Identification and Control of Membrane Bioreactor Biofouling Organisms Using Genetic Fingerprinting

Problem and Research Objectives

Membrane bioreactors (MBRs) are relatively new wastewater-treatment technologies promising exceptional treatment efficiency with a reduced surface-area footprint compared to conventional-treatment-process trains (Gander et al. 2000). A MBR uses an activated-sludge process in which the conventional secondary clarifiers are replaced by a membrane-separation process (either microfiltration or ultrafiltration).

Like other membrane systems—but to an even greater degree—MBRs are susceptible to biofouling (Chang et al. 2002). Biofouling is not a well-understood process but its effects increase operating pressures, reduce maximum flux (water passed through the membrane), increase recovery-cleaning requirements, and possibly reduce total membrane life (Cicek et al. 2001, LeClech et al. 2003). All of these effects of biofouling have adverse effects on either initial capital cost or ongoing operation-and-maintenance costs for MBRs. Because MBRs are quickly becoming the process of choice for water recycling there is a need to improve their cost efficiency by controlling the biofouling.

The primary research objectives were to 1) identify the microbial species present in MBRs under different operating conditions and 2) correlate the microbial species make-up in MBRs with biofouling conditions and water-quality parameters.

Methodology

This study included long-term operation of two different bench-scale MBRs. One MBR used a flat-sheet membrane technology provided by Enviroquip, Inc., utilizing Kubota membranes with 0.4 μm pore size. A second MBR used hollow-fiber technology provided by Ionics Corp. utilizing Mitsubishi membranes also with 0.4 μm pore size.

These bench-scale MBRs were operated using raw sewage pretreated only by passage through a 3mm-fine screen. Operating parameters that were varied include flux rate (flow per unit area of membrane, in this case 10 and 15 gallons per square foot of membrane per day [GFD]), solids retention time (SRT, in this case 5, 10, 20, and 40 days), organic/nutrient loading (raw sewage with/without supplemental organics), and state of oxygenation (high, low, or anoxic).

Steady-state operation was achieved under each set of conditions prior to proceeding to the next set of conditions. Operating and water-quality parameters that were monitored included trans-membrane pressure (TMP, continuous on-line measurement), soluble microbial products/extracellular polymeric substances (SMP/EPS) carbohydrate and protein fractions (cation exchange resin extraction, carbohydrates, and proteins), viscosity, particle size distribution (PSD), and soluble chemical oxygen demand (COD).

Microbial consortium samples from both mixed liquor and attached biofilms (cake layers) were collected from the bench-scale MBRs under various conditions. Samples of microbial populations in full-scale conventional activated-sludge systems and pilot-scale MBR systems were collected for comparisons. Genomic DNA for the total community was extracted en mass (using a culture-independent method which also yields unculturable organisms). Polymerase chain reaction (PCR) was used to produce 16S rRNA V3 region gene-amplification products.

Denaturing gradient gel electrophoresis (DGGE) was used to separate the 16S rRNA V3 region gene-amplification products to characterize the microbial community and monitor the dominant population.

The total number of DGGE bands provides an estimate of the microbial diversity within a given environment (a "genetic fingerprint"). The dominant microbial species were determined by DNA sequencing of genetic material taken from the DGGE bands. The sequenced DGGE bands were compared with the GeneBANK database to identify the bacteria responsible for biofouling.

Principal Findings and Significance

Total membrane flux resistance is easily calculated given the operating flux, viscosity, and TMP. Figures 1 and 2 show the total flux resistance during the various phases of the bench study for the Enviroquip, Inc., and Ionics Corp. MBRs, respectively. The slope of the total-resistance line can be considered the fouling rate.

For the Enviroquip, Inc., bench-scale MBR several observations can be made. First, at 10 GFD the fouling rate was essentially zero during the period of observation (meaning that the resistance held constant and fouling was minimal). Second, at 15 GFD there appear to be several different fouling rates. The fouling rate starts out low (about 2.8E10 $m^{-1}d^{-1}$), then apparently increases rapidly (to about 2.3E11 $m^{-1}d^{-1}$), slows down for a period (to about 2.9E10 $m^{-1}d^{-1}$), and then again rapidly increases (to about 2.3E11 $m^{-1}d^{-1}$). This phenomenon will require further investigation prior to reaching any possible conclusions. Third, when supplemental glucose was added to increase the feed strength by 50% for 7 days (with flux held at 15 GFD) the fouling rate did not appear to increase appreciably (to about 3.0E10 $m^{-1}d^{-1}$). Fourth, when the system was modified to eliminate the anoxic zone (with flux held at 15 GFD) the initial fouling rate seemed to decrease (to 6.4E09 $m^{-1}d^{-1}$). It was desired to correlate these phenomena with increases in the number of certain organisms—thereby implicating them indirectly as "fouling organisms." However this proved to be highly challenging and was not successful.

For the Ionics Corp. bench-scale MBR the fouling rate at 15 GFD (about 4.2E10 $m^{-1}d^{-1}$) was about three times as rapid as that at 10 GFD (about 1.4E10 $m^{-1}d^{-1}$). These fouling rates are all at SRT = 20 days.

During the relatively rapid increase in total resistance observed for the Enviroquip, Inc., bench-scale MBR at 15 GFD, the protein EPS and SMP in the mixed liquor were fairly steady but the permeate SMP showed an interesting trend (Figure 3). Figure 3 shows that the permeate SMP was fairly steady until a certain point (30–35 days into the 49-day run) when the value dropped off suddenly (meaning all SMP was retained). This is apparently an indication of severe fouling which could possibly be associated with specific bacteria species; however no such correlations were achieved. No trends in the protein fraction of EPS or mixed-liquor SMP that could be useful for predicting fouling were apparent in this data set.

Figures 4, 5, and 6 show the type of data obtained in the biofouling-genetics study. Each vertical lane represents a different sample of community DNA, each horizontal band

represents a different bacteria, and brighter bands indicate larger numbers of biomass (dominant species).

In Figure 4 it can be observed that there are differences in the dominant species of bacteria in the Huber MBR as compared to the Ionics Corp. MBR and that speciation changes over time in the MBRs.

Figure 5 shows the bench-scale Enviroquip, Inc., MBR under non-fouling conditions when the SRT was 20 days, the flux was 10 GFD, and no anoxic zone was included. In this case it can be observed that one dominant bacterium seems to wash out (upper band) and other bacteria either fluctuate, appear, or disappear. The biofilm sample (lane #6) taken at the end of this phase of experiments may be the most interesting since we can see that the dominant bacteria species are almost completely different from those in the mixed liquor, that there are fewer species present, and that three bacteria are highly concentrated (dominant).

Figure 6 shows the bench-scale Enviroquip, Inc., MBR under high-fouling conditions when the SRT was 20 days and the flux was 15 GFD. In this case there appears to be about three types of bacteria that are more dominant at the end when fouling was severe that were either less prevalent or were not present at the beginning. These bacteria, therefore, may be associated with fouling.

These bands were cut out for sequencing to identify the bacteria species. The data obtained from the sequencing was queried with GeneBANK to identify the bacteria. The results are shown in Figures 7 and 8.

Future work is necessary to further study these and other identified fouling bacteria species by reviewing their physiological/morphological characteristics to see if they can be biologically or chemically controlled to reduce their biofouling potential.

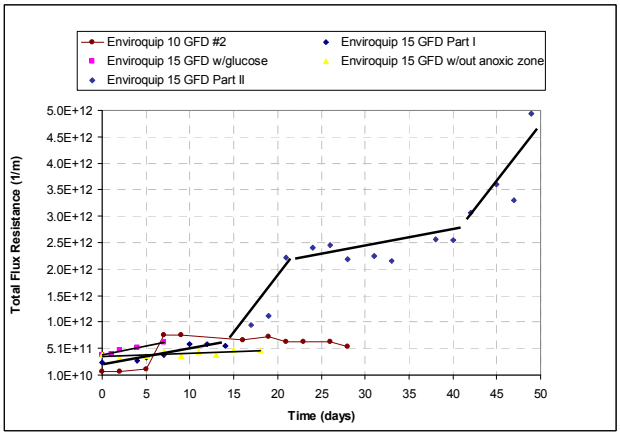

Figure 1. Fouling rates during operation of bench-scale Enviroquip, Inc., MBR

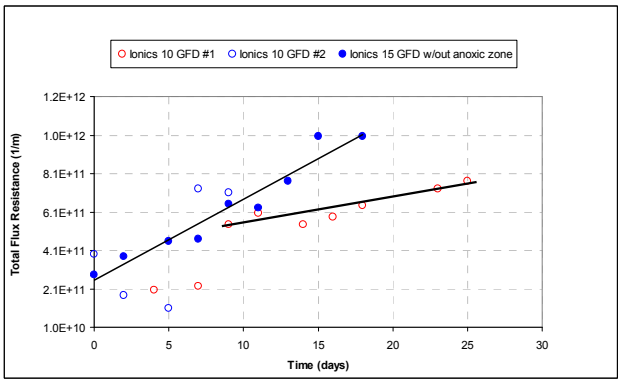

Figure 2. Fouling rates during operation of bench-scale Ionics Corp. MBR

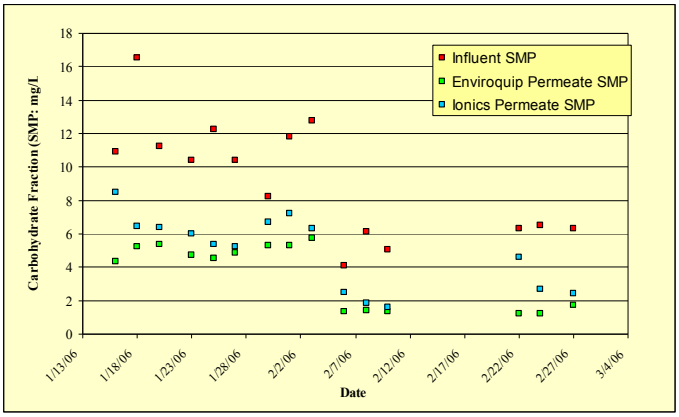

Figure 3. Comparison of permeate carbohydrate SMP from bench-scale MBRs

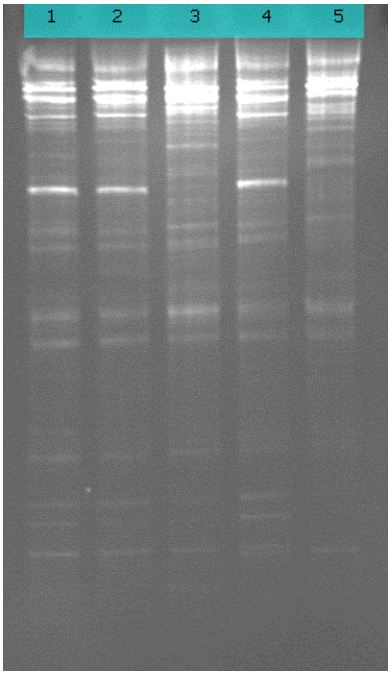

Figure 4. DGGE analyses of pilot MBR mixed-liquor samples. Lane 1: Huber 6/29/07, lane 2: Huber 7/6/07, lane 3: Ionics 6/29/07, lane 4: Huber 7/20/07, lane 5: Ionics 7/20/07

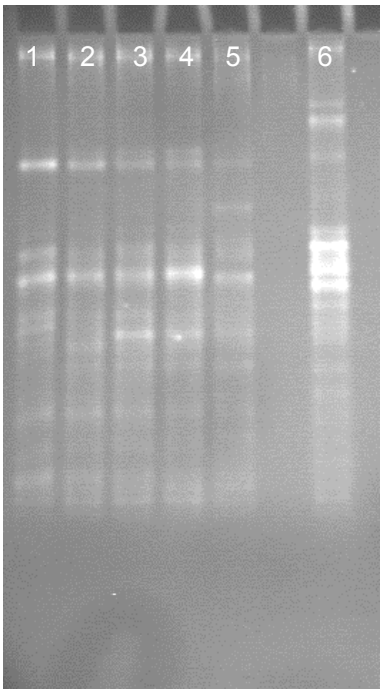

Figure 5. DGGE analyses of bench-scale Enviroquip, Inc., MBR mixed-liquor samples under non-fouling conditions. Lane 1: 5/24/07, lane 2: 5/31/07, lane 3: 6/7/07, lane 4: 6/14/07, lane 5: 6/21/07, lane 6: biofilm 6/28/07

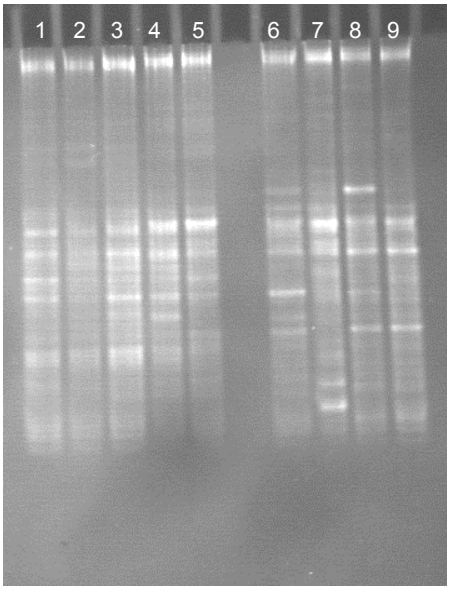

Figure 6. DGGE analyses of bench-scale Enviroquip, Inc., MBR mixed-liquor samples under high-fouling conditions. Lane 1: 3/8/07, lane 2: 3/15/07, lane 3: 3/22/07, lane 4: 3/29/07, lane 5: 4/5/07, lane 6: 4/19/07, lane 7: 4/26/07, lane 8: 5/3/07, lane 9: 5/10/07

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 Strand=Plus/Plus

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            in situ hybridization-microautoradiography
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1450)
  AUTHORS   Ginige,M.P., Keller,J. and Blackall,L.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-MAY-2003) Advanced Wastewater Management Centre,
            The University of Queensland, Ritchie Building (64A), Research
            Road, St Lucia, Brisbane, QLD 4072, Australia
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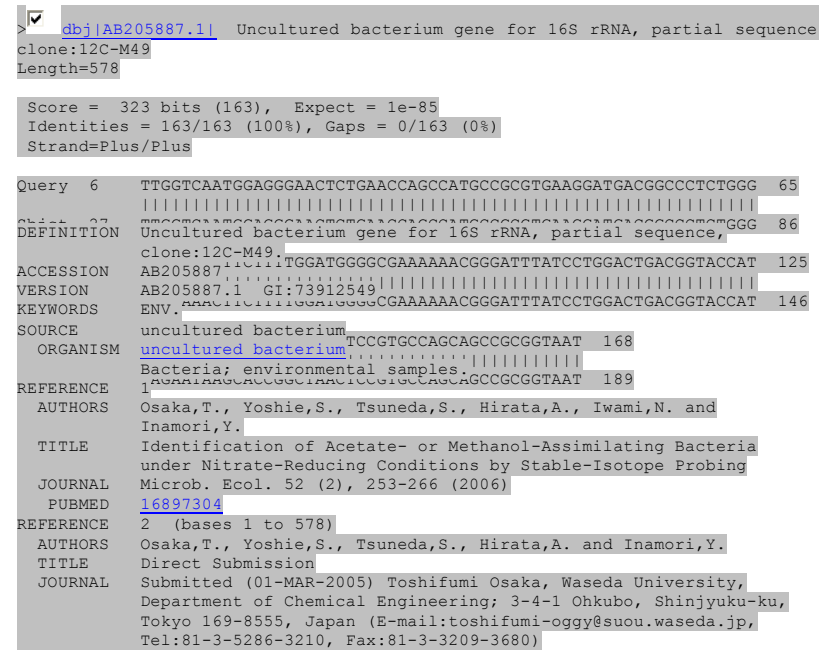
Figure 7. Denitrifying biofouling organism identified



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  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1
  AUTHORS   Osaka,T., Yoshie,S., Tsuneda,S., Hirata,A., Iwami,N. and
            Inamori,Y.
  TITLE     Identification of Acetate- or Methanol-Assimilating Bacteria
            under Nitrate-Reducing Conditions by Stable-Isotope Probing
  JOURNAL   Microb. Ecol. 52 (2), 253-266 (2006)
   PUBMED   16897304
REFERENCE   2  (bases 1 to 578)
  AUTHORS   Osaka,T., Yoshie,S., Tsuneda,S., Hirata,A. and Inamori,Y.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-MAR-2005) Toshifumi Osaka, Waseda University,
            Department of Chemical Engineering; 3-4-1 Ohkubo, Shinjyuku-ku,
            Tokyo 169-8555, Japan (E-mail:toshifumi-oggy@suou.waseda.jp,
            Tel:81-3-5286-3210, Fax:81-3-3209-3680)
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Figure 8. Nitrate-reducing biofouling organism identified

Publications Cited in the Synopsis

Chang, I.S., P. LeClech, B. Jefferson, and S. Judd. 2002. Membrane fouling in membrane bioreactors for wastewater treatment. *J. Environ. Eng*. 128:1018.

Cicek, N., J. Macomber, J. Davel, M.T. Suidan, J. Audic, and P. Genestet. 2001. Effect of solids retention time on the performance and biological characteristics of a membrane bioreactor. *Water Sci. Technol*. 43:43.

Gander, M., B. Jefferson, and S. Judd. 2000. Aerobic MBRs for domestic wastewater treatment: a review with cost considerations. *Sep. Purif. Technol*. 18:119.

LeClech, P., B. Jefferson, and S.J. Judd. 2003. Impact of aeration, solids concentration and membrane characteristics on the hydraulic performance of a membrane bioreactor. *J. Membr. Sci*. 218:117.