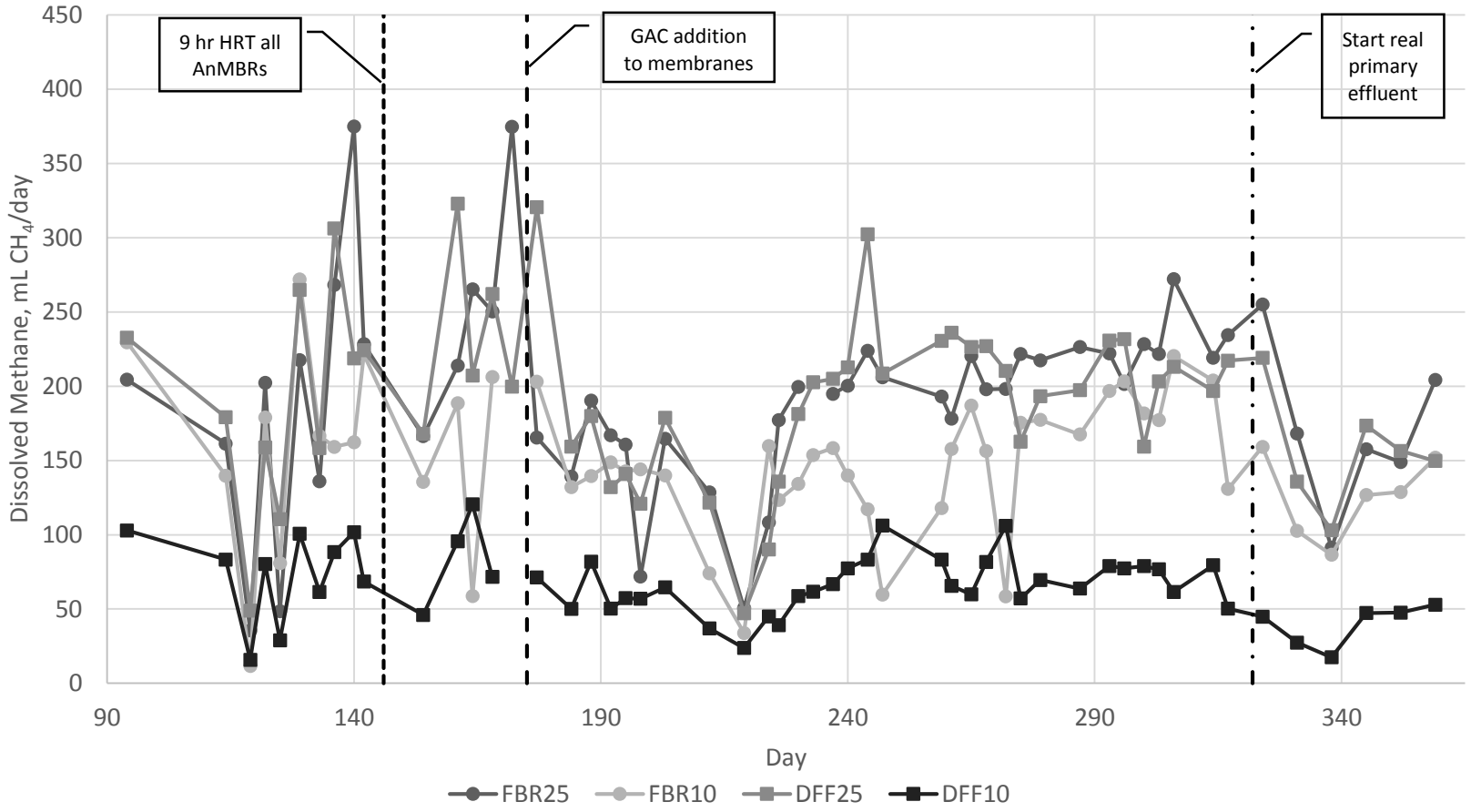

Figure A22 Dissolved methane loss in permeate.

APPENDIX E – PROTEIN AND CARBOHYDRATE

This Appendix includes all data collected for proteins and carbohydrates during the study. This information is provided as a supplement to performance data described in previous chapters.

Total protein analysis was conducted using a BCA Protein Assay Kit II (BioVision, Milpitas, CA) according the manufacturer's instructions.

Total carbohydrate analysis was conducted using a phenol-sulfuric acid method described in the following reference:

Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S., Lee, Y.C., 2005. Carbohydrate analysis by a phenol-sulfuring acid method in microplate format. Analytical Biochemistry, 339(2005), 69-72.

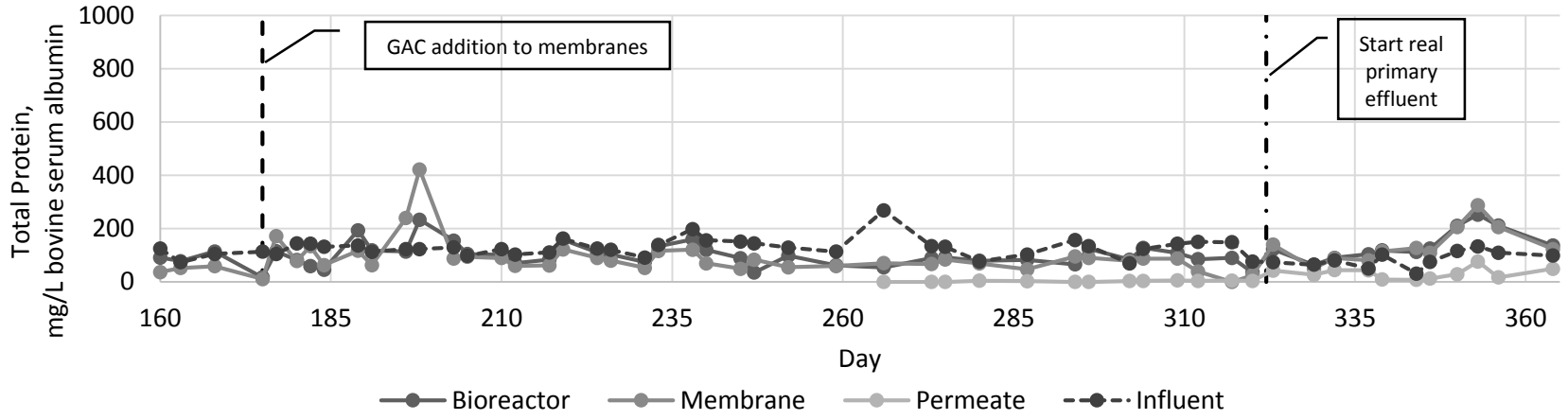

Figure A23 FBR25 influent, bioreactor compartment, membrane compartment, and permeate liquid total protein content.

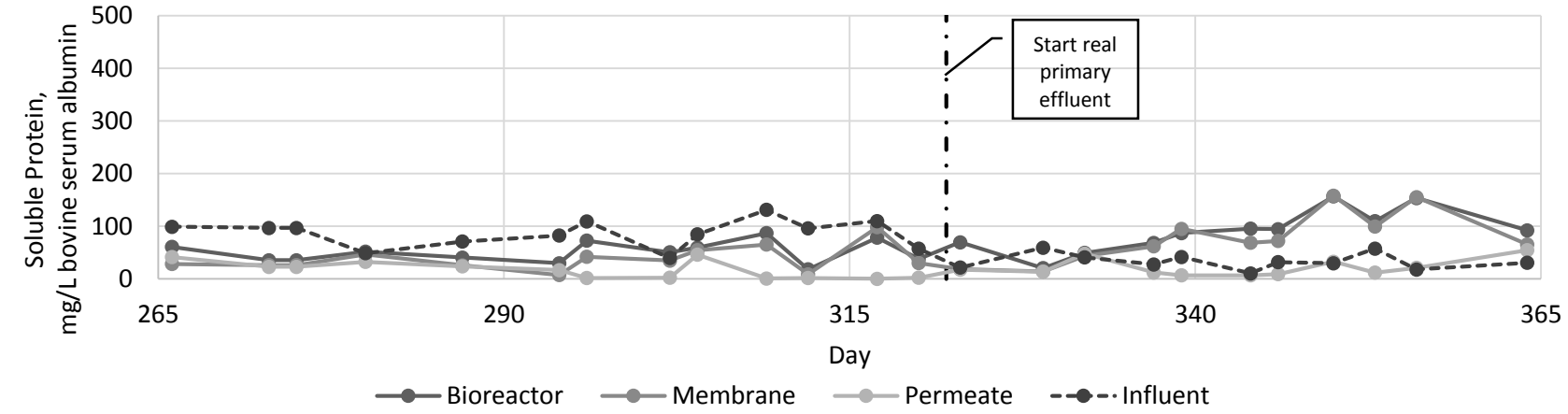

Figure A24 FBR25 influent, bioreactor compartment, membrane compartment, and permeate liquid soluble protein content.

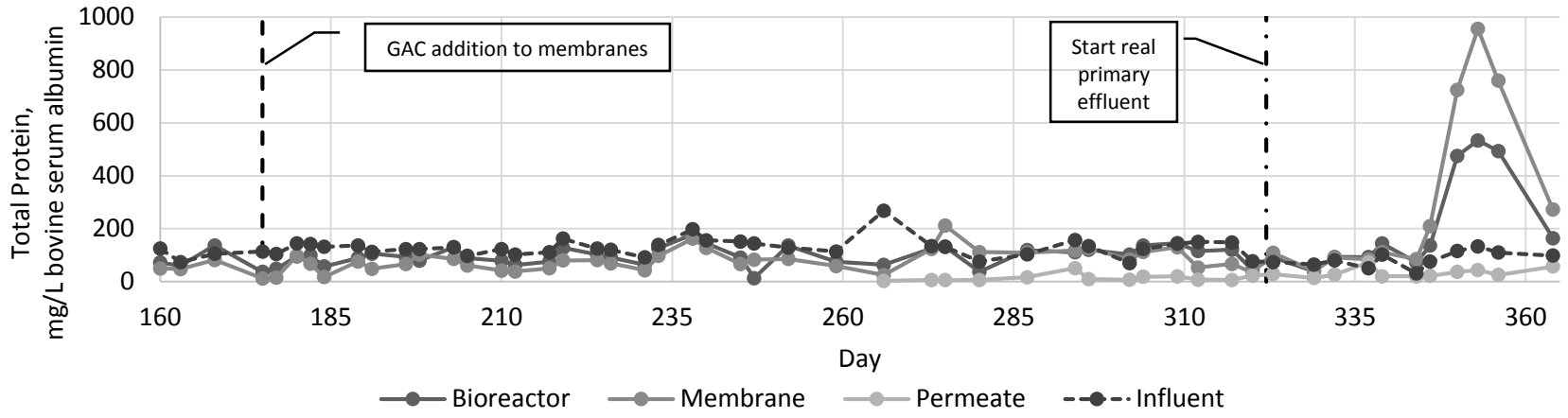

Figure A25 DFF25 influent, bioreactor compartment, membrane compartment, and permeate liquid total protein content.

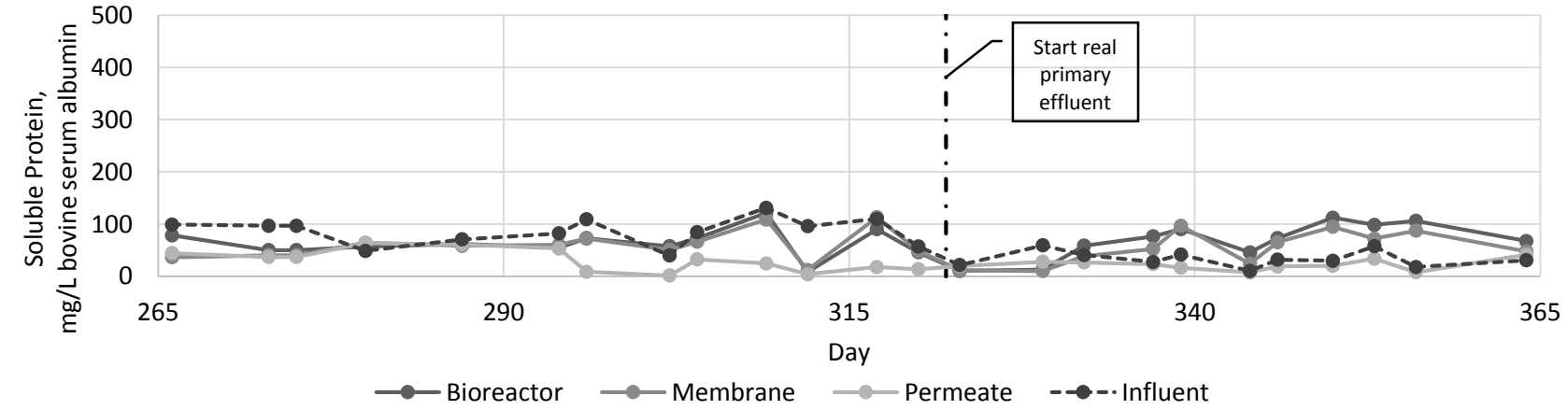

Figure A26 DFF25 influent, bioreactor compartment, membrane compartment, and permeate liquid soluble protein content.

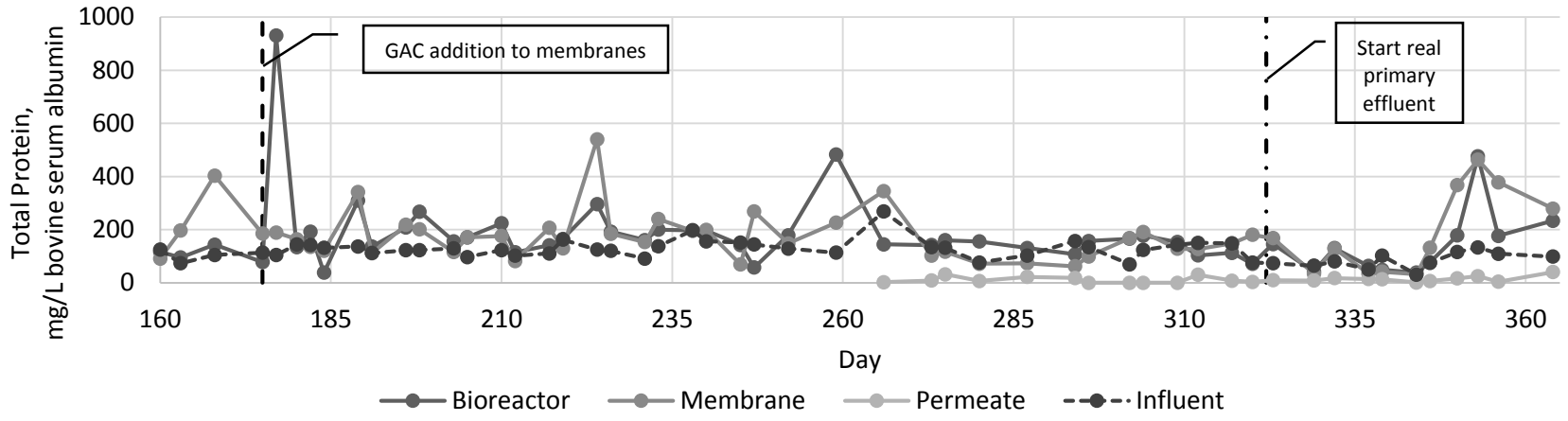

Figure A27 FBR10 influent, bioreactor compartment, membrane compartment, and permeate liquid total protein content.

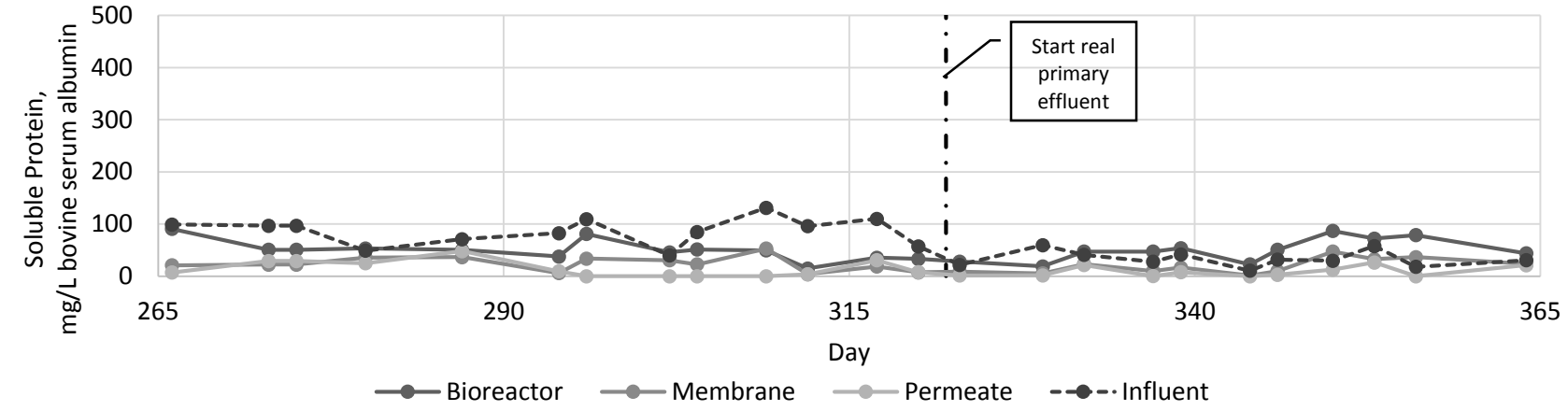

Figure A28 FBR10 influent, bioreactor compartment, membrane compartment, and permeate liquid soluble protein content.

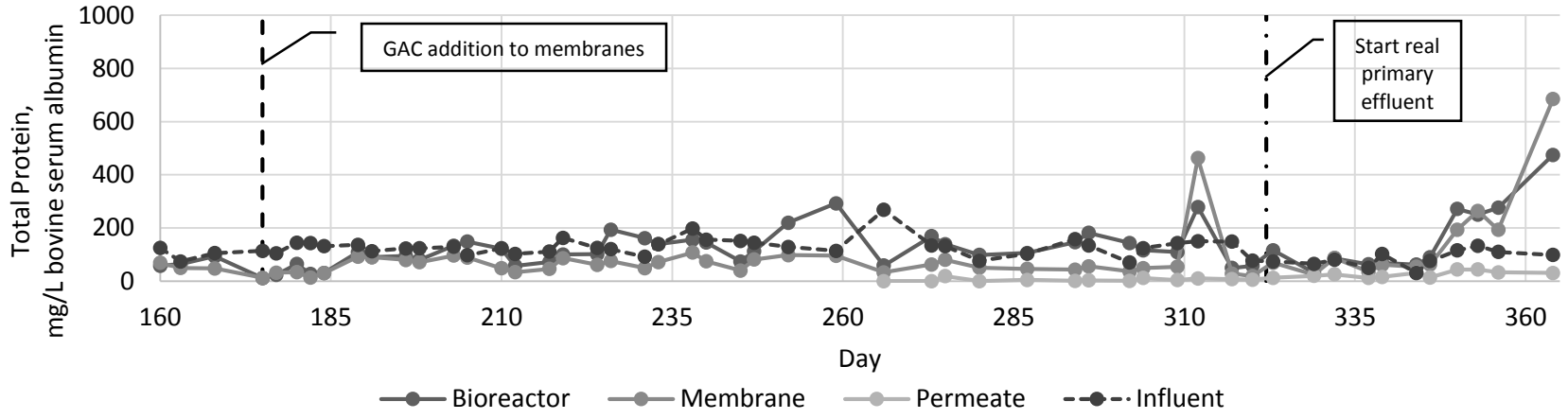

Figure A29 DFF10 influent, bioreactor compartment, membrane compartment, and permeate liquid total protein content.

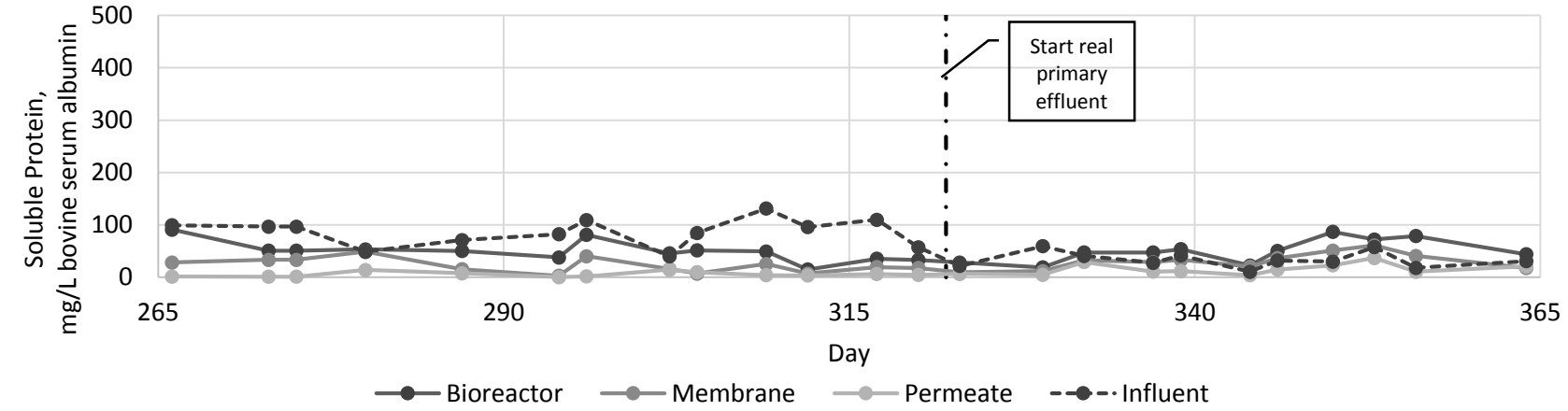

Figure A30 DFF10 influent, bioreactor compartment, membrane compartment, and permeate liquid soluble protein content.

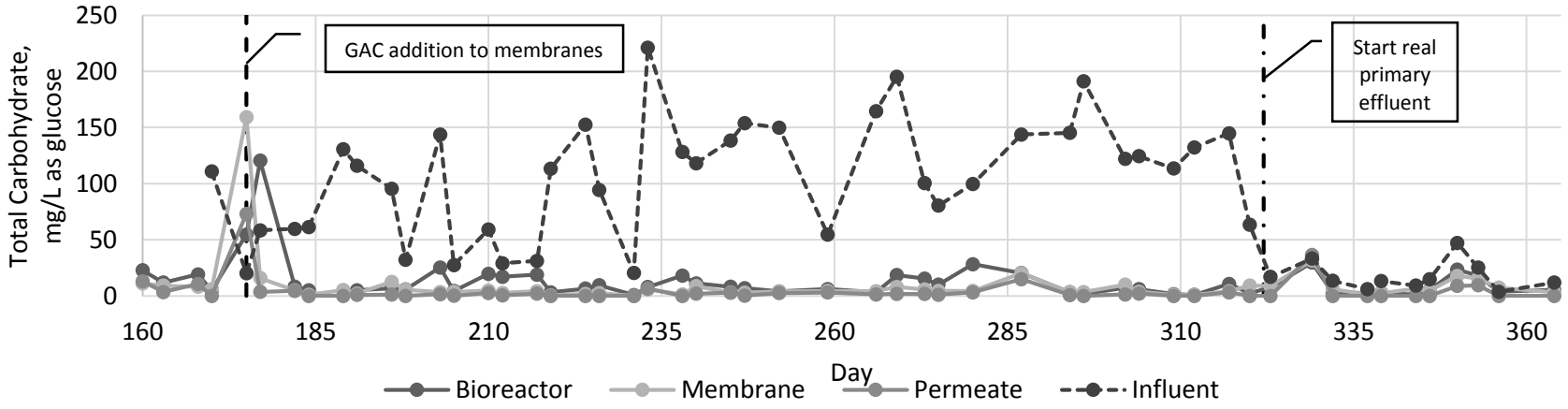

Figure A31 FBR25 influent, bioreactor compartment, membrane compartment, and permeate liquid total carbohydrate content.

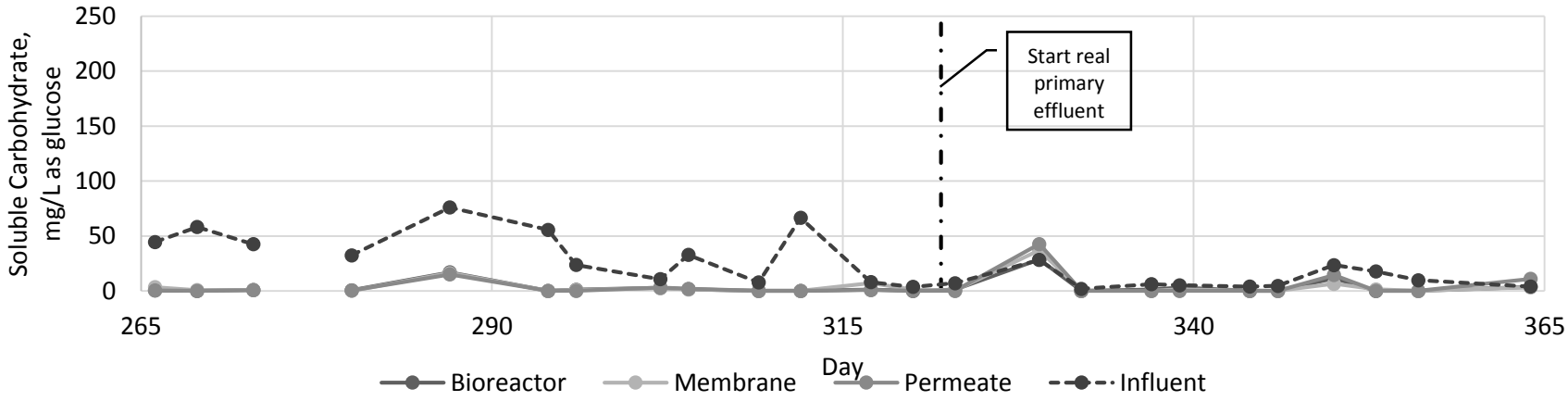

Figure A32 FBR25 influent, bioreactor compartment, membrane compartment, and permeate liquid soluble carbohydrate content.

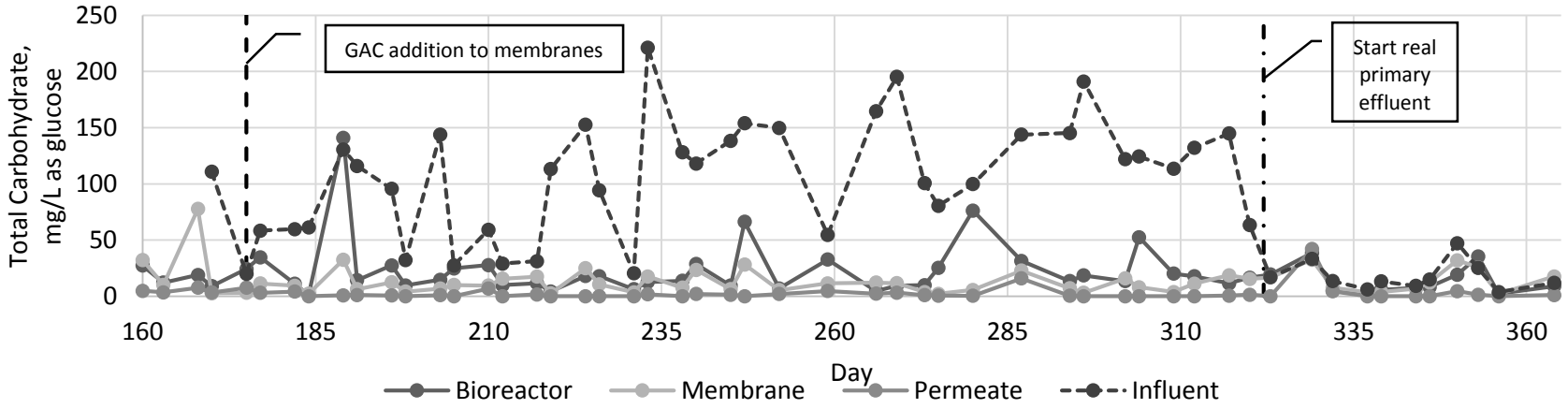

Figure A33 DFF25 influent, bioreactor compartment, membrane compartment, and permeate liquid total carbohydrate content.

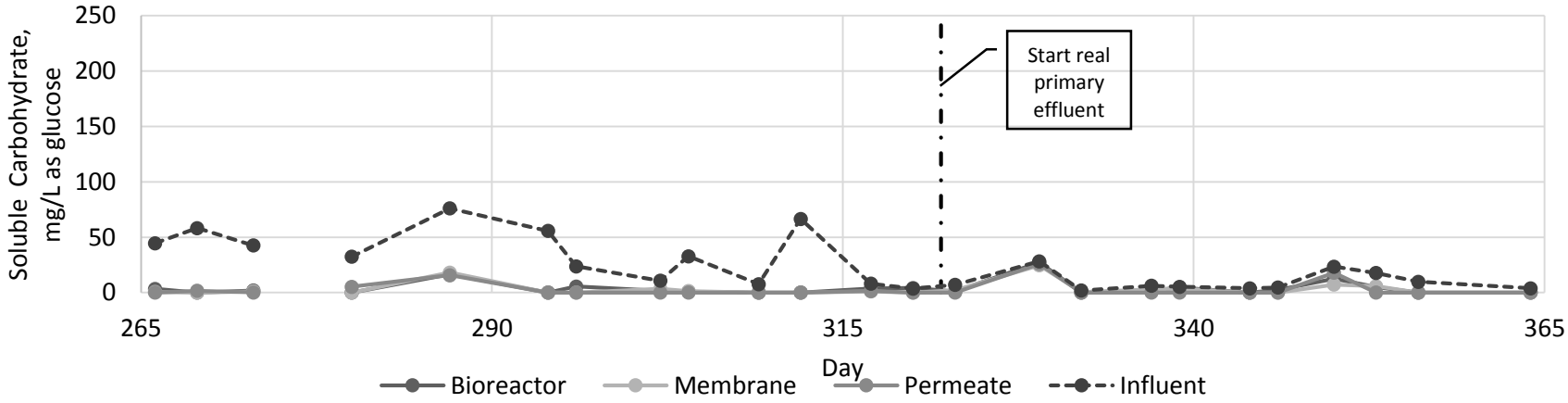

Figure A34 DFF25 influent, bioreactor compartment, membrane compartment, and permeate liquid soluble carbohydrate content.

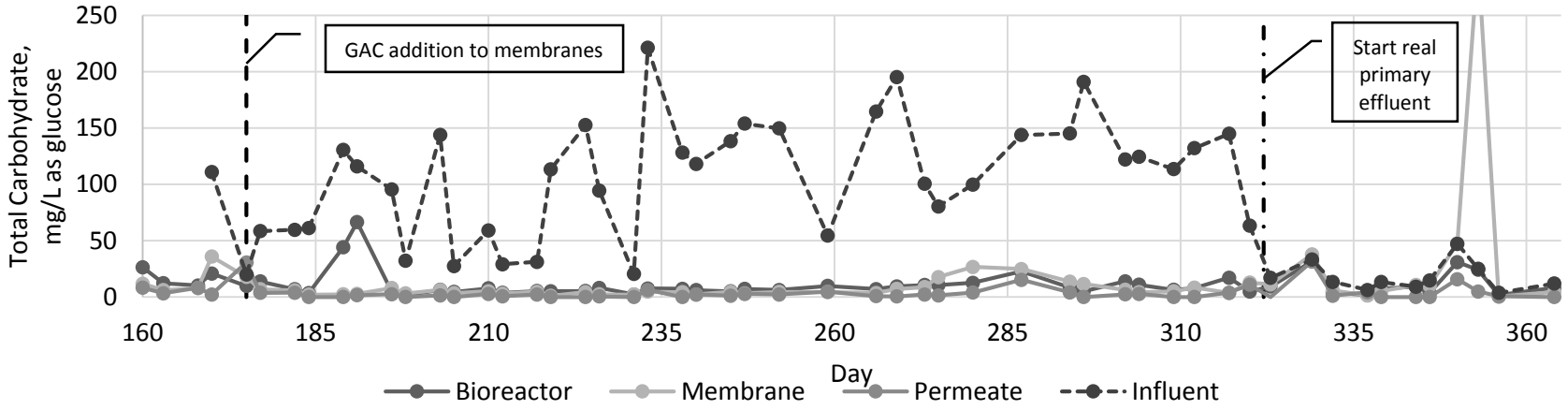

Figure A35 FBR10 influent, bioreactor compartment, membrane compartment, and permeate liquid total carbohydrate content.

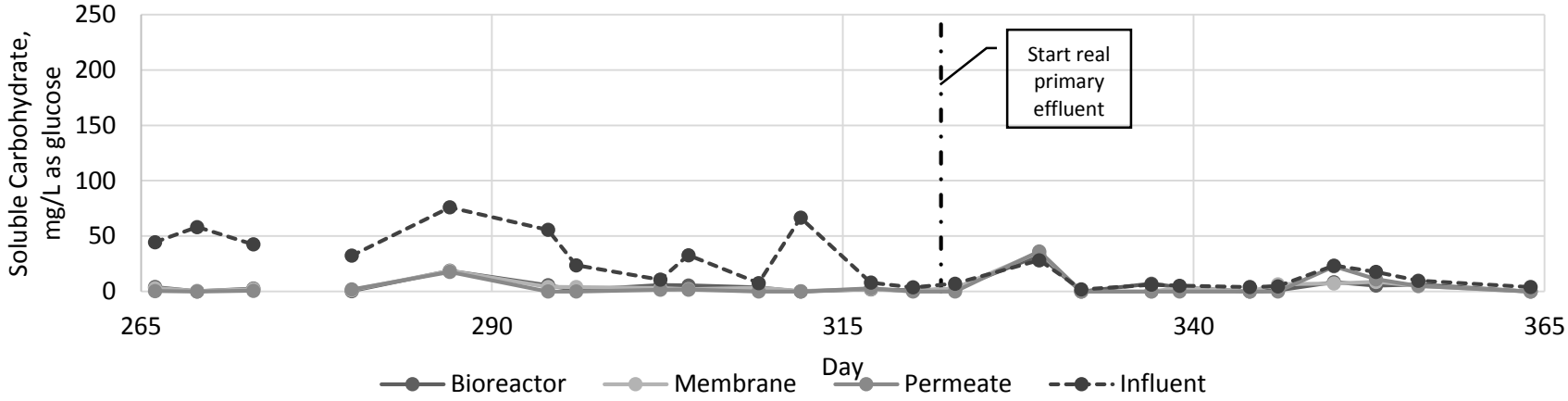

Figure A36 FBR10 influent, bioreactor compartment, membrane compartment, and permeate liquid soluble carbohydrate content.

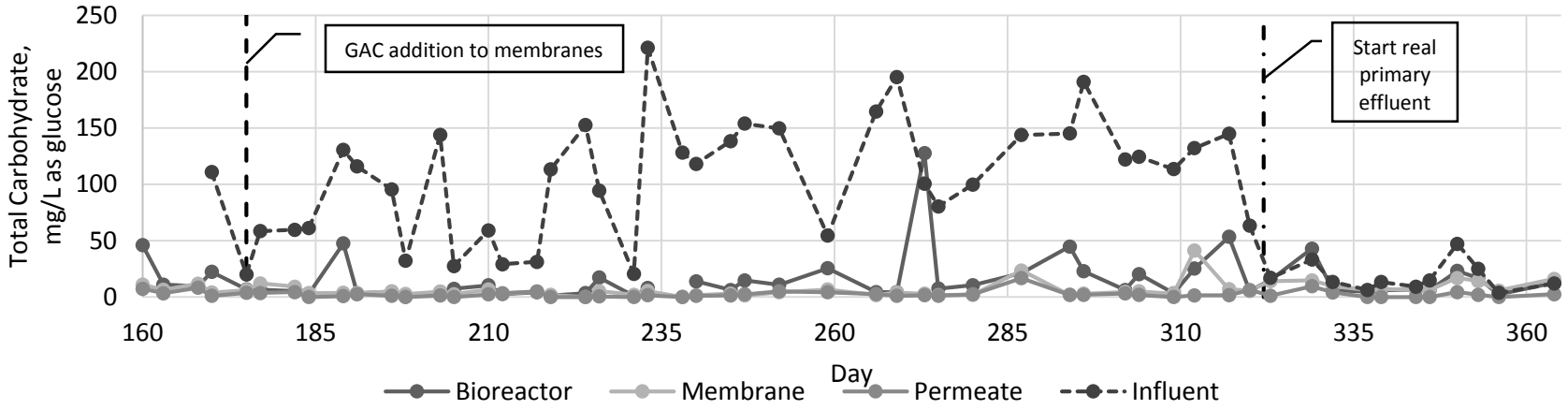

Figure A37 DFF10 influent, bioreactor compartment, membrane compartment, and permeate liquid total carbohydrate content.

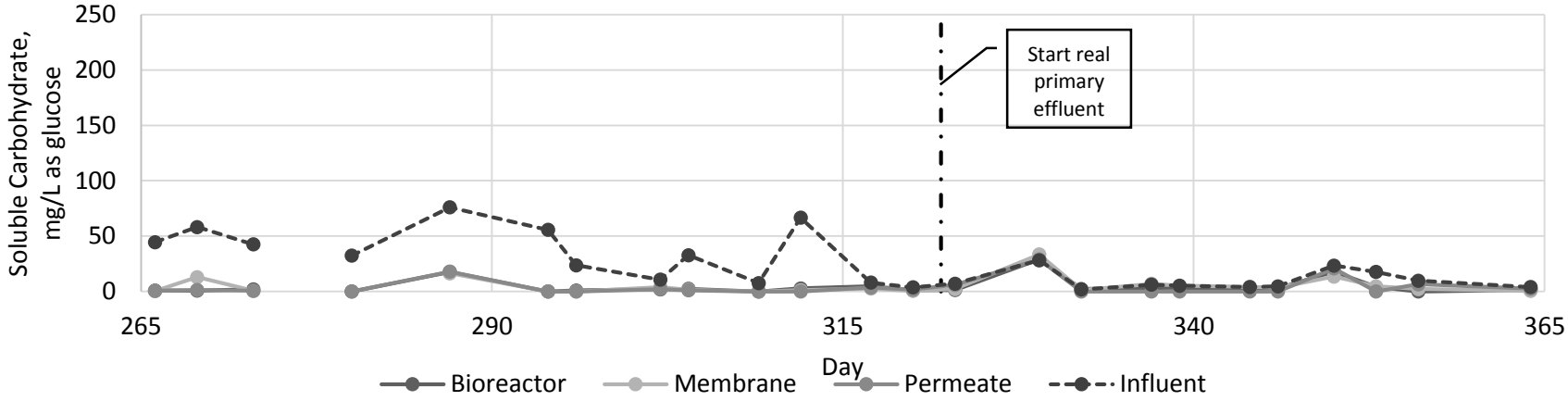

Figure A38 DF10 influent, bioreactor compartment, membrane compartment, and permeate liquid soluble carbohydrate content.

APPENDIX F – SUPPLEMENTARY INFORMATION FOR TABLE 3.4

Assumptions:
Flow: 40,000 m^3 (10 MGD)
BOD_5: 200 mg/L
COD: 400 mg/L ($COD:BOD_5 = 2$)

Primary setting:
Primary setter removes 30% BOD_5, 50% TSS, $COD:BOD_5 = 2$
To Digester:
(200 mg/L BOD_5)(30% removed)(40,000 m^3/d) = 2,400 kg/d BOD_5
(2,400 kg/d BOD_5)($COD:BOD_5 = 2$) = 4,800 kg/d COD

To Secondary Treatment:
(200 mg/L BOD_5)(70%)(40,000 m^3/d) = 5,600 kg/d BOD_5
(11,700 lb/day BOD_5)($COD:BOD_5 = 2$) = 11,200 kg/d COD

Aerobic treatment with nitrification:
A. Aeration (diffused air)
Assume 0.3 kWh/m^3 treated (Speece 1996)
(0.3 kWh/m^3)(40000 m^3/d) = 12,000 kWh/d

B. Biological nitrification
Assume 3,400 kWh/d required for 10 MGD (WEF 2009, Table C.4, p. 354)

C. Anaerobic digestion
Assume 1,700 kWh/d required for 10 MGD (WEF 2009, Table C.4, p. 354)

D. Belt filter press
Assume 500 kWh/d required for 10 MGD (WEF 2009, Table C.4, p. 354)

E. Energy recovered from digester biogass
Assume 3,500 kWh/d produced for 10 MGD (WEF 2009, Table C.4, p. 354)

Anaerobic treatment with ion exchange:
A. AnMBR
Experimental DFF bioreactor energy requirement ranged from 0.02 to 0.08 kWh/m^3
Estimate of low CFV membrane energy requirement, 0.23 kWh/m^3 (Le-Clech et al. 2006)
Therefore, AnMBR energy estimate of 0.25-0.31 kWh/m^3
(0.25-0.31 kWh/m^3)(40,000 m^3/d) = 10,100-12,300 kWh/d

B. Ion exchange nutrient removal
Assume ion exchange energy demand of 0.06 kWh/m^3 (Howe et al. 2012, Section 10.9)
(0.06 kWh/m^3)(40,000 m^3/d)(2 systems) = 4,800 kWh/d

C. Anaerobic digestion

Assume all solids from primary treatment are taken
Assume 50% reduction in VS coming from AnMBR compared to activated sludge
Therefore, assume digester receiving 75% of incoming solids compared to activated sludge
(1,700 kWh/d)(75%) = 1,300 kWh/d

D. Belt filter press

As in part C, assume process handling 75% of the solids load assumed for activated sludge
(500 kWh/d)(75%) = 350 kWh/d

E. Dissolved methane recovery

Assume air stripping for dissolved methane recovery at 0.05 kWh/m^3 (McCarty et al. 2011)
(0.05 kWh/m^3)(40,000 m^3/d) = 2000 kWh/d

F. Energy recovered from AnMBR biogas

From assumptions listed above: 11,200 kg/d COD to AnMBR
Assume effluent BOD_5=10 mg/L (from experimental results)
(10 mg/L BOD_5)(40000 m^3) = 400 kg/d BOD_5 remaining x2 = 800 kg/d COD
BOD removal = 11,200 kg/d – 800 kg/d = 10,400 kb/d COD removed
Assume 90% COD removed goes to CH_4
Assume 0.28 m^3 CH_4 produced per kg COD destroyed
(10,400 kg/d COD)(90% to CH_4)(0.28 m^3 CH_4/kg COD) = 2620 m^3 CH_4/d
Assume CH_4 has heating value of 37 MJ/m^3 (Khartchenko et al. 1997)
Assume 33% conversion to electricity (Kim et al. 2011)
(2620 m^3 CH_4/d)(37 MJ/m^3)(0.2778 kWh/MJ)(33%) = 8,900 kWh/d

G. Energy recovered from digester biogas

As in part C, assume process handling 75% of the solids load assumed for activated sludge. Therefore, assume 75% methane production compared to activated sludge scenario
(3,500 kWh/d)(75%) = 2,600 kWh/d

APPENDIX G – SUMMARY OF MEMBRANE FOULING CONTROL USING PAC

Introduction

The proposal for this project included investigating addition of powdered activated carbon (PAC) to the membrane compartments as a means to control fouling instead of GAC. This was based on previous evidence that PAC addition in flocculant biomass reactors using flat sheet membranes with gas sparging acted as an absorbing agent to reduce membrane foulants (Hu & Stuckey 2007; Akram & Stuckey 2008). Preliminary PAC testing performed on the DFF25 AnMBR in this study (polymeric membrane) had a negative impact on membrane fouling control, and as a result further analysis of PAC was suspended. This is a summary of the preliminary PAC testing that was performed.

Methods and results

The DFF25 membrane equalization tank was altered before PAC addition in order to isolate the membrane recirculation loop. This was done so that added PAC would not be transferred back to the bioreactor or wasted with excess bioreactor liquid not processed by the membrane. PAC selected was Darco G60 (Sargent-Welch, Skokie, IL), which has an approximate size distribution of d5 – 5.5 µm, d50 – 34 µm, d95 – 125 µm as indicated by Cabot Corp. (Billercia, MA). PAC was initially added at a dose of 1.7 g/L_R based on the dose used by Hu and Stuckey (Hu & Stuckey 2007), with the same low CFV used during GAC operation (0.018 m/s). Once PAC was added the membrane began to foul at an increased rate, with TMP reaching 1 bar after 28 h. The membrane was then removed and cleaned by flushing with a water jet. All tubing was also flushed to remove residual PAC. The system was then reconnected, a new dose of PAC

was added, and the system run again with similar results. PAC was also observed to be settling out in the membrane system and building up behind the hose clamp controlling TMP.

After this first negative result, efforts were then made to alter protocol by adjusting carbon dose and CFV operation. Different scenarios included:

- 25% original PAC dose (0.43 g/LR) at low CFV (0.018 m/s)
- 25% original PAC does at low CFV with a daily pulse (0.27 m/s for 30 s) to re-suspend particles
- 25% original PAC dose using wet sieved PAC (45 µm mesh) at low CFV with daily pulse
- 25% original PAC dose using wet sieved PAC at medium CFV (0.135 m/s)

All protocols yielded similar results for fouling time (Figures A39 and A40). Between each iteration the membrane was cleaned using the standard cleaning procedure using a water jet and soak in NaOCl solution and all tubing was flushed to remove PAC. Fresh PAC was added for each test.

Conclusions

All PAC scenarios resulted in similar or lower run-times compared to LXF operation without GAC. These preliminary results indicated that PAC was not well suited for this project. After discussing preliminary results with Xylem, Inc., further work with PAC was suspended from the project scope of work due to the additional projected time and cost anticipated to select an appropriate PAC and optimize dose/operation.

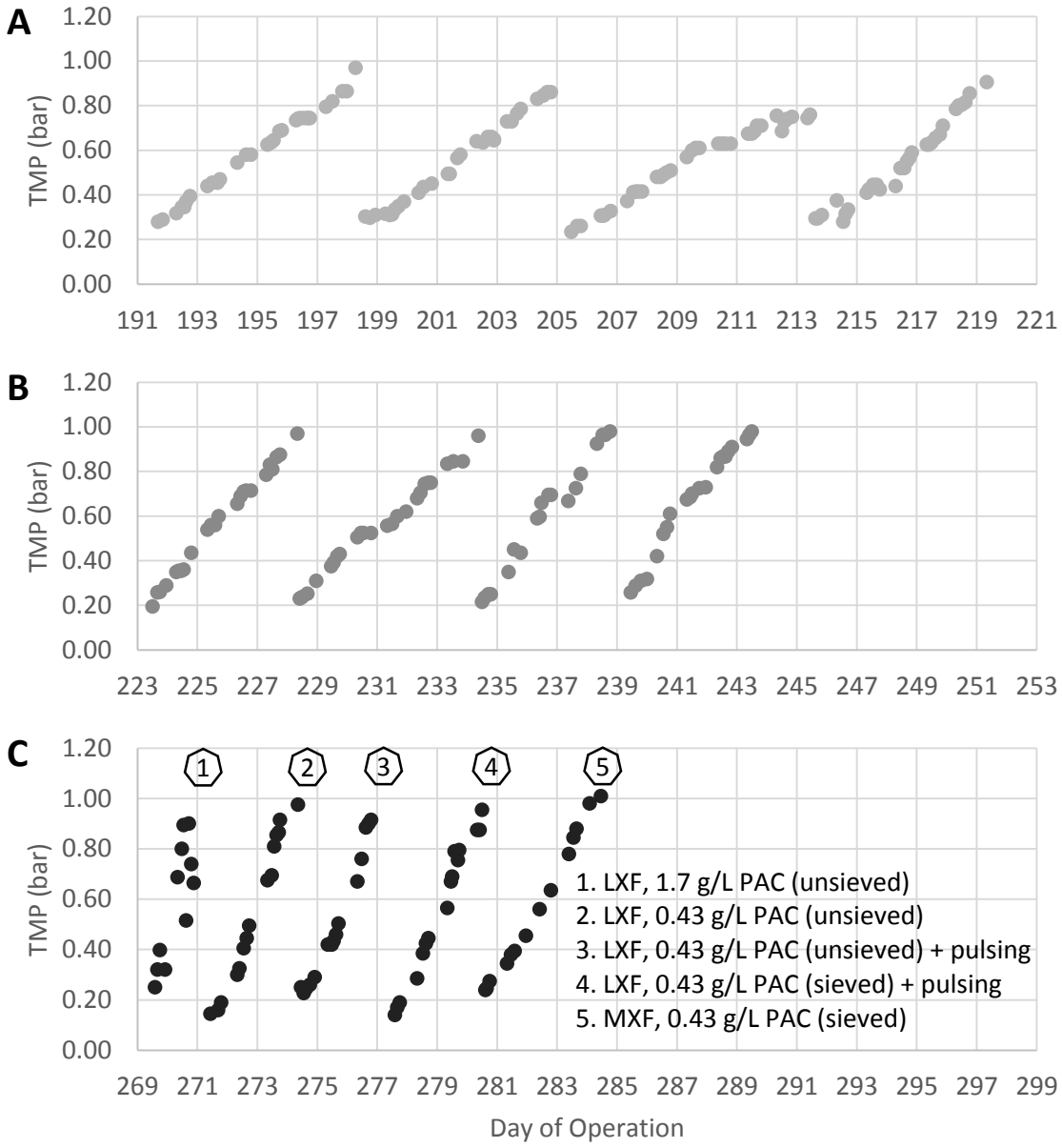

Figure A39 Comparison of DFF25 polymeric TMP change over time during A.) LXF+GAC, B.) LXF, and C.) PAC modes. LXF CFV = 0.024 m/s, MXF CVF = 0.135 m/s.

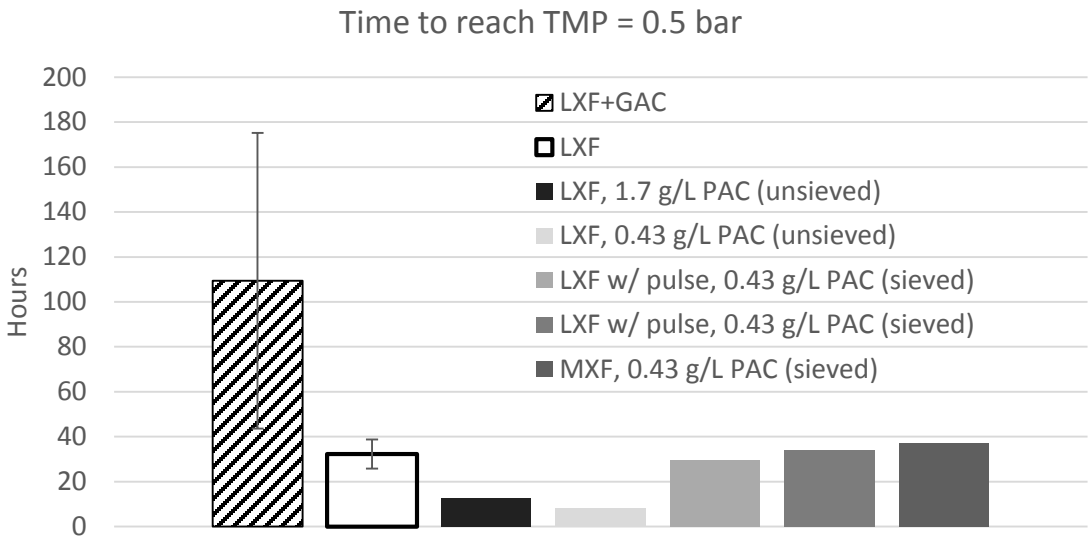

Figure A40 Comparison of DFF25 run-time during A.) LXF+GAC, B.) LXF, and C.) PAC modes. LXF CFV = 0.024 m/s, MXF CVF = 0.135 m/s.

References

Akram, A. & Stuckey, D.C., 2008. Flux and performance improvement in a submerged anaerobic membrane bioreactor (SAMBR) using powdered activated carbon (PAC). *Process Biochemistry*, 43, pp.93–102.

Hu, A.Y. & Stuckey, D.C., 2007. Activated carbon addition to a submerged anaerobic membrane bioreactor: effect on performance, transmembrane pressure, and flux. *Journal of Environmental Engineering*, 133(1), p.73.