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Psychrophilic anaerobic membrane bioreactor treatment of domestic wastewater

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ABSTRACT

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

1. Introduction

Because of the recent emphasis on sustainability in the water quality industry, various studies are exploring how domestic wastewater (DWW) treatment can be accomplished in an energy neutral or even energy positive fashion (Guest et al., 2009; McCarty et al., 2011). Current DWW treatment plants often recover energy in the form of methane-rich biogas produced during anaerobic digestion of primary sludge and biomass generated during conventional aerobic treatment. However, approximately 45% of the total biodegradable chemical oxygen demand (COD) in DWW is lost through oxidation to carbon dioxide (McCarty et al., 2011), and thus constitutes a lost resource. Furthermore, the energy

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requirements of aerobic treatment are typically much greater than the energy recoverable via anaerobic sludge digestion (Foley et al., 2010). To improve energy recovery, reduce costs, and minimize environmental impacts, mainstream anaerobic processes are being considered as replacements for conventional aerobic DWW treatment (van Haandel et al., 2006). In comparison to conventional aerobic treatment schemes with anaerobic sludge digestion, anaerobic mainstream DWW treatment has the potential to convert all biodegradable COD present in DWW to methane, generate substantially less residuals due to the much lower biomass yield of anaerobic microbes, and eliminate aeration requirements.

Although water conservation and source separation have the potential to change DWW characteristics and flow rates, DWW in the U.S. and in many other developed countries is still relatively low strength (average 5-day biochemical oxygen demand [BOD_5] varies from 110 to 350 mg/L in the U.S.) and is generated at high per capita flow rates (average production rate varies from 190 to 460 L/(capita d) in the U.S.) (Pons et al., 2004; Tchobanoglous et al., 2003). In addition, DWW temperatures are relatively low (average of 16 °C in the U.S.) and vary seasonally (Pons et al., 2004; Tchobanoglous et al., 2003). Given the common perception that anaerobic bioreactors must be heated to mesophilic (30–40 °C) or thermophilic (50–60 °C) temperatures to operate efficiently, it is not surprising that aerobic processes have been favored over anaerobic systems for the treatment of high volume and relatively cold DWW. Heating high volumes of DWW would not be economically feasible, especially since the potential energy recovery from low-strength DWW on a per volume basis is low (Lettinga et al., 2001; Martin et al., 2011). As a result, anaerobic treatment has not been used for mainstream DWW treatment except in regions with hot climates, which naturally benefit from elevated DWW temperatures (Aiyuk et al., 2006). In most temperate climates, efficient treatment at low temperatures would need to be demonstrated before widespread implementation of anaerobic DWW treatment could be considered.

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

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

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

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

2. Materials and methods

2.1. AnMBR configuration

The bench-scale AnMBR used in this study (Fig. 1) had a liquid volume of 5 L (total volume of 7 L) and contained two submerged membrane housings (manufactured by eMachineShop, Mahway, NJ). Each membrane housing incorporated two separate flat-sheet microfiltration polyethersulfone membranes (GE Osmonics, Greenville, SC) with a pore size of 0.2 μm and a total effective membrane area of 0.0387 m^2 (7.74 m^2/m^3). Because of the two separate membrane housings, two permeate streams, designated P1 and P2, were generated during operation. Intermittent mixing (1 min every 30 min) was provided by magnetic impeller (Applikon Biotechnology, Foster City, CA). Influent and permeate were pumped by peristaltic Masterflex L/S pumps (Cole-Parmer, Vernon Hills, IL). The bioreactor was equipped with a water

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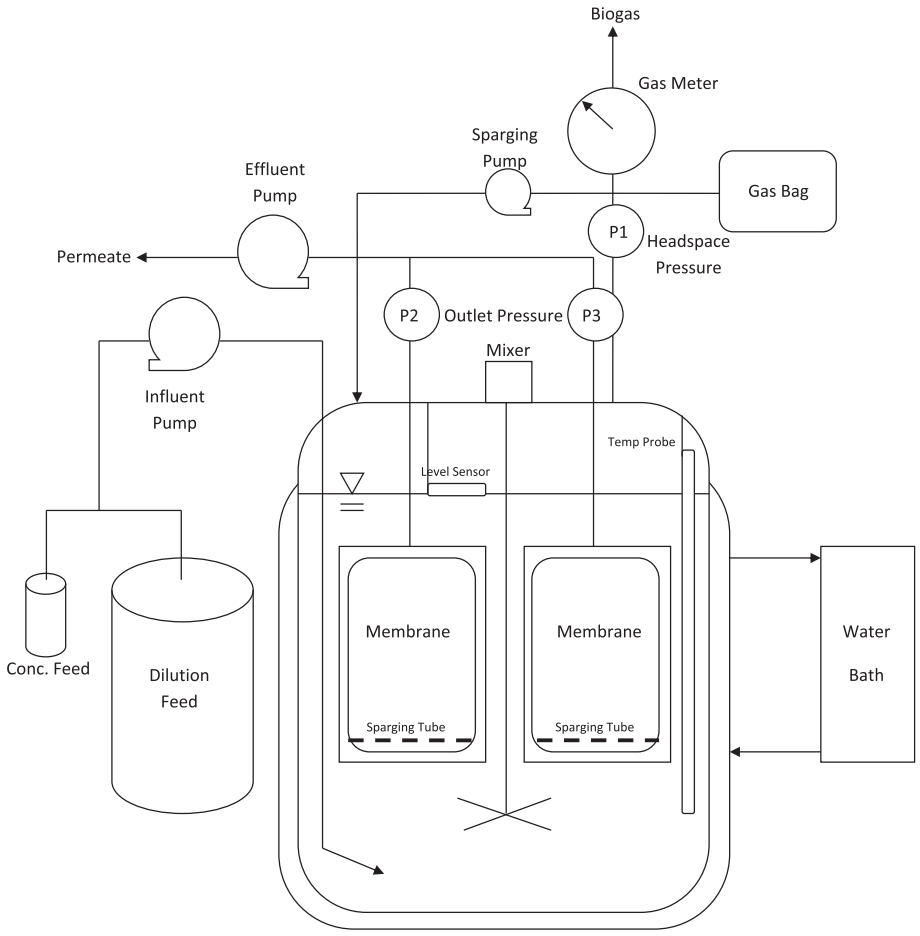

Fig. 1 – Schematic of bench-scale AnMBR.

jacket connected to a Polystat 6-L recirculating water bath (Cole-Parmer, Vernon Hills, IL) for temperature control. Pressures in the system were measured using pressure transducers (Omega Engineering, Stamford, CT) located in the headspace and on each permeate line. The bioreactor contained a level sensor and temperature probe (Applikon Biotechnology, Foster City, CA). The bioreactor headspace was connected to a biogas collection system with a 1-L Tedlar gas bag and mini diaphragm pump (KNF Neuberger, Trenton, NJ), which recirculated headspace biogas and dispersed it directly below each membrane through a horizontally placed sparging tube designed for fouling control. The bench-scale AnMBR was connected to a computer, which operated a control program (written in C++) and LabVIEW (National Instruments, Austin, TX) data acquisition software. The control program was responsible for operation of all pumps, biogas recirculation, and mixing. The LabVIEW application continuously monitored and recorded temperature, pressures, and feed flow rate.

2.2. *Inoculation and operational parameters*

The bench-scale AnMBR was inoculated with seed sludge from three sources: a mesophilic (35.5 °C) upflow anaerobic sludge blanket (UASB) reactor (Anheuser-Busch, St. Louis, MO), a mesophilic (32 °C) DWW treatment plant anaerobic sludge digester (Northfield Wastewater Treatment Plant, Whitmore Lake, MI), and a psychrophilic (0–23 °C; yearly temperature range was estimated based on a few data points) anaerobic lagoon used for the treatment of DWW (Maybee, MI). The system was inoculated with a total volatile suspended solids (VSS) concentration of 6000 mg/L, consisting of 2500 mg/L VSS of the UASB sludge, 2500 mg/L VSS of the anaerobic digester sludge, and 1000 mg/L VSS of the anaerobic lagoon sludge.

During the first operational period of 351 days, the bench-scale AnMBR was fed a synthetic wastewater that simulated DWW. The synthetic DWW was prepared as a concentrated solution adapted from the SYNTHES recipe presented by Aiyuk and Verstraete (2004) (Table S1). The original SYNTHES recipe had some divergences from reported medium strength U.S. DWW composition (Tchobanoglous et al., 2003), including elevated concentrations of phosphorus, nitrogen, and alkalinity. These concentrations were modified in the adapted recipe to formulate a DWW feed representative of medium strength U.S. DWW. The concentrated feed was prepared biweekly, acidified with hydrochloric acid to a pH of 3.5, and refrigerated at 4 °C to prevent biodegradation. After dilution with a basic buffer solution containing 3.57 mM sodium bicarbonate, 0.126 mM magnesium phosphate, 0.110 mM potassium phosphate, and 0.605 mM sodium hydroxide through in-line mixing, the synthetic feed had average measured total and soluble COD (SCOD) concentrations of 440 mg/L and 290 mg/L, respectively.

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The reactor temperature was maintained at 15.0 ± 0.1 °C throughout the study. The initial organic loading rate (OLR) during synthetic wastewater operation was 660 mg COD/(L d), which corresponded to a hydraulic retention time (HRT) of 16 h. The target membrane flux to achieve this HRT was 8 L/(m^2 h). At times, this target HRT was not reached due to reduced pump efficiency, resulting in a lower OLR. The OLR thus varied between approximately 440 and 660 mg COD/(L d) and the HRT varied between 16 and 24 h. Biomass was only removed from the AnMBR for sampling purposes, which resulted in an SRT of approximately 300 days.

Biogas sparging and permeate backflushing were employed to prevent membrane fouling. Biogas sparging was operated continuously at a flow rate of 4.67 L/min evenly distributed across the four membrane surfaces (specific gas demand of 7.24 m^3(m^2 h); superficial gas velocity of 13.9 m/h). Permeate backflushing was initialized by reversing the flow of the permeate pumps while keeping the flow rate constant (5.21 mL/min). During the first 185 days of operation, backflushing was performed for 30 s every 30 min. From days 186 through 351, backflushing was carried out for 4 min every 4 h to increase the duration of backflush events without decreasing permeate production, except as described below. In replicate experiments designed to study the contribution of backflushing to fouling prevention, membrane P1 was backflushed for 4 min every 4 h and membrane P2 was not backflushed. These experiments were carried out from days 231 through 269 and days 320 through 351.

During the second operational period, the bench-scale AnMBR was operated using actual DWW collected from the Dundee Wastewater Treatment Plant (Dundee, MI). A batch of primary influent was collected immediately after preliminary treatment (mechanical screen and grit removal) twice a week and stored at 4 °C. For consistency, wastewater was collected at approximately the same time on each collection day. Fresh membranes were installed at the start of the second operational period. Both membrane housings were backflushed for 4 min every 4 h. All other operational variables remained as described above except the OLR (170–393 mg COD/(L d)) which was lower relative to the first operational phase. During the first 50 days of this second operational period, unstable performance was observed and likely resulted from high and variable sulfate concentrations in the influent (160 ± 100 mg/L). These elevated and fluctuating sulfate concentrations were determined to be caused by the influent collection time coinciding with a once daily industrial facility wastewater discharge in close proximity to the Dundee Wastewater Treatment Plant. Unstable performance may have resulted from inhibitory compounds present in this industrial discharge. The influent collection time was changed and this resulted in lower and less variable influent sulfate concentrations for the next 40 days of AnMBR operation (65 ± 33 mg/L). Data are reported for these 40 days only.

2.3. Chemical assays and sampling

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

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

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

2.4. EPS extraction and quantification

Extracellular polymeric substances (EPS) were extracted by a cationic exchange resin method (Frolund et al., 1996) from biofilm samples removed from the AnMBR (with the membrane attached). Biofilm samples were removed from the AnMBR on days 276 and 320 of the first operational period and cut into 4 × 6 cm sections using a sterile scalpel. Additional biofilm sections were cut to determine volatile solids (VS) according to Standard Methods (2005). Biofilm samples for EPS extraction were immediately stored at −80 °C prior to extraction. Duplicate EPS extractions were performed for each membrane. EPS extraction was also performed on a fresh PES membrane section as a negative control. Proteins and carbohydrates in extracted EPS were quantified according to the Bradford assay (Bradford and Williams, 1976) and the Dubois method (Dubois et al., 1956), respectively.

2.5. Microbial analysis

Inocula biomass samples were individually stored at −80 °C upon AnMBR startup until further processing. Suspended and biofilm biomass samples were collected from the AnMBR 275

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days after startup and stored at −80 °C. DNA extractions were completed using a phenol chloroform extraction method (Urakawa et al., 2010). Additional DNA purification was done using the Wizard DNA Clean-Up System (Promega, Madison, WI) according to manufacturer's instructions. The V3, V4, and V5 variable regions of the 16S ribosomal RNA (rRNA) gene were targeted with bacterial pyrosequencing primers Bact-338F/Bact-909R and archaeal pyrosequencing primers Arch-340F/Arch-915 (Pinto and Raskin, 2012). A minimum of two uniquely barcoded primer pairs were used for amplification of each sample to provide replication in sequencing results. PCR products were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA). DNA was quantified using a spectrophotometer (NanoDrop, Wilmington, DE). After PCR purification and DNA quantification, bacterial and archaeal amplicons were separately pooled by equal mass (for each uniquely barcoded primer pair) and subsequently concentrated through PCR purification using the QIAquick PCR purification kit. Concentrated bacterial and archaeal amplicons were pooled at 40% bacterial amplicon mass and 60% archaeal amplicon mass. The resulting amplicon pool was concentrated through PCR purification using the QIAquick PCR purification kit and run on a 1% agarose gel. Gel extraction was performed using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) according to manufacturer's instruction. An additional PCR purification was done prior to submitting the amplicon pool to Engencore (University of South Carolina, Columbia, SC) for pyrosequencing of 1/8th pico-titer plate (Pinto and Raskin, 2012). The pooled amplicons generated approximately 20,000 reads and after quality screening 12,368 sequences remained (Table S2). The resulting sequences were classified using the Ribosomal Database Project (Maidak et al., 1997) and further analyzed with Mothur (Schloss et al., 2009) for operational taxonomic unit (OTU)-based clustering (average neighbor algorithm at 3% cutoff), principle co-ordinate analyses, and determination of weighted UniFrac distances (Liu et al., 2007).

3. Results and discussion

3.1. *Reactor performance*

To assess long-term treatment performance at a psychrophilic temperature of 15 °C, the bench-scale AnMBR was operated for 351 days treating simulated DWW. COD removal during this period averaged 92 ± 5% corresponding to an average permeate COD concentration of 36 ± 21 mg/L (Fig. 2). This level of COD removal was higher than the previously reported COD removal of approximately 85% for this temperature (Chu et al., 2005; Ho and Sung, 2010). The greater COD removal in the current study may have resulted from differences in membrane configuration (hollow fiber (Chu et al., 2005) and tubular (Ho and Sung, 2010) versus flat-sheet in the current study) and/or other differences. A study directly comparing the impact of different membrane configurations on COD removal in an AnMBR has yet to be done. Influent and permeate BOD_5 values were measured on day 269 of operation and averaged 227 and 18 mg/L, respectively (92% removal). The permeate COD concentration on this sampling day averaged 43 mg/L, slightly higher than the average permeate COD for the

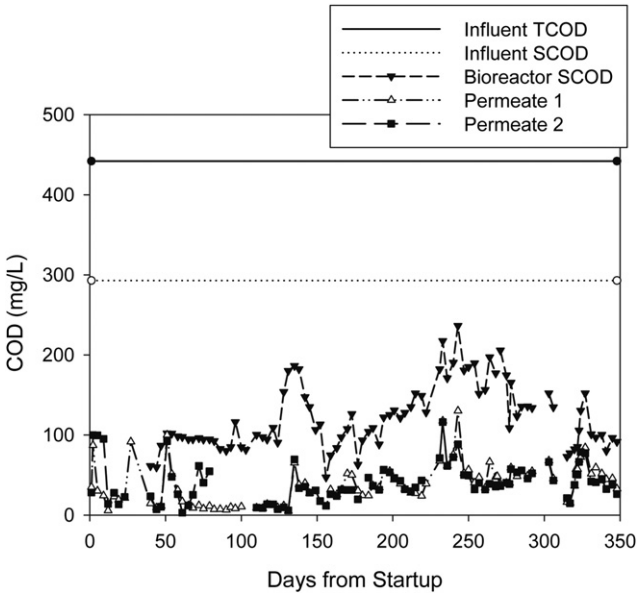

Fig. 2 – Average measured COD concentration in influent (total and soluble COD), bioreactor (soluble COD), and Permeate 1 (P1) and Permeate 2 (P2) (total COD).

351 days of operation. VFAs in the permeate were largely comprised of acetate (average concentration 18 ± 16 mg/L), with propionate present in lower concentrations (average concentration 4 ± 4 mg/L). Permeate concentrations of other VFAs, such as formate, isobutyrate, butyrate, isovalerate, and valerate, averaged ≤1 mg/L. The total VFA concentration in the permeate averaged 22 ± 20 mg/L as acetate whereas the total VFA concentration in the reactor averaged 28 ± 22 mg/L as acetate. Periodic spikes in permeate COD corresponded with spikes in permeate VFA concentrations, which typically occurred immediately after membrane replacement and unavoidable exposure of the system to oxygen. During the operational period of 351 days, bioreactor VSS gradually increased from 6000 mg/L to 10,600 mg/L, which corresponds to a yield <0.10 g VSS/g COD removed (the yield calculation takes into account biomass removal through sampling and membrane replacement).

Consistent differences in bioreactor and permeate soluble COD concentrations (Fig. 2) indicated substantial soluble COD removal across the membrane. This removal averaged 21 ± 8% of the total COD removal. It should be noted that differences in the physical removal capacity of the filters used in sample processing relative to the AnMBR membranes despite having the same pore size could have influenced this observation. However, other AnMBR studies have observed a similar phenomenon at a range of operational temperatures (Baek et al., 2010; Chu et al., 2005; Ho and Sung, 2009; Hu and Stuckey, 2006; Huang et al., 2011). Furthermore, Ho and Sung (2010) noticed an increase in membrane-mediated soluble COD removal with a decrease in operational temperature, but an explanation was not provided for why this temperature dependence may have occurred. The mechanism of soluble COD removal across the membrane may be related to microbial activity, size or charge exclusion, and/or adsorption. In

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two studies, specific methanogenic activity (SMA) experiments were performed with biofilm biomass, which suggested microbial activity in the membrane biofilm contributed to soluble COD removal across the membrane (Ho and Sung, 2010; Vyrides and Stuckey, 2011). The relative contribution of biological activity compared to other potential mechanisms has yet to be studied in detail. Regardless of mechanism, the removal of soluble COD across the biofilm is an important factor in achieving a high quality effluent during AnMBR treatment.

Approximately 40–50% of the total methane generated in the AnMBR was dissolved in the permeate and was thus discharged with the permeate rather than collected in the headspace (Fig. 3). The relatively high fraction of methane lost through the permeate is in part due to methane's increased solubility at psychrophilic temperatures. However, substantial methane oversaturation, approximately 1.5 times that predicted by Henry's law, was also responsible for this high methane loss through the permeate (Henry's law constant of 34,300 atm was used for the operational temperature; Tchobanoglous et al., 2003). Methane oversaturation has been observed in several non-membrane anaerobic bioreactor studies (Hartley and Lant, 2006; Pauss et al., 1990; Singh et al., 1996), which cited mass-transfer limitations as the likely cause. Conversely, Giménez et al. (2012) did not observe methane oversaturation when operating an AnMBR and contributed this observation to the use of biogas sparging creating equilibrium between the gas and liquid-phases. In the current study, the use of biogas sparging likely reduced mass-transfer limitations compared to conventional anaerobic bioreactors although methane oversaturation was still observed. It is possible that the pressure differential across the membrane plays a role in increasing permeate dissolved methane concentrations to the point of oversaturation. Further, methanogenic activity in the biofilm results in methane generation near the membrane surface and may contribute to permeate methane oversaturation, especially in combination with a pressure differential across the membrane. The oversaturated methane quantified in the permeate theoretically corresponds to 56% of the soluble COD removal that occurred across the membrane. It should be noted that the dissolved methane in the permeate was not included in the measured permeate COD, assuming our analyses results were consistent with those reported by Hartley and Lant (2006). Because methane has a global warming potential 25 times that of carbon dioxide (IPCC, 2007), management of permeate dissolved methane is necessary to limit greenhouse gas emissions (Smith et al., 2012). Furthermore, permeate dissolved methane represents a considerable fraction of the total energy available in DWW and its recovery may be necessary for energy neutral operation. The magnitude of potential direct greenhouse gas emissions from an AnMBR or other mainstream anaerobic treatment process is a direct result of the high volume of effluent containing dissolved methane generated and is only marginally increased by a lower operational temperature. Therefore, management of effluent dissolved methane is critical to limit greenhouse gas emissions from mainstream anaerobic processes regardless of temperature.

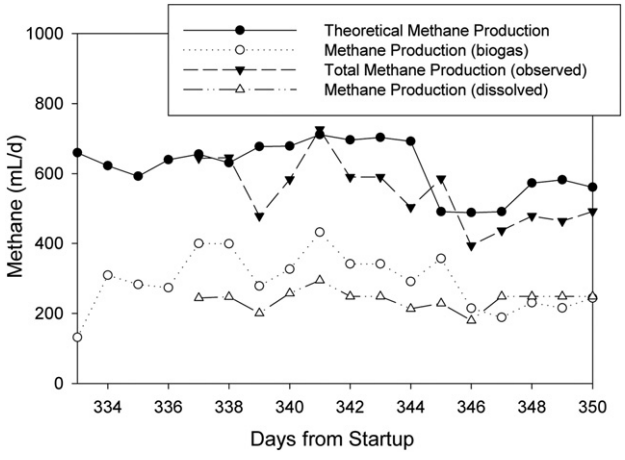

Fig. 3 – Methane production in the system (headspace, dissolved, and total observed) during 20 days of AnMBR operation compared to theoretical methane production. Theoretical methane production was calculated assuming 350 L of methane was generated per kg of COD removed (Grady et al., 2011) and by considering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

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

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

3.2. *Comparative membrane fouling experiment and biofilm EPS quantification*

To assess the role of biogas sparging and permeate backflushing in short- and long-term membrane fouling, comparative experiments were performed using the parallel

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membrane housings in the AnMBR. In a first type of experiment, permeate backflushing was practiced for only one membrane housing (P1), while biogas sparging was employed continuously on both membrane housings. Fresh membranes were installed at the beginning of the experiment. Over the course of the experiment (days 320–351), P1 did not show evidence of membrane fouling as the transmembrane pressure (TMP) and flux remained constant throughout the experiment (Fig. 4). However, P2 TMP increased to −45 kPa during the first 6 days of operation and then remained constant for the remainder of the experiment. P2 flux declined to approximately 3.5 L/m^{2}*h over the first 15 days of operation and did not change thereafter. During this fouling experiment, the difference in permeate COD concentrations between P1 and P2 averaged 10 ± 4 mg/L ($p < 0.05$). The more fouled membranes, P2, generated a higher quality permeate. This observation indicates that a correlation exists between membrane fouling and permeate quality. This experiment was reproduced (days 231–269) with a similar outcome. In a second type of experiment, biogas sparging was discontinued for both membrane housings, while permeate backflushing was continued to assess the role of biogas sparging in comparison to backflushing in membrane fouling control. Discontinuation of biogas sparging resulted in abrupt membrane fouling evidenced by a substantial increase in TMP (30–40 kPa) over the course of several hours. Taken together, these two types of experiments suggest that backflushing is necessary to avoid long-term membrane fouling, whereas biogas sparging is a prerequisite to having an operational AnMBR system. Furthermore, the combination of both fouling control measures enables better control of long-term fouling than when either is used individually.

Membranes with different levels of fouling were removed from the AnMBR and subjected to EPS extraction. Three of the four membranes were more fouled based on visual observation and higher TMP prior to membrane removal (−65 to −80 kPa), whereas one of the membranes was less fouled (TMP was −10 kPa). The membranes exhibiting greater fouling contained higher concentrations of EPS, measured as protein and carbohydrate mass per total organic mass (volatile solids) associated with the membrane, than less fouled membranes (Fig. 5). EPS may be a factor in the greater soluble COD removal observed with more fouling. However, fouled membranes also had considerably more attached biomass: 10 g VS/m^{2} for the less fouled membrane and an average of 135 ± 69 g VS/m^{2} for the fouled membranes, which corresponded to 8.7 ± 1.0% of the system's total VSS. Therefore, it is not possible to ascertain the relative contributions of EPS versus attached biomass on membrane fouling and soluble COD removal based on these data. Several AnMBR studies have considered EPS as a major contributor to membrane fouling (Chu et al., 2005; Gao et al., 2010) but its correlation with soluble COD removal in AnMBRs has not been assessed. EPS may increase adsorption of soluble organics or may correlate with increases in biofilm microbial activity as observed in aerobic filters (Gao et al., 2008). These potential mechanisms may be a factor in the greater soluble COD removal observed by membranes with more fouling.

3.3. *Microbial community analysis*

Analyzing the archaeal microbial communities in the biofilm and suspended biomass 275 days after AnMBR startup indicated *Methanosaeta* was the dominant genus in each sample representing 61.2 ± 5.1% and 66.7 ± 1.3% relative abundance

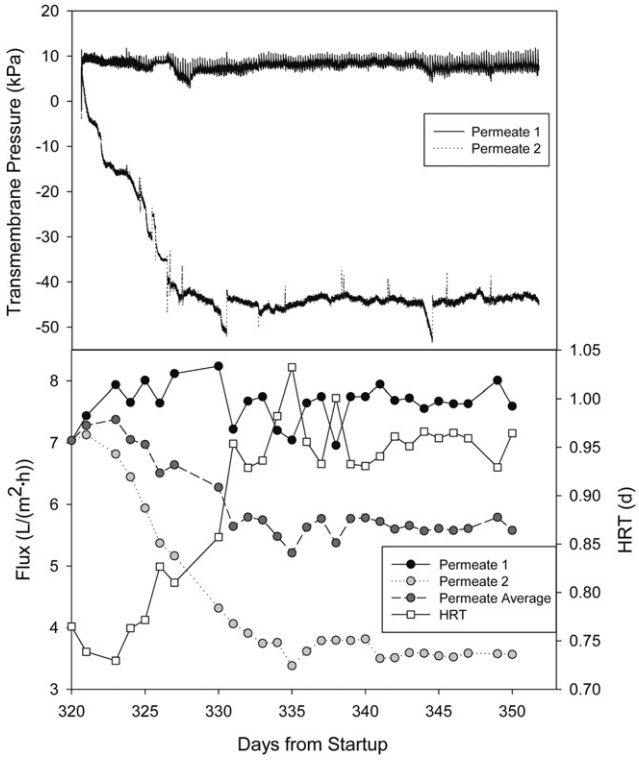

Fig. 4 – Transmembrane pressure for permeate 1 (P1) and Permeate 2 (P2) over time (top). Flux for Permeate 1 and Permeate 2 and HRT over time (bottom). During this operational period, days 320–351, Permeate 1 was backflushed for 4 min every 4 h operation while Permeate 2 was not backflushed.

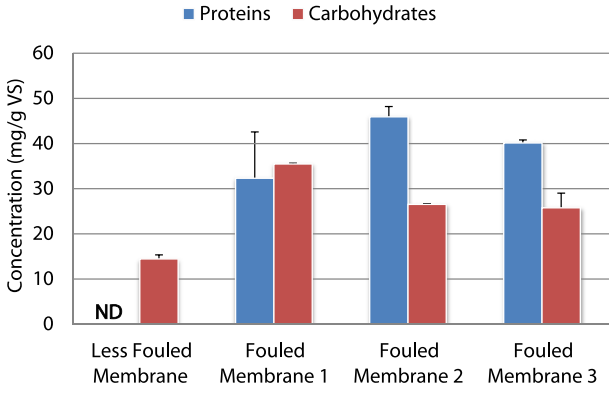

Fig. 5 – Concentration of proteins and carbohydrates in extracted EPS from membrane samples removed from the AnMBR. Less fouled membrane and fouled membrane 1 were removed from the AnMBR on day 276. Fouled membrane 2 and 3 were removed from the AnMBR on day 320. Error bars represent the standard deviation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

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(average and standard deviation obtained by using sequencing data generated using three different uniquely barcoded primer sets for each DNA extract), respectively, indicating that aceticlastic methanogens were abundant in the system (Fig. 6). Most hydrogenotrophic methanogens in the biofilm and suspended biomass belonged to the genera *Methanobacterium* and *Methanospirillum*. The relative abundance of these genera was considerably different in the biofilm and suspended biomass communities: *Methanobacterium* constituted 10.7 ± 2.2% and 21.5 ± 2.2% of the relative abundance in the biofilm and suspended biomass samples, respectively. In contrast, *Methanospirillum* represented 19.6 ± 3.0% and 8.2 ± 1.1% relative abundance in the biofilm and suspended biomass samples, respectively. Kinetic values observed by Schauer et al. (1982) and Karadagli and Rittmann (2005) suggest that *Methanospirillum* spp. have a higher substrate affinity than *Methanobacterium* spp., whereas their maximum specific growth rates are similar. Substrate concentrations are likely lower in the biofilm in comparison to suspended biomass creating conditions in the biofilm favorable to methanogens with higher substrate affinity. However, these kinetic parameters were not determined at psychrophilic temperatures and may not be appropriate to describe the present study. Alternatively, the propensity of *Methanospirillum* to grow in filaments (Beveridge et al., 1991) may have resulted in the observed higher relative abundance of *Methanospirillum* over *Methanobacterium* in the biofilm.

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

Bacteroidetes were the dominant bacterial phylum in the biofilm and suspended biomass samples (Fig. 7). This contrasts with the work by Gao et al. (2010) in which *Bacteroidetes* were observed in the suspended biomass but not in the fouling layer of an AnMBR operated for treatment of a synthetic domestic wastewater at a temperature of 30 °C. In addition to *Bacteroidetes*, *Proteobacteria* and *Firmicutes* showed a high relative abundance in both AnMBR biomass samples. Known syntrophic bacteria belonging to the genera *Smithella* and *Syntrophorhabdus* were found in both the suspended biomass and biofilm at relatively low abundances (<1%) indicating the presence of syntrophic interactions between hydrogenotrophic methanogens and syntrophs. The 'semi-syntrophic' class *Anaerolineae* of the bacterial phylum *Chloroflexi* (Narihiro et al., 2012) were also detected at 0.8% and 1.8% relative abundance in the biofilm and suspended biomass, respectively. Gao et al. (2010) speculated that candidate division OP11 specifically contributed to membrane fouling as this phylum was abundant (37–63% of bacteria) in the fouling layer in their study. Candidate division OP11 was detected in only the biofilm biomass in our study but at very low abundance (<0.1%) and therefore likely did not play a role in membrane fouling in the current study. Different operational parameters such as temperature may have caused this apparent inconsistency. A comparison between the biofilm and suspended biomass bacterial communities OTUs indicated a total of 193 and 145 OTUs in the biofilm and suspended biomass, respectively, with 84 OTUs shared.

Comparing the bacterial and archaeal communities in the AnMBR and the inocula indicated that the AnMBR communities showed the highest level of similarity with the mesophilic inocula (Fig. 8). The bacterial communities in the AnMBR biofilm and suspended biomass were most similar to each other, and showed a high degree of similarity to the mesophilic anaerobic digester inoculum. The AnMBR archaeal communities were most similar to the mesophilic UASB inoculum.

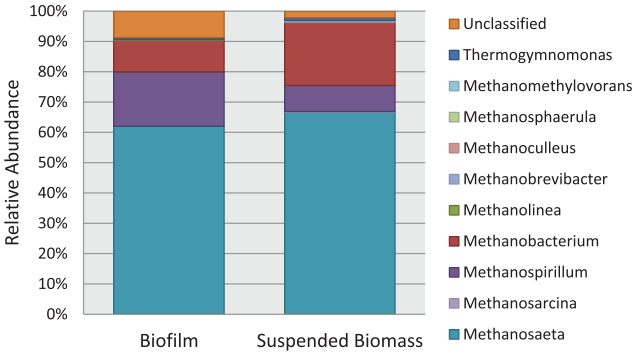

Fig. 6 – Classification at the genus level of the archaeal communities in the biofilm and suspended biomass samples taken 275 days after startup.

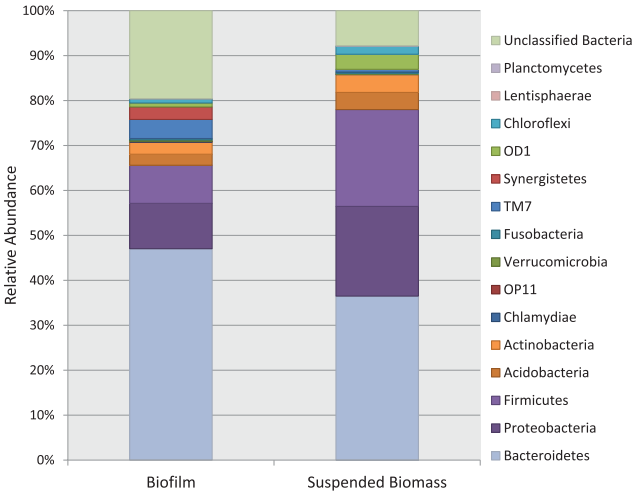

Fig. 7 – Classification at the phylum level of the bacterial communities in the biofilm and suspended biomass samples taken 275 days after startup.

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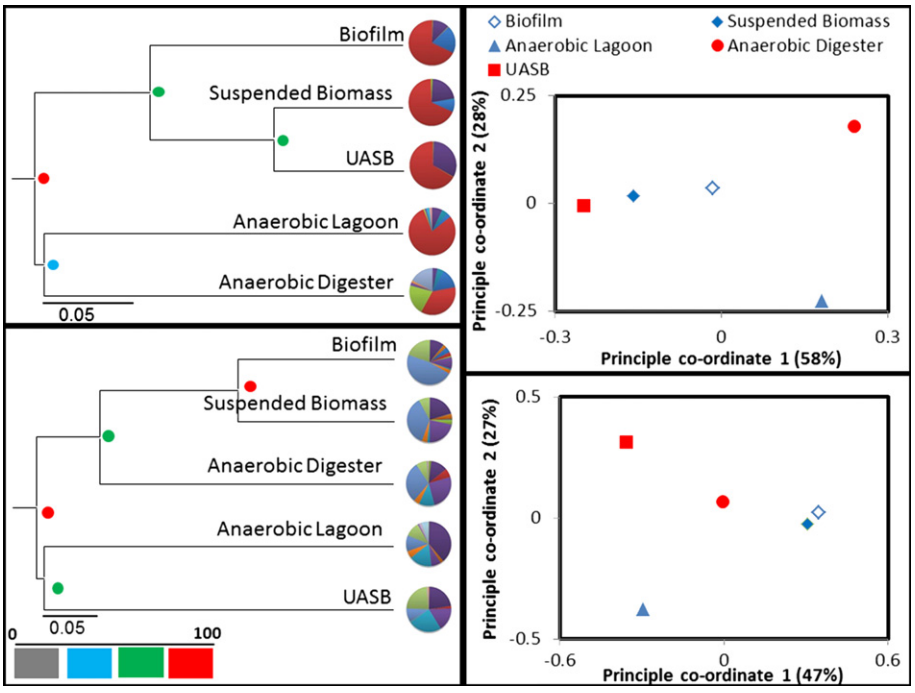

Fig. 8 – Comparison of the AnMBR communities in biofilm and suspended biomass samples (275 days after startup) with the three inocula using dendrograms of the weighted UniFrac distance metric (archaea top left; bacteria bottom left) and principle co-ordinate analyses (archaea top right; bacteria bottom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

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

4. Conclusions

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

- A high quality effluent was generated during AnMBR treatment at a psychrophilic temperature of 15 °C: 92 ± 5% COD removal and 36 ± 21 mg/L average permeate COD during simulated DWW operation; 24 ± 3 mg/L average permeate BOD_5 during actual DWW operation.
- Dissolved methane in the permeate represented a substantial portion of the total methane generated in the system (approximately 40–50% of total methane generated over time).

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- Membrane fouling was successfully managed using biogas sparging and permeate backflushing. Comparative fouling experiments suggested that the combination of the two fouling control measures was important.
- Pyrosequencing of the AnMBR and inocula microbial communities demonstrated that mesophilic inocula are suitable for psychrophilic AnMBR seeding.

Collectively, these conclusions indicate that AnMBRs are a viable candidate technology for innovation within DWW treatment with the ability to produce similar quality effluents as aerobic treatment, while concurrently recovering useful energy and producing considerably less residuals. However, full-scale implementation of the technology will require further research to overcome the existing operational concerns, such as membrane fouling, energy intensity of fouling control, and the presence of relatively high concentrations of dissolved methane in the permeate. Future research efforts should specifically focus on a better understanding of membrane fouling and how to control fouling with minimal energy input (i.e., reduced sparging rates, intermittent sparging, or alternative low-energy fouling control strategies) while maximizing flux. However, the biofilm's role in treatment appears important and could be negatively impacted by operation at higher fluxes. Finally, development of efficient dissolved methane recovery processes is necessary to maximize energy recovery and avoid direct greenhouse gas emissions. In conclusion, although AnMBRs appear promising for DWW treatment, significant advancements and large scale demonstration are necessary for AnMBRs to be considered over more established treatment technologies.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2012.12.028.

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