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Influent wastewater microbiota and temperature influence anaerobic membrane bioreactor microbial community

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HIGHLIGHTS

- Reactor configuration and temperature influence AnMBR microbial community.
- Continuous seeding with wastewater microbiota caused community shifts in AnMBRs.
- Hydrogenotrophic methanogenesis became dominant at 10 °C.
- AnMBRs seeded with identical biomass developed similar archaeal communities.

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ABSTRACT

Sustainable municipal wastewater recovery scenarios highlight benefits of anaerobic membrane bioreactors (AnMBRs). However, influences of continuous seeding by influent wastewater and temperature on attached-growth AnMBRs are not well understood. In this study, four bench-scale AnMBR operated at 10 and 25 °C were fed synthetic (SPE) and then real (PE) primary effluent municipal wastewater. Illumina sequencing revealed different bacterial communities in each AnMBR in response to temperature and bioreactor configuration, whereas differences were not observed in archaeal communities. Activity assays revealed hydrogenotrophic methanogenesis was the dominant methanogenic pathway at 10 °C. The significant relative abundance of *Methanosaeta* at 10 °C concomitant with low acetoclastic methanogenic activity may indicate possible *Methanosaeta-Geobacter* direct interspecies electron transfer. When AnMBR feed was changed to PE, continual seeding with wastewater microbiota caused AnMBR microbial communities to shift, becoming more similar to PE microbiota. Therefore, influent wastewater microbiota, temperature and reactor configuration influenced the AnMBR microbial community.

1. Introduction

Sustainable municipal wastewater recovery scenarios have highlighted anaerobic biotechnology 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 °C (Seib et al., 2016a; Smith et al., 2015). These results indicate that historical anaerobic biotechnology challenges including poor operation at low temperature with low strength wastewater, and high effluent organic concentration can be overcome (Lettinga et al., 2001).

While AnMBR technology shows great promise, remaining challenges require further investigation including high energy requirements for membrane operation (Seib et al., 2016a) 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).

In engineered microbial systems, community structure and diversity are considered important factors to achieve process stability (Briones and 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

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maintain system function in the event of environmental stress (i.e. pH change, substrate change, toxicity, etc.) (Briones and Raskin, 2003; Fernandez et al., 2000). 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 and Wilsey, 2001). Communities with higher richness and Shannon-Weaver index values are more diverse (Stirling and Wilsey, 2001). A high evenness score indicates unique community members are evenly distributed, which is beneficial for functional redundancy (Fernandez et al., 2000). Additionally, ordination techniques including non-metric multidimensional scaling (NMDS) and principal component analysis (PCA) have been useful to compare microbial community differences in separate systems (Bialek et al., 2011; Bocher et al., 2015).

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). 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 et al., 2006).

While the importance of microorganisms in biological systems is evident, 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 treating 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 and bioreactor configuration (Bialek et al., 2011; O'Reilly et al., 2009), bacterial communities are typically 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 (McKeown et al., 2009; O'Reilly et al., 2009; Siggins et al., 2011).

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, 2015). Both studies evaluated completely mixed submerged AnMBRs with gas sparging treating synthetic municipal 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 (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 suggests 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 (Seib et al., 2016b).

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 °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 at low temperatures.

2. Methods

2.1. AnMBR configurations

Two different AnMBR configurations utilizing different biofilm technologies and membrane types were used as previously described (Seib et al., 2016b). 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) (2.3 L working volume) combined with a ceramic membrane (1 L working volume). The FBR contained 0.6 mm × 1.7 mm (12 × 30 mesh) coconut-based 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).

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., 2016b). For the first 320 days, all AnMBRs were fed synthetic primary effluent wastewater (SPE) as previously described (Seib et al., 2016b). 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 (South Shore Water Reclamation Facility, Oak Creek, WI, USA) and stored at 4 °C before use (Table 1). After an initial startup period (day 1–79), total system hydraulic residence time (HRT) in all AnMBRs was 9 h from day 80 to 145. On day 146, HRT was adjusted to the minimum time necessary to achieve <10 mg/L BOD_5 in AnMBR permeate in each system. Membranes were operated with flux ranging from 5.9 to 7.4 L/m^2 h and chemically cleaned using NaClO and HNO_3 solutions when transmembrane pressure increased above 0.5 bar (Seib et al., 2016b).

2.3. Analytical procedures

Influent and permeate BOD_5, TCOD, NH_3-N, TKN, PO_4^{-3}, TP, TSS, and VSS concentrations were determined using standard methods (APHA et al., 1999). Bioreactor bulk liquid volatile fatty acid (VFA) concentrations were determined by gas chromatography with a flame ionization detector (FID) (Agilent 7890A, Santa Clara, CA, USA). Methane concentration in biogas was determined using gas chromatography with a thermal conductivity detector (TCD) (Agilent 7890A, Santa Clara, CA, USA).