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Membrane fouling in an anaerobic membrane bioreactor: Differences in relative abundance of bacterial species in the membrane foulant layer and in suspension

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ABSTRACT

A laboratory anaerobic membrane bioreactors (AnMBRs) (10 L volume) was operated at 30 °C and fed with artificial sewage containing 30% protein at COD loading rate 5.1 kg/m^3-d to investigate membrane fouling with two membranes. Biomass attached to the membrane surface and formed a foulant layer on the membrane. The foulant layers from polyvinylidene fluoride ultrafiltration membranes coated with PEBAX (cPVDF) and an uncoated polyetherimide (PEI) ultrafiltration membranes were analyzed and compared to suspended biomass in the reactor, using terminal restriction fragments (T-RFs) of the 16S rRNA gene and a clone library. One species of OP11 bacteria was present at high relative abundance in the foulant layers of both membranes. By contrast, *Bacteroidetes* and *Firmicutes* (LGC) species were present at low relative abundance in the foulant layers but high relative abundance in the suspended biomass. Similar differences were observed for other species. The results suggest that some minority species like OP11 play a direct role in fouling by attaching to the membrane surface while others, including some that likely play a major role in the metabolism of influent organics, play a less important or indirect role. In the AnMBR, the EPS was predominately proteinaceous. EPS and microbial cells of the foulant layer contributed to membrane fouling. The results also indicate that fouling of PEI was faster than cPVDF and this reaffirm the importance of the membrane material in fouling.

1. Introduction

Membrane bioreactors (MBRs) represent a promising technology for wastewater treatment because biomass is separated from the treated water by filtration through a membrane, eliminating the operational and ecological issues associated with gravity separation, and producing a particle-free effluent. Biomass concentrations within MBRs can be higher (up to 20 g/L) than the levels attained by gravity settling (up to 6 g/L), enabling higher volumetric removal rates and a smaller footprint. As with other dispersed growth systems, the biomass wasting rate can be adjusted to select for or against different microbial groups based on their specific growth rates and to adjust the ratio of organic matter oxidized to organic matter assimilated. Operation at a low biomass wasting rate – i.e., a long solids retention time – favors slow-growing microorganisms, such as methanogens [1] or nitrifying bacteria [2]. It also increases the fraction of organic matter oxidized, and decreases the fraction assimilated [3]. MBR treatment thus offers operational control and an effluent suitable for direct reuse or for further treatment by reverse osmosis [4]. While aerobic MBRs are now widely used in full-scale systems, anaerobic MBRs (AnMBRs) are still under development. Recent research on AnMBRs has focused on energy-savings, costs, and methane recovery [5]. To date, there have been few in-depth investigations of membrane fouling in AnMBRs, especially when treating low-strength wastewater such as domestic sewage.

Membrane fouling is considered as a major operational challenge for MBR process and has been investigated comprehensively for aerobic MBR. The fouling decreases permeate flux and membrane lifespan. Potential foulants include inorganic precipitates, soluble microbial products (SMP), extracellular polymeric substances (EPS), and cells [6–9]. SMP refers to soluble proteins,

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polysaccharides, and humic-like materials [10,11]. EPS refers to insoluble carbohydrates, proteins, lipids and nucleic acids within a highly hydrated gel matrix [12]. These materials decrease the permeate flux by decreasing the effective size of membrane pores and increasing frictional resistance to permeate flow. In the literature, the terms "EPS" and "SMP" are often loosely used, and at times used interchangeably. In this study, we use operational definitions: SMP are defined as the organic matter that passes through a 0.45 μm filter, and EPS as the centrifuged insoluble organic material that becomes soluble when heated at 80 °C for 30 min [13,14].

Several research groups have investigated the effects of EPS and SMP on membrane fouling [15–18]. It is commonly held that membrane fouling is due to both gel layer formation and pore blockage, and what biomass composition plays an important role [18], but the relative significance of protein and carbohydrate EPS and SMP is not clear. In some aerobic MBRs, protein was identified as the main foulant [19,20], while carbohydrate was the main foulant in others [21,22].

While the role of different microbial species in foulant layer formation has received little attention, it is reasonable to expect microorganisms to differ in their relative contributions to membrane fouling given their differences in the nature and quantity of EPS and SMP, susceptibility to adhesion at different membrane surfaces, and capacity to colonize and form biofilms, In anaerobic reactors, the microbial community is diverse, which consists of hydrolytic-fermentative bacteria, acetogens, acetate- and hydrogen-utilizing methanogens, and, sulfate-reducing bacteria when sulfate is present [23–25]. The relative abundance of these groups depends upon the composition of the wastewater and reactor design and operation. It has been reported that methanogens produce low levels of polysaccharide EPS, accounting for only about 2% of the total biomass [26]. Hydrolytic-fermentative bacteria, on the other hand, can produce high levels. In a mixed culture of *Selenomans ruminantium* and *Clostridium butyricum*, for example, polysaccharide EPS comprised up to 30% of the biomass [27].

The objective of this study was to determine how the relative abundance of microbial species in the foulant layer differs from that of the biomass suspension, and to identify related factors (EPS, SMP, and membrane material) that contribute to membrane biofouling. Accordingly, membrane flux and microbial community structure were monitored over a 2-month period. We also compared the community composition of the membrane foulant layer to that of the bioreactor suspension and visualized morphological changes of the microbial community.

2. Materials and methods

2.1. Anaerobic membrane bioreactor operation and sampling

The present study was performed with a laboratory-scale AnMBR equipped with pH control (pH 7.0) and an automated gas production monitoring system. The reactor was inoculated with mesophilic anaerobic digester sludge from the San Jose Water Pollution Control Plant (San Jose, California) and fed with a complex organic particulate artificial sewage containing ~500 mg COD/L from ground cat food (Nestle Purina PetCare Company, St. Louis, MO 63164) containing 30% protein and supplemented with ~30 mg NH_4^+-N/L. The AnMBR was acclimated to the synthetic sewage for more than 6 months prior to initiation of the present study. The temperature was maintained at 30 °C by recirculating warm water through tubing coils wrapped around the reactor. The AnMBR consisted of an upflow anaerobic reactor made of a glass column (10 L in volume, ID 10.6 cm × H 124 cm) coupled to two side-stream Rayflow® flat-sheet membrane modules (Rhodia Orelis, Cranbury, NJ) arranged in parallel (Fig. 1). Each module contained about

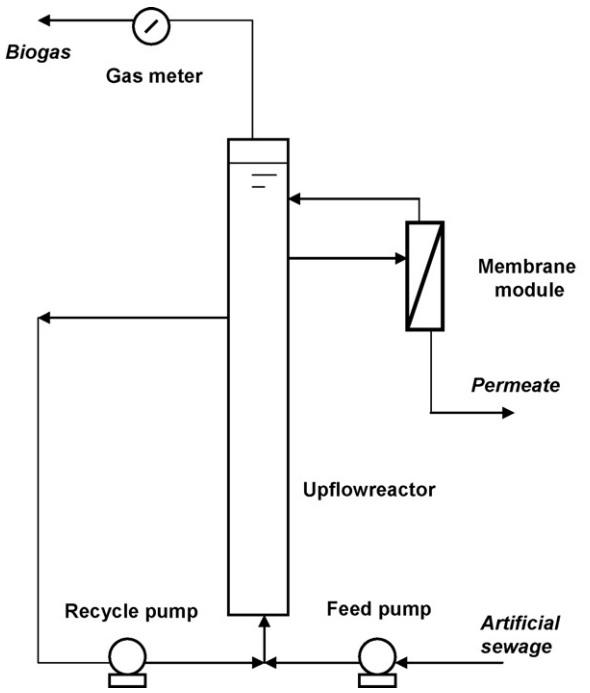

Fig. 1. Schematic diagram of AnMBR.

260 cm^2 of membrane area with a cross flow path channel height of 1.5 mm. The hydraulic retention time (HRT) and solids retention time (SRT) were 1 and 50 days, respectively. The long SRT was chosen to enable disintegration and hydrolysis of the complex particulate organic material in the synthetic sewage. During this study, the reactor system was operated for 82 days. On day 0, fresh polyvinylidene fluoride (PVDF) ultrafiltration membranes coated with 1% PEBAX 1657, a polyether block amide, hereafter referred to as cPVDF membranes (~100 kDa) were installed in both membrane modules. On day 62, the cPVDF membranes were removed for analysis and replaced by polyetherimide (PEI) ultrafiltration membranes (~30 kDa). Both membrane samples were provided by Membrane Technology & Research, Inc. (Menlo Park, CA).

The fouled membranes were coated with a yellow foulant layer covered by a loose dark brown cake. The dark cake was gently scraped and rinsed off with deionized water. The underlying yellow foulant was removed by 60 min of sonication (solid state/ultrasonication FS14, Fisher Scientific) in deionized water. The resulting rinse-water suspension was centrifuged at 12,000 × *g* for 15 min to obtain solids for characterization.

2.2. Samples for DNA extraction and PCR

Genomic DNA was extracted using FastDNA® SPIN kit for soil (BIO 101, Carlsbad, CA). Briefly, centrifuged solid samples were transferred into a 2-mL tube containing lysing matrix E. After addition of 978 μL of sodium phosphate buffer and 122 μL of MT buffer (from the kit), the tube was mixed by vortex action for 15 min at the maximum speed setting (Vortexer 2 and Scientific Industries Inc.). The sample was then processed according to the manufacturer's protocol.

The 16S rRNA gene was PCR-amplified from the genomic DNA using primer sets 8F-FAM and 1392R [28]. Each 50 μL PCR mixture contained 0.25 μM of each primer, 1X Fail-Safe PCR buffer F (Epicentre, Madison, WI), 1.25 units of AmpliTaq LD Taq polymerase (Applied Biosystems, Inc., Foster City, CA), and 30–100 ng of genomic DNA. Amplification was conducted on a Gene Amp 9700 Thermocycler (Applied Biosystems) following the standard