Novel Application of Oxygen-Transferring Membranes to Improve Anaerobic Wastewater Treatment

Anthony S. Kappell, Michael J. Semmens, Paige J. Novak, Timothy M. LaPara

Department of Civil Engineering, University of Minnesota, 122 Civil Engineering, 500 Pillsbury Drive SE, Minneapolis, MN 55455; telephone: +612-624-6028; fax: +612-626-7750; e-mail: lapar001@umn.edu

Received 1 April 2004; accepted 4 June 2004

Published online 10 January 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/bit.20219

Abstract: Anaerobic biological wastewater treatment has numerous advantages over conventional aerobic processes; anaerobic biotechnologies, however, still have a reputation for low-quality effluents and operational instabilities. In this study, anaerobic bioreactors were augmented with an oxygen-transferring membrane to improve treatment performance. Two anaerobic bioreactors were fed a synthetic high-strength wastewater (chemical oxygen demand, or COD, of 11,000 mg l^{-1}) and concurrently operated until biomass concentrations and effluent quality stabilized. Membrane aeration was then initiated in one of these bioreactors, leading to substantially improved COD removal efficiency (> 95%) compared to the unaerated control bioreactor (~ 65%). The membrane-augmented anaerobic bioreactor required substantially less base addition to maintain circumneutral pH and exhibited 75% lower volatile fatty acid concentrations compared to the unaerated control bioreactor. The membrane-aerated bioreactor, however, failed to improve nitrogenous removal efficiency and produced 80% less biogas than the control bioreactor. A third membrane-augmented anaerobic bioreactor was operated to investigate the impact of start-up procedure on nitrogenous pollutant removal. In this bioreactor, excellent COD (> 90%) and nitrogenous (> 95%) pollutant removal efficiencies were observed at an intermediate COD concentration (5,500 mg l^{-1}). Once the organic content of the influent wastewater was increased to full strength (COD = 11,000 mg l^{-1}), however, nitrogenous pollutant removal stopped. This research demonstrates that partial aeration of anaerobic bioreactors using oxygen-transferring membranes is a novel approach to improve treatment performance. Additional research, however, is needed to optimize membrane surface area versus the organic loading rate to achieve the desired effluent quality.

Keywords: biofilms; hybrid bioreactor; membrane aeration; nutrient removal

INTRODUCTION

Over the last 3 decades, anaerobic biological treatment of high-strength industrial wastewaters has become an established pollution control technology with increasing acceptance worldwide (Lettinga, 1995). Despite the numerous advantages of anaerobic biological treatment, however, these systems still have a reputation for lower-quality effluents and operational instabilities. Several researchers have reported excellent organic pollutant removal efficiencies (>85%) using different anaerobic process technologies (Nachaiyasit and Stuckey, 1995, 1997; Singh et al., 1996; Behling et al., 1997; Barber and Stuckey, 1999), whereas others have reported removal efficiencies much lower than those typical of aerobic systems (<65%) (Lettinga et al., 1983; Barbosa and Sant'Anna, 1989; Boopathy and Tilche, 1992; Fox and Venkatasubbiah, 1996). The removal of nitrogenous pollutants in anaerobic bioreactors also poses a particularly difficult challenge. One solution is to follow anaerobic treatment with an aerobic bioreactor, but this strategy is simultaneously too cumbersome and too costly for widespread application.

Membrane-aerated biofilm reactors (MABRs) are a relatively new technology for removing organic and nitrogenous pollutants from wastewater (Yamagiwa et al., 1998; Hibiya et al., 2003; Semmens et al., 2003; Terada et al., 2003). MABRs utilize a microporous or silicone membrane to transfer oxygen to a biofilm that actively grows on the membrane surface. These membrane-aerated biofilms are unique in character compared to standard biofilms grown on inert substrata. Nutrient concentrations (both organic and nitrogenous) are highest at the biofilm-liquid boundary and decrease with depth into the biofilm. In contrast, oxygen penetrates the biofilm from the membrane, often generating an aerobic zone near the membrane and an anoxic zone at the biofilm-liquid boundary (Casey et al., 1999). Recent research has demonstrated that nitrifying bacteria and aerobic heterotrophic bacteria can colonize the aerobic portion of the biofilm (Schramm et al., 2000; Hibiya et al., 2003; Terada et al., 2003; Cole et al., 2004), while denitrifying bacteria and other anaerobes colonize the anaerobic portion (Cole et al., 2004). MABRs are advantageous, therefore, because they can simultaneously perform both nitrification and denitrification within a single bioreactor.

Correspondence to: Timothy M. LaPara

Contract grant sponsors: the National Science Foundation; the National Oceanic and Atmospheric Administration (NOAA)

Contract grant numbers: BES 0123394; USCOC/NA16RG1046

In this study, we examined the impact of oxygen-transferring membranes on the performance of laboratory-scale anaerobic bioreactors treating a synthetic high-strength wastewater. Because oxygen only penetrates a short distance from the membrane surface (typically < 500 μm) (Cole et al., 2004), our hypothesis was that a thin aerobic layer could exist in the biofilm near the membrane that would be suitable for nitrification and aerobic heterotrophic oxidation; the majority of the bioreactor, however, would remain sufficiently reduced such that fermentative and methanogenic activity would continue. Anaerobic bioreactors augmented with a membrane transferring oxygen, therefore, would be a novel single-stage system capable of some level of nitrogenous pollutant removal while retaining much of the cost-effectiveness of anaerobic wastewater treatment bioreactors.

MATERIALS AND METHODS

Bioreactors

Bioreactors were constructed of polycarbonate and had a liquid volume of 5 liters plus a headspace volume of 2 liters. Bioreactors were mixed using a magnetic stir plate and stir bar. Sterile feed medium was added for 1 hr each day while the reactor liquid was simultaneously removed; this procedure maintained a mean hydraulic retention time of 30 days. Reactor pH was maintained using pH controllers that fed 1.0 M sodium bicarbonate. Biogas escaped from the bioreactors through a plastic ball valve and was collected in an inverted graduated cylinder and quantified using the water displacement method. Bioreactors were operated at 22 ± 2°C.

Flat-sheet dual-sided membranes (3M, St. Paul, MN) were synthesized using a thermally induced phase separation process (Mrozinski, 1988). Membranes had a thickness of 74 μm, a bubble point pore size of 0.21 μm, and a total surface area of 0.1 m^2. About 90% of the membrane surface was covered with a polypropylene nonwoven material to enhance bacterial attachment and prevent biofilm sloughing. Membranes were supplied with air at a flow rate of 200 ml min^{-1} at atmospheric pressure. The control bioreactor contained a membrane module that was filled with water and capped to prevent gas transfer.

The feed medium was designed to represent a high-strength wastewater and contained the following (per liter of deionized water): 7 g glucose, 3 g tryptone, 2.3 g ammonium chloride, 680 mg potassium phosphate, 710 mg sodium phosphate, 5 mg ferrous chloride, and 1 mL SL7 trace mineral solution (Biebl and Pfennig, 1981). The medium had an average chemical oxygen demand (COD) of approximately 11,000 mg l^{-1}, an ammonium-nitrogen concentration of 600 mg l^{-1}, and an organic nitrogen content of 1,000 mg l^{-1} (as nitrogen). A synthetic ammonia wastewater was also used to grow actively nitrifying biofilms on the membranes used in two of the reactors; this synthetic ammonia wastewater was similar to feed medium described above except that glucose and tryptone were excluded.

Bioreactor Operation

Two different experimental protocols were used in this study to assess the impact of partial membrane-aeration on anaerobic bioreactors. The first approach was to insert an oxygen-transferring membrane into a preexisting anaerobic bioreactor. Two anaerobic bioreactors were operated for almost 100 days to establish a pseudo-steady state with respect to COD removal and to biomass density. During this period, nitrifying biofilms were grown on the membranes by incubating them in organic-free ammonia wastewater. Membranes coated with nitrifying bacteria were then installed in both bioreactors. After an additional time period for the bioreactors to restabilize, membrane aeration was initiated in only one of the bioreactors. Following another 50 days of operation, ammonium chloride was removed from the feed media to evaluate whether excess ammonia was inhibiting nitrification.

The second operational approach was to install the membrane initially in the bioreactor and to aerate an organic-free ammonia wastewater to establish a nitrifying biofilm on the membrane. The bioreactor was inoculated with anaerobic biomass from the bioreactors described above and the organic content of the wastewater was then increased in two steps to COD concentrations of 5,500 and 11,000 mg l^{-1}. The goal of this experiment was to discern the impact of start-up procedure and COD loading on treatment performance.

Analytical Methods

All samples were collected from the bioreactors prior to the daily addition of sterile wastewater. Biomass in the bulk fluid was measured as optical density at 600 nm with a 1 cm path length using a Beckman DU 530 UV/Vis spectrophotometer (Beckman Coulter, Fullerton, CA). Liquid samples were filtered through a 0.2 μm pore size syringe filter (Ion Chrom Acrodisc; Gelman Sciences, Baton Rouge, LA) for COD analysis or a 0.45 μm syringe filter (Gelman Sciences) for the analysis of volatile fatty acids, nitrite, and nitrate. COD was quantified according to a miniaturized closed-reflux colorimetric method (LaPara et al., 2000). Ammonia was measured using an ammonia-specific electrode (Hach, Loveland, CO). Total kjeldahl nitrogen was quantified using a Digesdahl Digestion Apparatus (Hach) and the Nessler reaction (Daniels et al., 1994). Nitrite and nitrate were measured using a Metrohm 761 Compact Ion Chromatograph with a Metrohm 766 IC sample processor (Metrohm, Herisau, Switzerland). Oxidation-reduction potential was measured using a platinum redox combination electrode using Zobell solution for electrode calibration. Oxidation-reduction potentials were consistently quantified as < −250 mV and dissolved oxygen was continuously below detection (data not shown).

Individual concentrations of the volatile fatty acids (VFAs) were measured by gas chromatography (HP 5890; Hewlett-Packard, Palo Alto, CA) using a flame ionization detector. Samples were first acidified using 0.6 M oxalic acid and then injected onto a 6 ft × 4 mm I.D. glass column packed with 80/120 Carbopak B-DA/4% Carbowax 20M (Supelco 11889; Sigma-Aldrich, St. Louis, MO). Ultrahigh-purity (UHP)/zero-grade nitrogen was the carrier gas at a flow rate of 16 ml min^{-1}. Methane concentrations in the reactor headspace were measured by gas chromatography using a thermal conductivity detector. Samples were collected from the reactor headspace using a gas-tight syringe (Valco Instrument, Houston, TX) and injected onto an HP molecular sieve column (13× packed). UHP/zero-grade helium was the carrier gas at a flow rate of 20 ml min^{-1}.

RESULTS

Membrane Augmentation of Established Anaerobic Bioreactors

Two anaerobic bioreactors required approximately 70 days to reach a pseudo-steady state with respect to biomass concentration (Fig. 1A) and to soluble COD removal efficiency (60–70%; Fig. 1B). After membranes were installed (day 98) and membrane aeration was initiated (day 119), biomass levels in the experimental reactor soon stabilized at a concentration that was 1.4-fold higher than the control reactor. During this same time period, the effluent soluble COD concentration of the experimental reactor rapidly declined to approximately 500 mg l^{-1}, while the control reactor exhibited removal efficiencies that were similar to that prior to the installation of the membrane. When ammonium chloride was removed from the feed medium on day 178, the quantity of biomass in the experimental bioreactor rapidly declined while the biomass concentrations in the control reactor were not substantially affected. Biomass concentrations were similar in both reactors by day 210. The effluent soluble COD concentrations simultaneously increased to more than 1,500 and 5,500 mg l^{-1} in the experimental and control bioreactors, respectively.

The effluent ammonium-nitrogen concentrations from both bioreactors were similar prior to the initiation of membrane aeration on day 119 (Fig. 2). Following the installation of the membranes, the ammonium-nitrogen concentration in the experimental reactor was consistently 1.2-fold higher than the control reactor. After the removal of ammonium chloride from the feed media on day 178, substantial decreases in the effluent ammonium-nitrogen concentrations were observed. Neither nitrite nor nitrate was detected in the effluent of either reactor throughout the experiment (data not shown).

The pH of both reactors was controlled by adding sodium bicarbonate (1.0 M) to maintain a pH of at least 6.7.

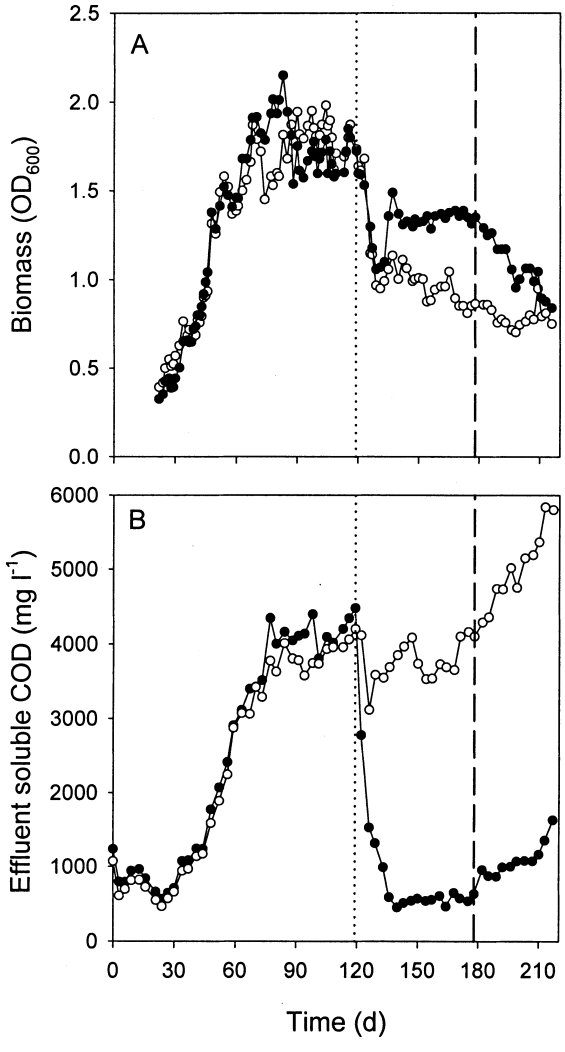

Figure 1. Biomass concentrations in the bulk fluid measured as optical density (**A**) and the effluent soluble COD concentrations (**B**) of a membrane-augmented anaerobic bioreactor (●) and a control anaerobic bioreactor (○) measured as optical density. The dotted and dashed lines indicate the dates when membrane aeration was initiated (day 119) and ammonium chloride was removed from the feed media (day 178), respectively.

The amount of sodium bicarbonate added daily to each reactor was similar prior to membrane aeration (Fig. 3). Following the initiation of membrane aeration, however, the experimental reactor required no sodium bicarbonate addition to maintain a pH near 7.5 (data not shown), whereas similar amounts of base were required for the control reactor prior to the installation of the membranes.

Effluent VFA concentrations were measured in both reactors following the initiation of membrane aeration (Fig. 4). The total effluent VFA concentrations in the control reactor were more than threefold higher than in the experimental reactor (Fig. 4A). There were also substantial differences between the concentrations of specific VFAs in the experimental and control reactors (Fig. 4B and C). Acetic acid and propionic acid concentrations were at least 2- and 10-fold higher, respectively, in the control reactor as compared to the experimental reactor. Following the

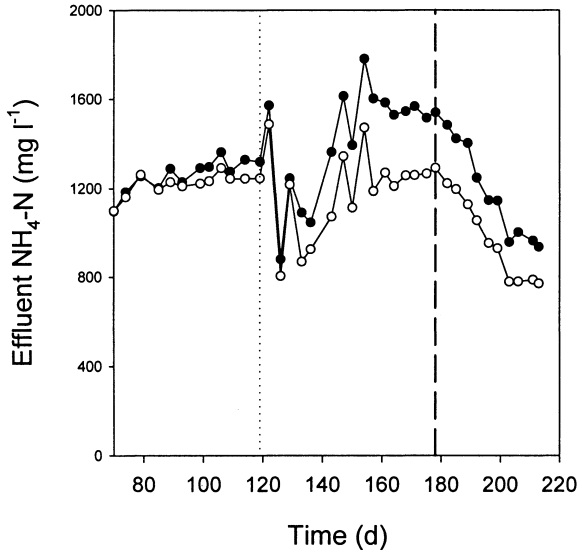

Figure 2. The effluent ammonium-nitrogen concentrations of a membrane-augmented anaerobic bioreactor (●) and a control anaerobic bioreactor (○). The dotted and dashed lines indicate the dates when membrane aeration was initiated (day 119) and ammonium chloride was removed from the feed media (day 178), respectively.

removal of ammonium chloride from the feed media, the butyric acid concentration rapidly increased in the control bioreactor (Fig. 4C); a less substantial increase in butyric acid concentration was also observed in the experimental bioreactor (Fig. 4B).

The volume of biogas produced each day from the reactors was highly variable. Both bioreactors exhibited similar behavior until the membranes were installed on day 98 (Fig. 5A). Following the perturbation caused by the installation of the membranes, the daily biogas production

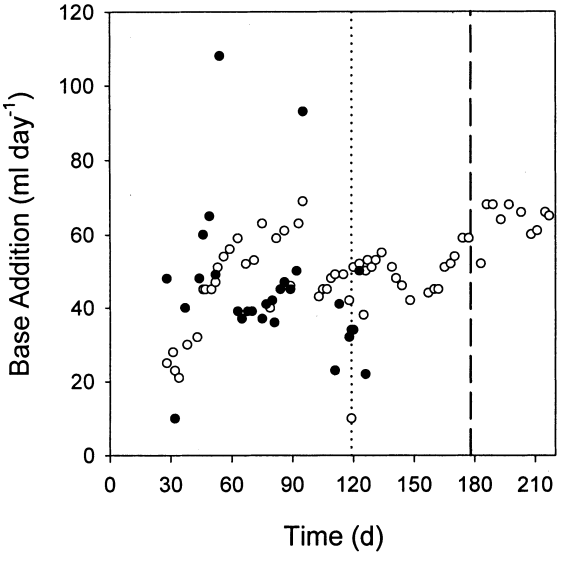

Figure 3. The volume of 1 M sodium bicarbonate added to either a membrane-augmented anaerobic bioreactor (●) or a control anaerobic bioreactor (○).

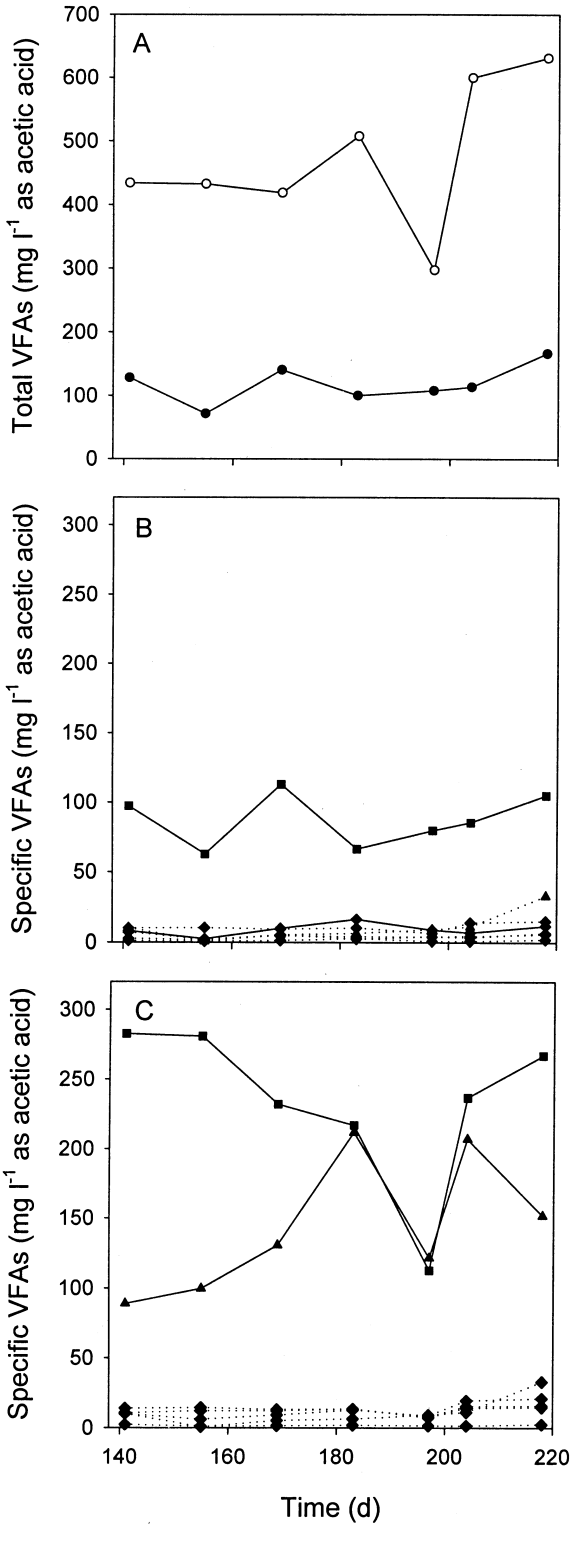

Figure 4. **A:** The total concentration of volatile fatty acids in the effluents from a membrane-augmented anaerobic bioreactor (●) and a control anaerobic bioreactor (○) following the initiation of membrane aeration. **B:** The concentrations of individual fatty acids in the effluent of a membrane-augmented anaerobic bioreactor. **C:** The concentrations of individual fatty acids in the effluent of a control anaerobic bioreactor. ■, acetic acid; ▲, butyric acid; ♦, all other organic acids.

rates in the control reactor recovered to preperturbation levels by day 126. In contrast, the amount of biogas produced by the experimental reactor remained substantially lower following the initiation of membrane aeration (day 119). The methane content of the biogas produced by both bioreactors initially increased and remained high (60–80%) prior to membrane installation (Fig. 5B). Following the perturbation caused by membrane installation, the methane content of the control reactor biogas was 50–60%; after the removal of ammonium chloride from the feed media, the methane content declined to approximately 40%. In contrast, the methane content of the experimental reactor was approximately 15% following the initiation of aeration. A substantial increase in the methane content of the biogas from the experimental reactor began on day 150. After the ammonium chloride was removed from the feed media on day 178, the volume of biogas produced by both reactors was similar but the methane content of the biogas from the experimental bioreactor was about 1.5-fold higher than that in the control bioreactor biogas.

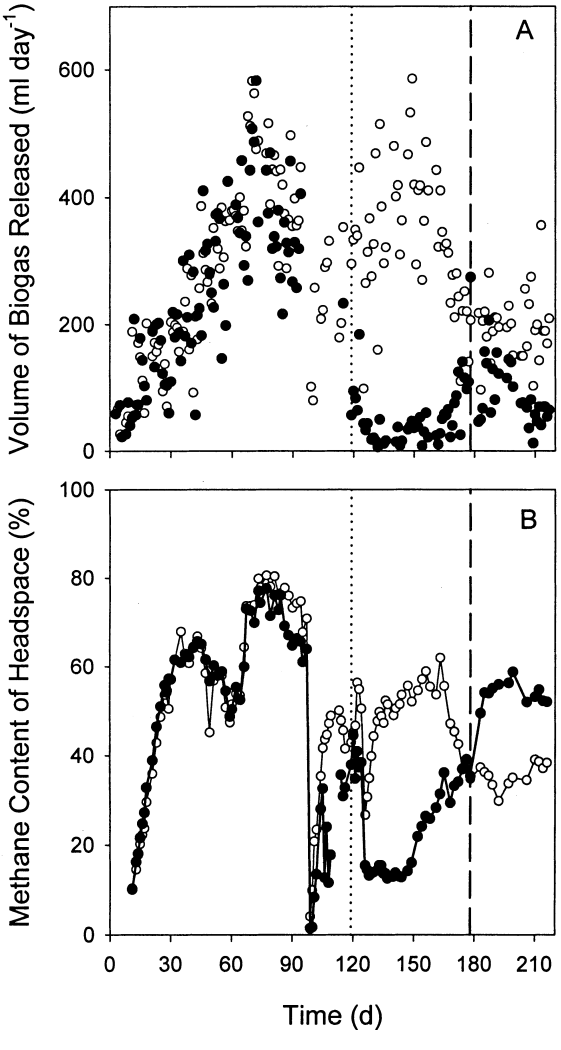

Figure 5. **A:** The quantity of biogas released from a membrane-augmented anaerobic bioreactor (●) and a control anaerobic bioreactor (○). **B:** The methane content of the headspace of a membrane-augmented anaerobic bioreactor (●) and of a control anaerobic bioreactor (○).

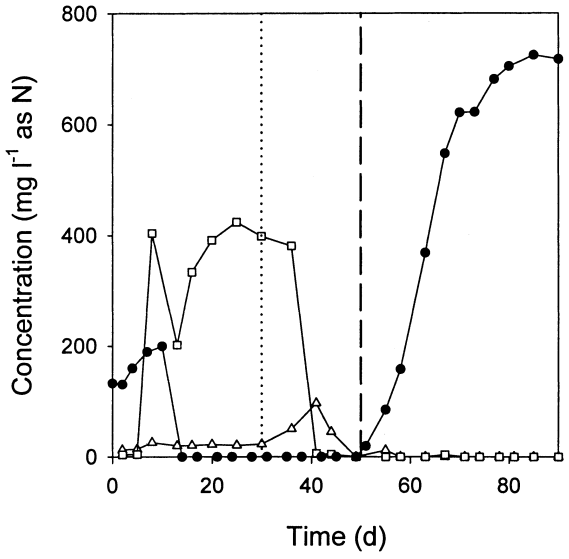

Figure 6. The concentrations of nitrogenous species in a membrane-augmented anaerobic bioreactor. The dotted and dashed lines indicate the dates when the organic content of the feed medium was increased to 0.5 × strength (COD = 5,500 mg l^{-1}) and 1.0 × strength (COD = 11,000 mg l^{-1}), respectively. ●, ammonium-nitrogen; □, nitrite-nitrogen; △, nitrate-nitrogen.

Start-Up of a Membrane-Augmented Anaerobic Bioreactor

Based on the results from these two bioreactors, a different start-up procedure was used for the operation of the third

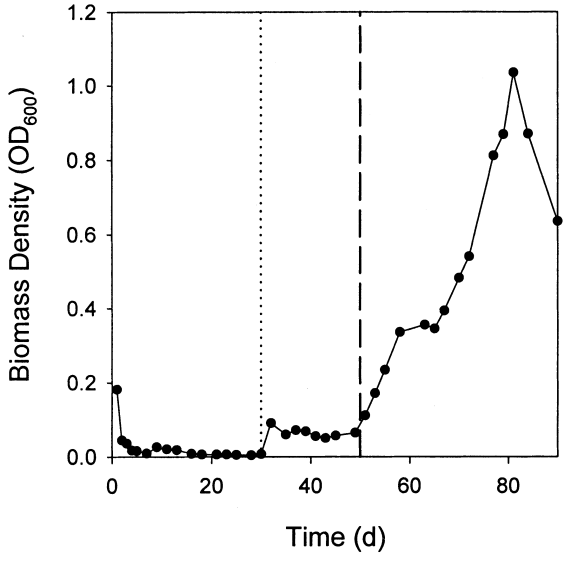

Figure 7. Biomass concentrations in the bulk fluid of a membrane-augmented anaerobic bioreactor measured as optical density. The dotted and dashed lines indicate the dates when the organic content of the feed medium was increased to 0.5 × strength (COD = 5,500 mg l^{-1}) and 1.0 × strength (COD = 11,000 mg l^{-1}), respectively.

bioreactor. In this third bioreactor, ammonium-nitrogen concentrations in the effluent initially increased but then declined to below detection within 15 days (Fig. 6). The effluent nitrite-nitrogen concentrations increased as the ammonium-nitrogen concentrations decreased, reaching a plateau of approximately 400 mg l^{-1}. The effluent nitrate-nitrogen concentration averaged 20 mg l^{-1} during this period. On day 30, organic compounds were added to the feed medium (COD = 5,500 mg l^{-1}), and the reactor was reinoculated with an anaerobic microbial culture from the previously operated anaerobic reactors. Nitrite-nitrogen soon decreased to less than the detection limit and nitrate-nitrogen increased slightly before decreasing to less than the detection limit on day 50. The composition of the feed medium was increased to full strength on day 50 (influent COD = 11,000 mg l^{-1}). At this time, ammonium-nitrogen concentrations in the effluent increased rapidly until the end of the experiment (Fig. 6). Nitrite-nitrogen and nitrate-nitrogen concentrations in the effluent were at or near the detection limit (<1 mg l^{-1}) throughout this time period.

Biomass levels in the bulk fluid were low or near the detection limit until organic compounds were included in the feed medium (Fig. 7). Biomass levels remained stable (OD_{600} = 0.05–0.07) for the next 20 days until the organic content of the feed medium was increased to full strength, at which time the biomass levels rapidly increased until the end of the experiment. Effluent soluble COD concentrations were monitored after day 30; COD removal efficiencies were >90% throughout the experiment (data not shown). Biogas production occurred intermittently after the strength of the feed medium was increased on day 50 (Fig. 8A). The methane content of the biogas simultaneously increased, reaching a peak of about 15% on day 65 (Fig. 8B).

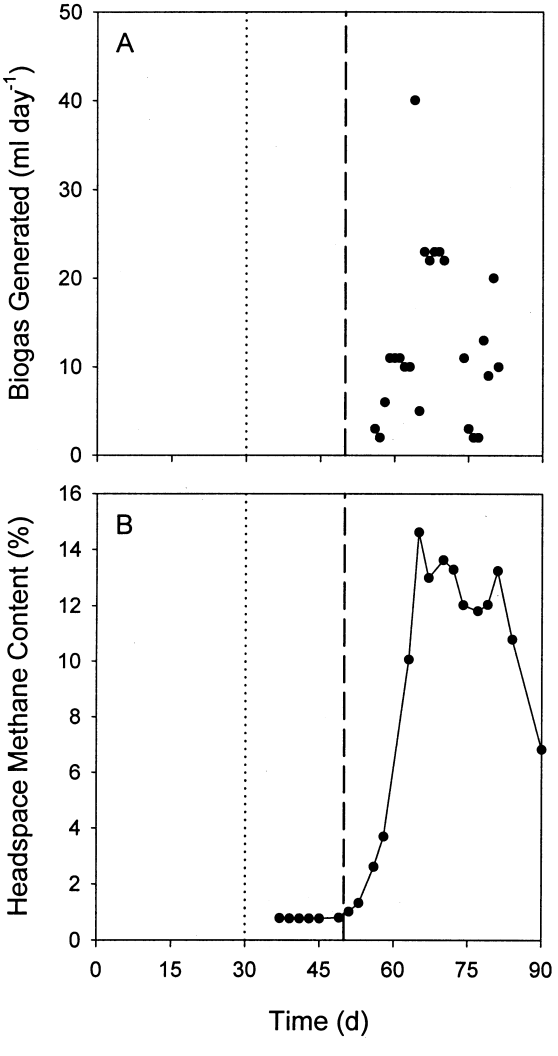

Figure 8. **A:** The quantity of biogas released from a membrane-augmented anaerobic bioreactor. **B:** The methane content of the headspace of a membrane-augmented anaerobic bioreactor.

DISCUSSION

Previous researchers have demonstrated that membrane-aerated biofilms can simultaneously support both aerobic and denitrifying conditions within a single biofilm (Hibiya et al., 2003; Semmens et al., 2003; Terada et al., 2003; Cole et al., 2004). In this study, a novel application of oxygen-transferring membranes was presented in which the gradient of oxidation-reduction potential within the membrane-aerated biofilm was substantially greater, transitioning from aerobic conditions near the membrane to methanogenic conditions at the interface between the biofilm and the bulk liquid. Our hypothesis for this research was that the membrane-augmented anaerobic bioreactor would exhibit improved COD removal efficiency and partial nitrogenous pollutant removal while maintaining some fermentative and methanogenic biological activity. Our results partially support this hypothesis; the membrane-augmented anaerobic bioreactor had substantially better COD removal efficiency and methane production was adversely affected, but improved nitrogenous pollutant removal was not observed. From a practical perspective, these results suggest that partial membrane aeration of otherwise anaerobic bioreactors could be a useful biotechnology for the treatment of wastewaters where better effluent quality and operational stability are more desirable than methane production. For these situations, the installation of membranes into existing anaerobic bioreactors would be a relatively inexpensive option compared to the additional of more reactor volume to increase treatment capacity in these systems.

The membrane-augmented bioreactor maintained much lower volatile fatty acid concentrations, which are key intermediates in the anaerobic conversion of organic compounds to methane and carbon dioxide (McCarty and Smith, 1986). The volume of biogas produced decreased significantly in the membrane-augmented bioreactor, suggesting that some of the organic substrate was oxidized to CO_2 via aerobic heterotrophic metabolism in the membrane-aerated biofilm. Although biogas production

was lower in the membrane-augmented bioreactor, methanogenesis was not completely inhibited by continuous oxygen delivery.

Higher biomass concentrations helped the membrane-augmented anaerobic bioreactor achieve better performance. Although a portion of this biomass was in the suspended form, the majority of additional biomass was attached to the membrane. The thickness of this biofilm likely became excessively thick, however, which may explain why soluble COD removal efficiency in the experimental bioreactor deteriorated toward the end of the first experiment. The performance of thick biofilms is limited by substrate diffusion, resulting in decreased substrate uptake (Casey et al., 2000). Excessive biofilm accumulation has been previously inferred as the cause of reduced COD and nitrogen removal efficiencies (Semmens et al., 2003). In this study, the decline in bioreactor performance coincided with the removal of ammonium chloride from the feed media, suggesting that this perturbation could have also been responsible for the reduction in membrane-augmented anaerobic bioreactor performance. Nonetheless, additional research is needed to control biofilm thickness to maintain high substrate diffusion rates into the biofilm. Preliminary work suggests that biofilm thickness can be controlled by optimizing the composition of the membrane and by mechanical shearing (data not shown).

Although previous researchers have demonstrated that membrane-aerated biofilms can support both nitrification and denitrification (Yamagiwa et al., 1998; Casey et al., 1999; Semmens et al., 2003; Terada et al., 2003), our membrane-augmented anaerobic bioreactors failed to achieve nitrogenous pollutant removal concomitant with methane production. Of particular concern is that heterotrophic bacteria can outcompete nitrifying bacteria for oxygen when organic substrate is available because nitrifying bacteria grow much slower than aerobic heterotrophs (Rittmann, 1987). The portion of the biofilm closest to the membrane, therefore, needs to be oxygen-rich but have very low organic substrate concentrations to favor the growth of nitrifying bacteria. Although the ammonia and organic substrate concentrations within the biofilm could not be quantified, our results suggest that in situ conditions selected against the growth of nitrifying bacteria stemming from the high COD concentrations in the bioreactors. We speculate that additional membrane surface area would help promote nitrification, although this may further inhibit methanogenesis. This hypothesis is supported by our third bioreactor experiment in which efficient nitrogenous removal was inhibited by an increase in the COD loading to the bioreactor. Model predictions (Shanahan and Semmens, 2004) of membrane-aerated biofilm behavior under these conditions were also consistent with the results observed herein (data not shown).

Membrane-aeration significantly affected biogas production in these experiments. Oxygen is generally considered toxic to methanogens, even at low concentrations, although several methanogenic microorganisms have shown limited tolerance to oxygen (Zitomer and Shrout, 1998). Although the volume of biogas produced was consistently lower in the membrane-augmented anaerobic bioreactor, the partial pressure of methane in the biogas increased toward the end of the experiment. This suggests that oxygen toxicity did not directly impact methane production, but that a fraction of the organic compounds was metabolized aerobically, reducing the amount of substrate available for anaerobic metabolism. Further support of this interpretation was provided by the measurement of oxidation-reduction potentials in the bulk reactor liquid, which were within the acceptable range for methanogenesis (data not shown).

In conclusion, anaerobic bioreactors augmented with a small specific surface area of oxygen-transferring membrane offer several attractive performance features for wastewater treatment. Better COD removal is achieved while maintaining lower concentrations of volatile fatty acids. Methane production decreased, however, suggesting that a fraction of the organic substrate was oxidized aerobically. Nitrogenous pollutant removal was achieved only at an intermediate COD loading rate, suggesting there is a relationship between organic loading rate, membrane surface area, nitrogen removal, and methane production. Based on the results of this study, it seems unlikely that a sufficiently large oxidation-reduction potential gradient can exist within a membrane-aerated biofilm such that both nitrification and methanogenesis can occur in a single bioreactor. Additional research is needed to examine the effects of reactor design, organic and nitrogen loading rates, membrane-specific surface area, and biofilm thickness on process behavior with respect to nitrogen removal and methane production.

The authors thank 3M for providing the membranes used in this study and the Metropolitan Council Environmental Services for performing the volatile fatty acid analyses. They also thank David Stuckey for helpful discussions regarding the design of their experiments.

References

Barber WP, Stuckey DC. 1999. The use of the anaerobic baffled reactor (ABR) for wastewater treatment: a review. Wat Res 33:1559–1587.

Barbosa RA, Sant'Anna GL. 1989. Treatment of raw domestic sewage in an UASB reactor. Wat Res 23:1483–1490.

Behling E, Diaz A, Coline G, Herrera M, Gutierrez E, Chacin E, Fernandez N, Forster CF. 1997. Domestic wastewater treatment using a UASB reactor. Bioresour Technol 61:239–245.

Biebl H, Pfennig N. 1981. Isolation of members of the family *Rhodospirillacaea*. In: Starr MP, Stolp HG, Troper HG, Balows A, Schlegel HG, editors. The prokaryotes. Berlin: Springer-Verlag. p 267–273.

Boopathy R, Tilche A. 1992. Pelletization of biomass in a hybrid anaerobic baffled reactor (HABR) treating acidified waste-water. Bioresour Technol 40:101–107.

Casey E, Glennon B, Hamer G. 1999. Review of membrane aerated biofilm reactors. Resour Conserv Recyl 27:203–215.

Casey E, Glennon B, Hamer G. 2000. Biofilm development in a membrane-aerated biofilm reactor: effect of flow velocity on performance. Biotechnol Bioeng 67:476–486.

Cole AC, Semmens MJ, LaPara TM. 2004. The stratification of activity and bacterial community structure in biofilms grown on membranes transferring oxygen. Appl Environ Microbiol 70:1982–1989.

Daniels L, Hanson RS, Phillips JA. 1994. Chemical analysis. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR, editors. Methods for general and molecular bacteriology. Washington, DC: American Society for Microbiology. p 512–554.

Fox P, Venkatasubbiah V. 1996. Coupled anaerobic/aerobic treatment of high-sulphate wastewater with sulphate reduction and biological sulphide oxidation. Wat Sci Technol 34:359–366.

Hibiya K, Terada A, Tsuneda S, Hirata A. 2003. Simultaneous nitrification and denitrification by controlling vertical and horizontal microenvironment in a membrane-aerated biofilm reactor. J Biotechnol 100:23–32.

LaPara TM, Alleman JE, Pope PG. 2000. Miniaturized closed reflux, colorimetric method for the determination of chemical oxygen demand. Waste Management 20:295–298.

Lettinga G, Roersma R, Grin P. 1983. Anaerobic treatment of raw domestic sewage at ambient temperatures sing a granular bed UASB reactor. Biotechnol Bioeng 25:1701–1723.

Lettinga G. 1995. Anaerobic digestion and wastewater treatment systems. Antonie van Leeuwenhoek 67:3–28.

McCarty PL, Smith DP. 1986. Anaerobic wastewater treatment. Environ Sci Technol 20:1200–1206.

Mrozinski JS. 1988. Microporous materials incorporating a nucleating agent and methods for making same. US Patent No. 4,726,989.

Nachaiyasit S, Stuckey DC. 1995. Microbial response to environmental changes in an anaerobic baffled reactor (ABR). Antonie van Leeuwenhoek 67:111–123.

Nachaiyasit S, Stuckey DC. 1997. The effect of shock loads on the performance of an anaerobic baffled reactor (ABR): I, step changes in feed concentration at constant retention time. Wat Res 31:2737–2747.

Rittmann BE. 1987. Aerobic biological treatment. Environ Sci Technol 21:128–136.

Schramm A, de Beer D, Gieseke A, Amann R. 2000. Microenvironments and distribution of nitrifying bacteria in a membrane-bound biofilm. Environ Microbiol 2:680–686.

Semmens MJ, Dahm K, Shanahan J, Christianson A. 2003. COD and nitrogen removal by biofilms growing on gas permeable membranes. Wat Res 37:4343–4350.

Shanahan JW, Semmens MJ. 2004. A multi-population model of membrane-aerated biofilms. Environ Sci Technol 38:3176–3183.

Singh KS, Harada H, Viraraghavan T. 1996. Low-strength wastewater treatment by a UASB reactor. Bioresource Technol 55:187–194.

Terada A, Hibiya K, Nagai J, Tsuneda S, Hirata A. 2003. Nitrogen removal characteristics and biofilm analysis of a membrane-aerated biofilm reactor applicable to high-strength nitrogenous wastewater treatment. J Biosci Bioeng 95:170–177.

Yamagiwa K, Yoshida M, Akira I, Ohkawa A. 1998. A new oxygen supply method for simultaneous organic carbon removal and nitrification by a one-stage biofilm process. Wat Sci Technol 37:117–124.

Zitomer DH, Shrout JD. 1998. Feasibility and benefits of methanogenesis under oxygen-limited conditions. Waste Management 18:107–116.