Grant title: Characterizing and Controlling Membrane Biofouling

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Problem and Research Objective. The application of membranes to separate particulate and suspended materials from water streams is an evolving technology. Membrane unit operations have many advantages as compared to conventional treatment technology that rely upon clarifiers and filtration to remove suspended materials. The primary disadvantages of membrane unit operations include capital costs for the membranes and operating costs associated with routine membrane cleaning. Biofouling is a serious problem for the operation of membrane unit operations because it results in decreased transmembrane fluxes. For this project, we hypothesize that preventing the initiation of biofilm formation on membrane surfaces is the best approach for eliminating biofouling. To scientifically test this hypothesis, we investigated the fundamental mechanisms of biofilm initiation on membrane surfaces.

The overall objective of this collaboration is to identify approaches to eliminate fouling of membrane surfaces due to the action of biological components. To accomplish this objective, our research team examined the initiation of biofilm formation on membrane surfaces through a synergistic study of the physicochemical properties of select membranes; the impact of various water streams on the physiochemical properties of select membranes; the biochemical interactions between microorganisms and select membranes; and the role of microbial ecology in the initiation of biofilm formation on membrane surfaces.

Four Tasks were undertaken:

TASK ONE existing laboratory-scale membrane unit operations were challenged with water (e.g., distribution system pipe loop) and wastewater (e.g., mixed liquor from activated sludge and/or membrane bioreactor systems) samples as well as mock environmental samples (e.g., clean water dosed with pure cultures of specific microorganisms or commercially available humic acids);

TASK TWO biofouling of membranes was quantified using an improved version of an existing conceptual and mathematical model relating transmembrane flux to fouling;

TASK THREE selective accumulation of microorganisms on membrane surfaces was documented using available genome-enabled molecular biotechnologies; and

TASK FOUR broad dissemination will be achieved through organizing and conducting a preconference workshop at State of Ohio Water Environment Association meeting with a focus on characterizing and preventing membrane biofouling.

Methodology.

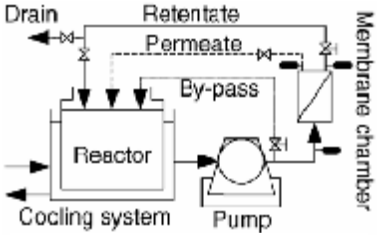

Fig 1. Schematic of lab-scale membrane system.

With prior USGS funding, we designed, constructed, and operated a laboratory-scale membrane reactor system shown in Figure 1. Flat sheets (72 square centimeters) of Osmonics PVDF microfiltration (0.3 micron nominal pore size) or ultrafiltration (30,000 Dalton molecular weight cut off) membrane were operated at 20^{o}C with a transmembrane pressure of 100 kPa and constant cross flow velocities of 0.3, 1, 2, 3.5, or 4.5 m/sec. During the period of operation, transmembrane flux was measured continuously. Reduced transmembrane flux observed over time was presumed to be linked to membrane fouling using a resistance in series model. To complete Task One, the existing membrane system were challenged with water samples collected from existing full-scale and laboratory-scale bioreactors (e.g., Mill Creek wastewater treatment plant located at 1600 Gest St, Cincinnati, Ohio) as well as samples of water collected from full-scale municipal drinking water production plants on the Ohio River (e.g., Miller Treatment plant in California, Ohio and the US EPA Test and Evaluation Facility located at 1600 Gest St, Cincinnati, Ohio). These water samples were placed in the reactor tank and maintained at a constant temperature. Each water sample was characterized according to Standard Methods for the Examination of Water and Wastewater (including determinations of Total Solids, Suspended Solids, Volatile Solids, Volatile Suspended Solids, Total Particle Counts, and Cell Abundance by staining). Vacuum filtration was performed with samples of representative membranes listed in Table 2. Optimal filtration times (defined as steady-state permeate flux) were determined experimentally and were approximately 5 hours (with a flow rate of 20 liters/square meter of membrane/hr).

The membranes tested in this study are presented in Table 1.

Table 1. Membranes proposed to be tested in Task One.

Company	**Materials**	**Interaction with H_2O**	**Pore size or MWCO**
Osmonics	Nitrocellulose	Hydrophilic	0.1 μm
	Cellulose acetate	Hydrophilic	0.22 μm, 20K
	Polycarbonate	Slightly hydrophobic	0.1, 0.2 μm
	Polypropylene	Hydrophobic	0.1, 0.22 μm
	Polysulfone	Hydrophobic	30K, 60K
	Polyamide	Hydrophobic	4K
	Polyethersulfone	Hydrophobic	5K, 20K
	Polyvinylidene fluoride	Hydrophobic	0.3 μm, 30K
Mitsubishi Rayon[1]	Polyethylene	Hydrophobic	0.1 μm

In addition to testing environmental samples, we used standard aseptic techniques to prepare pure cultures of representative microorganisms including *Escherichia* and *Acinetobacter*. Batches of pure cultures were prepared on standard media, and diluted to

a concentration of less than 1,000 mg volatile suspended solids per liter (e.g., wastewater) to one million colony forming units per one hundred milliliters (e.g., surface water) using phosphate buffered saline. Filtration tests were performed with these pure cultures using the same standard operating procedure followed for the environmental samples. To test the impacts of EPS on irreversible biofouling, we dosed synthetic EPS compounds into the membrane flow cell. Nucleic acid (reagent grade DNA), polysaccharide (reagent grade alginate), and protein (reagent grade casein) were examined.

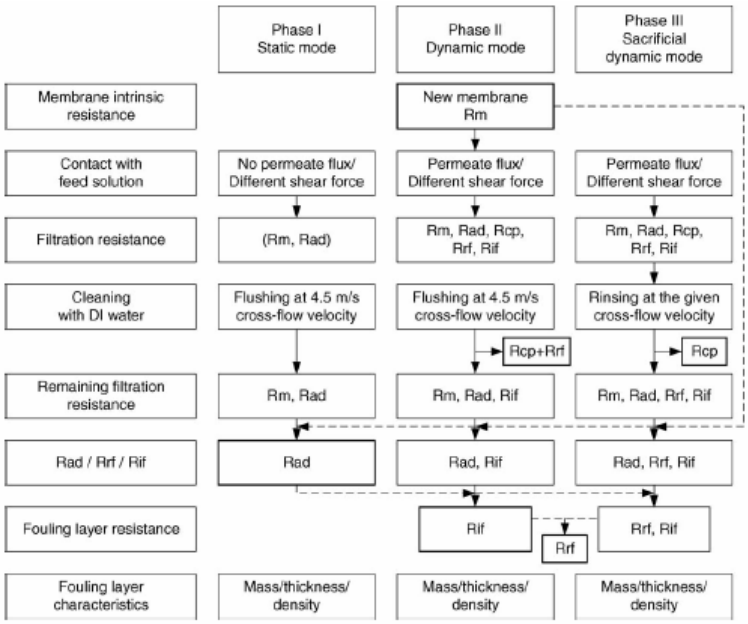

Fig. 2. Flow chart outlining the procedure for membrane characterization to determine intrinsic (R_m), reversible (R_{rf}), and irreversible (R_{if}) membrane fouling based upon adsorption (R_{ad}), concentration polarization (R_{cp}), resistance after flushing ($R_{t,f}$), and resistance after rinsing ($R_{t,r}$).

Membrane biofouling was characterized using a well-described resistance in series model coupled with the characterization protocol presented in Figure 2. Biofouling was defined as irreversible resistance (that cannot be removed by flushing with a cross flow velocity of 4.5 m/sec). Irreversible resistance was calculated as the resistance after flushing minus the intrinsic resistance minus the resistance due to adsorption.

In prior work, we used 16S ribosomal DNA targeted molecular biology tools to fingerprint microbial communities irreversibly bound to membrane surfaces, and we compared these fingerprints with the identity of the microbial populations present in suspension. As shown in Figure 3, for Osmonics PVDF microfiltration membrane, biofouling of the membrane surface was due to the irreversible adhesion of specific microbial populations (e.g., *Asticcacaulis* spp.) that were not present in abundance in suspended form (e.g., predominance of *Zoogloea* spp. in suspension). This result was unanticipated as prior engineering assumptions indicated that all microorganisms were equally likely to cause irreversible biofouling. For this project we used existing 16S rDNA-targeted techniques available in our laboratory including Amplified Ribosomal DNA Restriction Analysis (ARDRA) as well as cloning and sequencing.

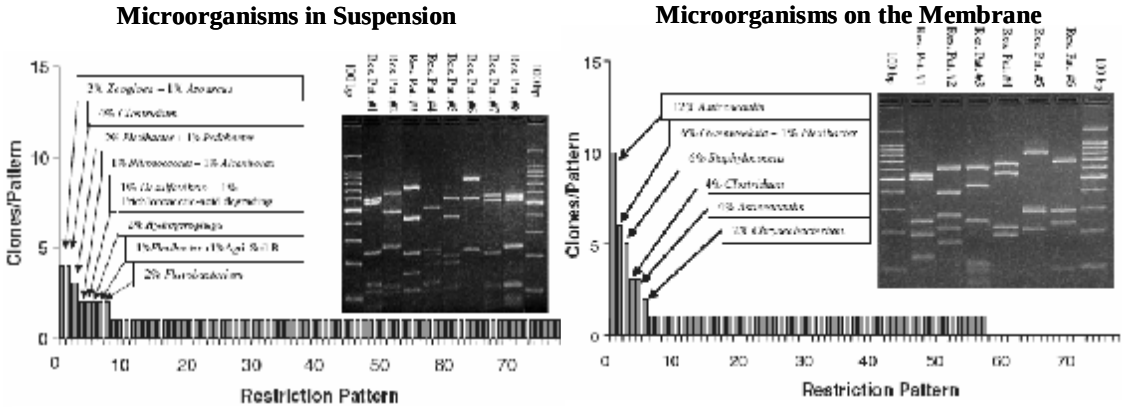

Fig. 3. Composition of the microbial communities in suspension and irreversibly attached to membrane surfaces during vacuum filtration with Osmonics PVDF microfiltration membrane.

Briefly, genomic DNA was extracted using commercial Soil DNA Extraction kits (MoBio, Inc.) following the manufacturer's instructions. Polymerase chain reaction (PCR) targeting 16S rDNA will be performed using primers S-D-Bact-0011-a-S-17 (5' GTTTGATCCTGGCTCAG) and S-D-Bact-1492-a-A-21 (5' ACGGYTACCTTGTTACGACTT) with Takara Ex Taq. PCR products were verified using 1% agarose gel electrophoresis. Cloning will be performed using the Topo TA Cloning Kit (Invitrogen, Inc.) according to the manufacturer's instructions. Plasmid inserts were amplified by PCR using primers M13 (-21) (5' TGTAAAACGACGGCCAGT) and M13 Reverse (5' CAGGAAACAGCTATGAC). Restriction digests for ARDRA were performed using the endonuclease MspI according to the manufacturer's instructions (Promga). ARDRA were performed using agarose gel electrophoresis and an available Kodak digital gel documentation system. For sequencing, individual clones were subcultured and plasmid DNA was extracted using QIAprep Spin Miniprep Kit (QiaGen) according to the manufacturer's instructions. Semi-automated DNA sequencing was performed on a fee-basis by the DNA Core Laboratory of the University of Cincinnati using primer S-D-Bact-0338-a-S-18 (5' ACTCCTACGGGAGGCAGC). Sequencing results were analyzed using BLAST as well as an available Linux workstation running ARB. These experiments were used to identify the predominant microbial populations present on the membrane surface as well as in suspension. By comparing the composition of these two microbial communities, we identified which membrane surfaces provide selective environments to accumulate specific microbial populations.

All experiments were performed using existing laboratory-facilities available at the University of Cincinnati.

Principal Findings and Significance.

In previous work, an experimental procedure and model were developed to estimate irreversible biofouling from laboratory-scale membrane component testing data. This model and experimental procedure was extended to examine biofouling of biomass from a variety of sources including: activated sludge biomass from full-scale municipal sewage treatment plants; natural biomass from surface waterways; biomass from laboratory-scale

membrane bioreactor sewage treatment plants; and pure cultures of select microorganisms. The biomass was further characterized by a number of experimental parameters including: composition of extracellular polymeric substances (EPS); floc size; and zeta potential. In addition, genome-enabled molecular biology-based techniques were used to identify and quantify predominant bacterial populations present in the mixed liquor suspended growth biomass as well as in adhered sessile biofilms.

The major findings included the following observations:

- The identity of members of microbial communities irreversibly bound to membrane surfaces can be very different from the identity of membranes of microbial communities found in suspended growth bioreactors.
- Members of the Alpha-subclass of the Proteobacteria including *Brevundimonas* and *Asticcacaulis* are more likely to irreversibly bind to membranes.
- Members of the Gamma-subclass of the Proteobacteria including *Acinetobacter* are more likely to irreversibly bind to membranes.
- Some members of the Low G+C content of the Gram Positive Bacteria including *Staphylococcus* spp. are more likely to irreversibly bind to membranes.
- Students and adult practitioners of environmental engineering can be introduced to the benefits of genome-enabled molecular biology-based techniques for identifying and quantifying microbial populations in environmental samples through one-day long workshops and seminars which include hands-on activities accompanying lecture-discussion formats for information dissemination.
- Bioreactor operation in a plug-flow mode resulted in a microbial community which was less likely to produce irreversible biofouling as compared to a microbial community developed in a bioreactor operated in a completely-mixed mode.
- *Acinetobacter calcoaceticus* and *Gordonia amarae* are more likely to result in irreversible biofouling as compared to a similar abundance of *Escherichia coli* suggesting that specific bacterial populations may be more prone to the initiation of irreversible biofouling.
- Mathematical modeling of the competition of six theoretical bacterial populations competing for three growth limiting nutrients in a steady-state biofilm of constant thickness with variable density showed that different biofilm conditions favored different amounts of diversity in the bacterial community.
- Bioreactor operating conditions which influenced the diversity of the bacterial community were different for three base cases including: fully penetrated; internal mass transfer resistance; and external mass transfer resistance.

In summary, the results of this project suggest that specific bacterial populations are responsible for the initiation of biofouling in membrane bioreactors. Furthermore, operating conditions selected by engineers in the design of membrane bioreactors could influence microbial community composition and its physical properties. Thus, the ability to reduce biofouling in membrane bioreactors can be influenced by the operating conditions selected by the engineers during the system design phase.

Future work should examine if engineering design decisions can influence biofouling in long term studies through selective control of microbial community composition. Furthermore, studies of molecular microbial ecology should be conducted to specifically elucidate the mechanism whereby this potential level of control can be used.