A Hybrid Membrane-Biofilm Process for Concurrent Nitrification and Denitrification of Wastewater

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Introduction

Total nitrogen (TN) removal from wastewater is an increasingly important treatment objective, yet many wastewater treatment facilities were designed with low solids retention times, and cannot achieve biological nitrification, i.e., conversion of ammonium to nitrate. For nitrifying plants, upgrading to denitrification, i.e., reduction of nitrate to nitrogen gas, often requires the use of exogenous electron donors, increasing operating costs.

We proposed a new process, the hybrid (attached and suspended-growth) membrane biofilm process (HMBP), for TN from wastewater. The HMBP (Figure 1) incorporates air-supplied hollow-fiber membranes into an activated sludge tank, achieving nitrification with a membrane-aerated biofilm. By maintaining completely-mixed conditions, the BOD concentration is kept low, thus denitrifying heterotrophs are mainly in the suspended phase (Downing and Nerenberg 2007). This allows for TN removal with a single tank, short bulk-liquid solids retention times (bSRTs), and minimal or no exogenous electron donor addition. Also, by utilizing passive aeration via the membranes, rather than bubbled aeration, and maximizing BOD use for denitrification, up to 70 percent of a plant's energy needs for aeration can be saved (Semmens 2005).

The HMBP combines the benefits of the integrated fixed-film activated sludge (IFAS) process, a hybrid process where nitrifiers are retained on suspended or fixed carriers, with membrane aerated biofilm reactors (MABRs), a fixed-film process that can concurrently nitrify and denitrify, but performs poorly when influent BOD is present.

A potential added benefit of membrane aeration is shortcut TN removal, which has been suggested for MABR systems (Hibiya, Terada et al. 2003; Terada, Hibiya et al. 2003; Terada, Yamamoto et al. 2006). Shortcut TN removal is the reduction of nitrite rather than nitrate during denitrification, which results in a reduced carbon source requirement of 40% and oxygen requirements by 25% (van Loosdrecht and Salem 2006). Nitrite accumulation is based on selection of ammonia oxidizing bacteria (AOB) over nitrite oxidizing bacteria (NOB), thus limiting the amount of nitrite oxidized to nitrate. Conditions that favor AOB over NOB include high temperature (Hellinga, Schellen et al. 1998), high free ammonia (FA) (Anthonisen, Loehr et al. 1976), high pH (Villaverde, Garcia-Encina et al. 1997), and low dissolved oxygen (DO) (Munich, Lant et al. 1996). Inhibition of NOB via low DO may occur when membrane-aerated biofilms operate in an anoxic environment, where the outer biofilm regions have low or zero oxygen concentrations. AOB can outcompete NOB at lower oxygen concentrations, as they have a lower K value for oxygen (Schramm, DeBeer et al. 1998). In a nitrifying MABR, microsensor studies indicated that nitrite was the main product of nitrification (Schramm, De Beer et al. 2000). MABRs achieving TN removal were shown to have low levels of nitrate in the biofilm (Hibiya, Terada et al. 2003), and an oxygen mass balance indicated the majority of oxygen transferred to a MABR was used for ammonia oxidation, with little oxygen remaining for nitrite oxidation (Terada, Hibiya et al. 2003).

We examined performance of the HMBP at several nitrogen and organic loading rates. Microsensor and FISH measurements were used to provide new insights into the structure and function of the HMBP biofilm, including evidence of short-cut denitrification (Downing and Nerenberg 2008).
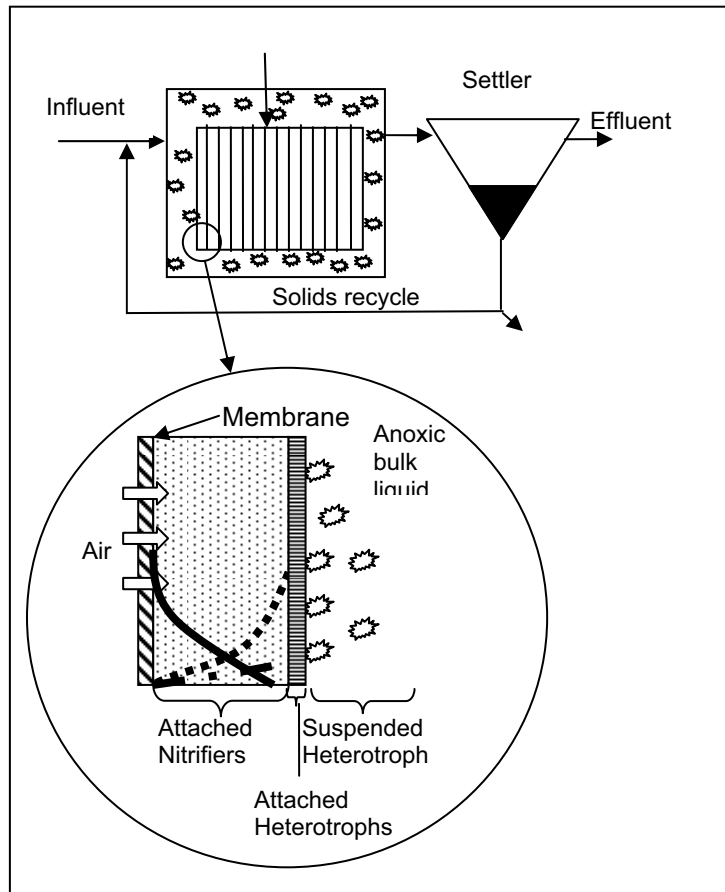
Figure 1. Process schematic of the hybrid membrane biofilm process (HMBP). Gradients of oxygen (━), ammonia (•••), and BOD (•━•) are shown through the biofilm.

Methods

Bench-scale reactor

The bench-scale HMBP reactor was designed following Downing and Nerenberg (2007), and was intended to simulate an activated sludge system. The reactor influent flow rate was 7.9 or 15.7 mL/min, and the sludge recycle ratio was 1.0. The activated sludge tank volume was 6 liters, resulting in a hydraulic retention time (HRT) of 10 or 20 hours. A bulk liquid sludge retention time (bSRT) of 2.5 days was maintained. The settler volume was 5 liters. The aeration tank included an axial mixer with a 3.5-inch blade operated at 1000 rpm. An open rectangular chamber contained a bank of hollow fiber membranes, referred to as the membrane bank. The membranes were a composite, with microporous polyethylene encasing a dense, polyurethane core (HFM200TL, Mitsubishi Rayon, Japan). The membrane outside diameter was 280 μm, and the total surface area of fiber was 1,900 cm^2 or 1,400 cm^2, depending on the loading rate being

tested. The membranes were pressurized with compressed air at 35 kPa. Measurement of suspended solids in the influent, reactor tank, effluent, and settled sludge determined the volume of wasted sludge necessary to maintain the 2.5 day sbSRT.

A synthetic wastewater was prepared from distilled water amended with 1.386 g Na_2HPO_4, 0.849 g KH_2PO_4, 0.05 g $MgSO_4^.7H_2O$ per liter, as well as a trace mineral solution (Nerenberg, Rittmann et al. 2002). $(NH_4)_2SO_4$ was added to achieve either 20 or 12 mg/L NH_3-N. Various BOD concentrations were achieved via magnesium acetate addition. BOD was calculated from the acetate by multiplying the acetate concentration by 1.08 mg BOD/mg acetate.

Analytical methods

For the bench-scale studies, nitrate, nitrite, and acetate were analyzed by ion chromatography (IC2500 with AS11/AG11 column, Dionex Corp, Sunnyvale CA.). The eluant was sodium hydroxide. NH_3-N was measured using a colorimetric method (High Range, Hach, Loveland, CO). pH was monitored using a glass electrode pH meter. Bulk liquid dissolved oxygen (DO) concentration was measured using a DO probe (YSI Model No. 55/25 FT). Suspended solids were measured according to *Standard Methods in Water and Wastewater*(Rand, Greenberg et al. 1978).

Microsensors

Microsensor measurements were completed at the bench-scale. Oxygen profiles through the biofilm were measured with Clark type oxygen microelectrodes (ox10, Unisense A/S, Aarhus, Denmark). Ammonium, nitrite, and nitrate profiles were measured using LIX microsensors. These microsensors were constructed as described elsewhere (De Beer and van den Heuvel 1988; De Beer and Sweerts 1989; De Beer, Schramm et al. 1997). Measurements were taken at intervals of 20 μm through the biofilm when influent concentrations of ammonium were 20 mgN/L and BOD were 150 mg/L. A micromanipulator was used to position the microsensors through the biofilm (Unisense A/S, Aarhus, Denmark). A total of three profiles were taken at three different locations for each sensor, resulting in nine profiles each for oxygen, ammonium, nitrite, and nitrate. Net fluxes into, or out of, the biofilm for ammonium, nitrite, and nitrate were calculated as described elsewhere (Schramm, De Beer et al. 1999).

FISH

The ecology of the biofilm was analyzed using FISH. Oligonucleotide probes used were: Nso190, which targets the majority of AOB (Mobarry, Wagner et al. 1996); NIT3, which targets *Nitrobacter* spp. (Wagner, Rath et al. 1996); NSR1156, which targets *Nitrospira* spp. (Schramm, DeBeer et al. 1998); and EUB338, which targets general bacteria (Amann, Krumholz et al. 1990). Membranes with attached biofilm were removed from the reactor, fixed in 4% paraformaldehyde for 60 minutes at 4^oC, embedded in OCT compound, and sectioned using a cryostat, as described elsewhere (Schramm, De Beer et al. 2000). Hybridizations were performed as previously described (Manz, Amann et al. 1992).

Results

Bench-scale

A major advantage of the HMBP, as compared to previous membrane aeration systems, is the ability to treat increased BOD loadings without losing nitrification efficiency. As shown in Table 1, the HMBP has a similar nitrification rate as MABR systems. However, at high BOD loading rates, the HMBP maintains the same nitrification rate, whereas MABR systems have been shown to have a decreasing nitrification rate at increasing BOD loadings (Walter, Haase et al. 2005).

Table 1. Nitrification rates in the bench-scale HMBP and previous MABR systems

Nitrogen Loading $gN\ m^{-2}\ day^{-1}$	Organic Loading $gBOD\ m^{-2}\ day^{-1}$	Nitrification Rate $g\ m^{-2}\ day^{-1}$	Denitrification %	Reference
2	8	0.4	>90	Timberlake et al., 1988
3	13	2.2	39	Yamagiwa et al., 1994
1.9	8.3	1.71	>90	Semmens et al, 2003
2	7	0.77	>95	Hibiya et al, 2003
1.65	3.3	1.1	24%	This study
	6.6	1.1	29%	
	9.9	1.1	67%	
2.24	4.5	1.1	10%	This study
	9.0	1.1	40%	
	12.4	1.1	70%	
	16.8	1.1	80%	
1.46	16.8	1.0	99%	This study

The bulk BOD concentration was shown to have a major impact on nitrification in the HMBP (Figure 2). This may be explained by greater BOD penetration into the biofilm, allowing heterotrophic bacteria to compete for oxygen from the membranes. Although the BOD spikes were below 2.5 mg/L, the BOD source was acetate, which is readily degradable and consequently can stimulate growth at very low concentrations. A typical municipal wastewater contains more complex BOD, and significant effects on nitrification would not be expected at these low concentrations.

The mechanisms for nitrification and denitrification in the HMBP were also explored at the bench scale using microsensors. Shortcut nitrification, where nitrite is the main product of nitrification rather than nitrate, has been suggested in previous MABR studies (Satoh, Ono et al. 2004; Terada, Yamamoto et al. 2006). The use of microsensors during these experiments quantified the amount of nitrite and nitrate produced by the biofilm and consumed by the suspended heterotrophs. Results of microsensor profiles are summarized in Figure 3.

Under the three loading conditions in which microsensor measurements were taken, nitrite was the dominant form of oxidized nitrogen produced by the biofilm, confirming shortcut nitrogen removal. Effluent BOD was below 0.2 mg/L for the first two loading conditions, but spiked to approximately 2.5 mg/L for the final loading condition. This spike in effluent BOD resulted in decreased nitrification rates due to competition of AOB and heterotrophs in the biofilm.
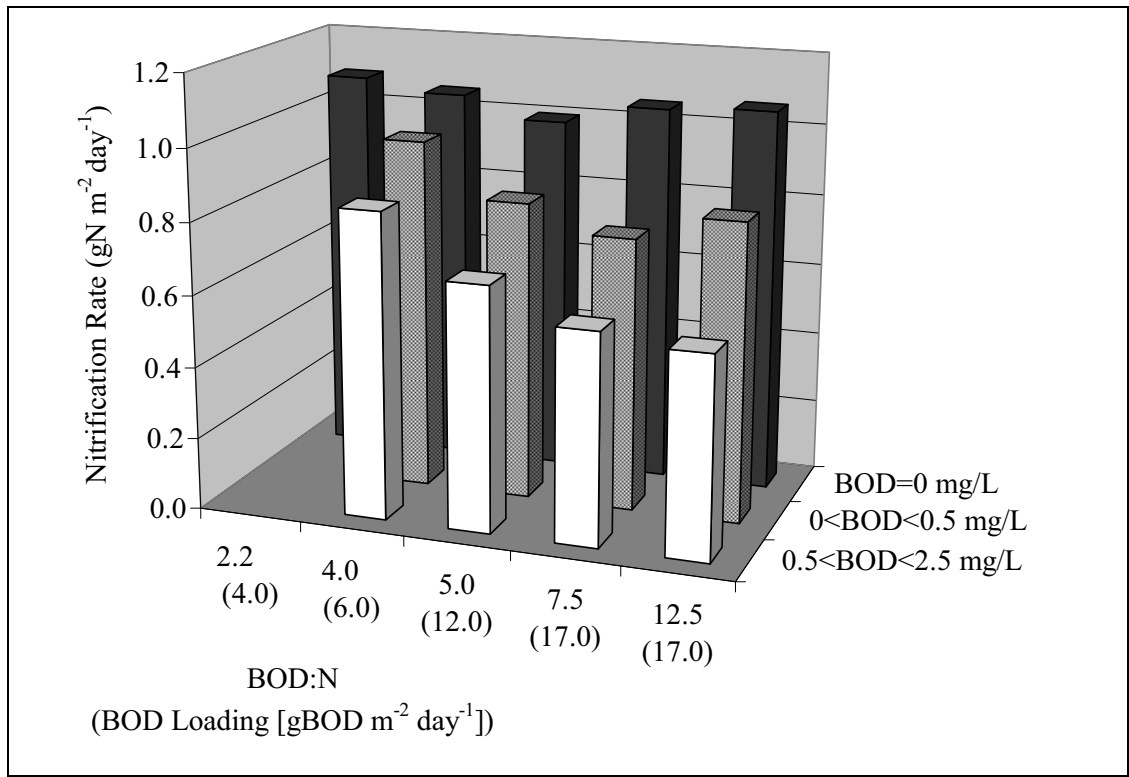

Figure 2. Impact of effluent BOD concentration of average nitrification rates at varying influent BOD:N ratios. BOD concentrations indicated on the right axis are for the bulk-liquid

All denitrification occurred in the bulk liquid under BOD to nitrogen (BOD:N) loading ratios of 5 and 7.5, where 70% and 80% denitrification was achieved, respectively. Under a BOD:N loading ratio of 12.5, full denitrification was consistently achieved. Under this condition, about 25% of the denitrification occurred in the biofilm, with 75% occurring in the bulk liquid. The increased biofilm denitrification is due to the increased bulk liquid BOD concentration, which allows effective diffusion of BOD into the biofilm.
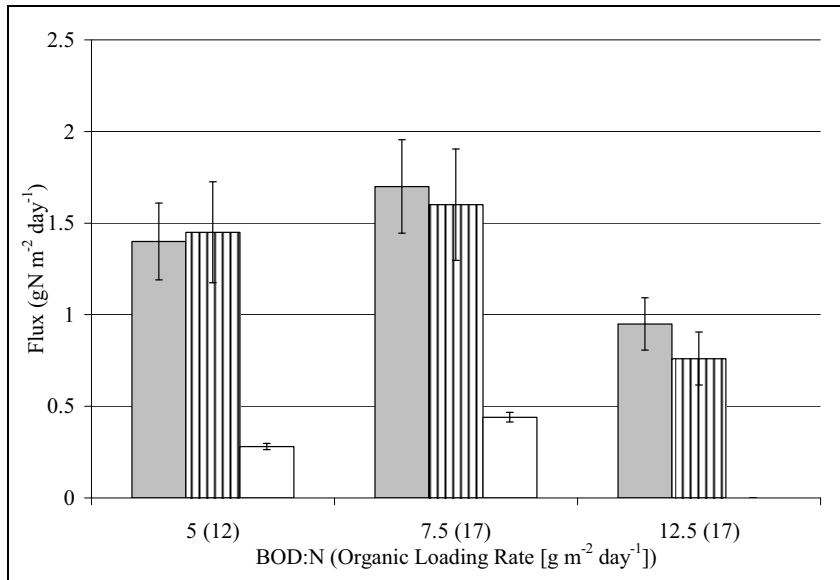
Figure 3. Rates of nitrification (solid), nitrite production (vertical lines), and nitrate production (white) by the biofilm in the bench-scale HMBP at varying loading conditions, as calculated from biofilm microgradients.

FISH results from the bench-scale study indicated a uniquely stratified biofilm. With 150 mg/L BOD and 20 mg/L NH_4^+-N in the influent, AOB were present throughout the biofilm, whereas two distinct layers of NOB and heterotrophs were present (Figure 4). Starting from the membrane biofilm interface, a mixture of AOB and NOB were observed, consistent with Schramm et al. (2000). At a distance of approximately 50 μm from the membrane biofilm interface, NOB populations decreased, and only AOB were present. A section of biofilm at 50 μm to 75 μm from the membrane contained exclusively AOB. The outer edge of the biofilm was composed of putative heterotrophic bacteria. Ecology studies of MABR system revealed some denitrifying heterotrophs throughout the biofilm thickness (Cole, Semmens et al. 2004). The existence of a strictly nitrifying layer in the HMBP results in increased nitrification rates by limiting heterotrophic competition in the aerobic portions of the biofilm.
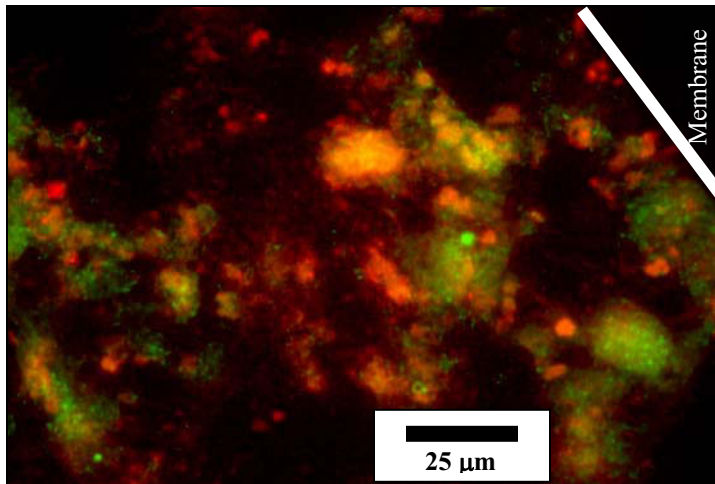
Figure 4. FISH image with an ammonium loading of 2 gN m^{-2} day^{-1} (20 mgN/L) and an organic loading of 17 gBOD m^{-2} day^{-1} (150 mg/L). Biofilm thickness was approximately 120 μm. AOB are shown in red/orange, while other bacteria are shown in green. Green cells near the membrane surface were proven to be NOB in separate hybridizations, while green cells near the biofilm edge are putative heterotrophs.

FISH results also indicated an increasing heterotrophic presence in the biofilm under the last loading condition (BOD:N of 12.5 in the influent). As previously stated, increased effluent BOD occurred on a more regular basis at this loading rate. The increased effluent BOD allowed BOD to diffuse into the biofilm. This increased BOD concentration in the biofilm results in the increased heterotrophic presence.

Conclusions

The HMBP achieves sustained nitrification rates despite increasing BOD loadings. The results suggest that nitrite is the main product of nitrification, and is preferentially reduced by heterotrophic bacteria. Given the HMBP's ability to achieve short-cut denitrification with short bSRTs, to aerate passively, and to maximize use of endogenous donors for denitrification, it has great potential to contribute to sustainable wastewater treatment. It also may be ideal to retrofit into existing plants that were designed with short SRTs and are unable to achieve nitrogen removal.

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