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ORIGINAL PAPER

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High efficiency degradation of tetrahydrofuran (THF) using a membrane bioreactor: identification of THF-degrading cultures of *Pseudonocardia* sp. strain M1 and *Rhodococcus ruber* isolate M2

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Abstract A mixed microbial culture capable of growing aerobically on tetrahydrofuran (THF) as a sole carbon and energy source was used as the inoculum in a 10 l working volume membrane bioreactor. Following start-up, the reactor was operated in batch mode for 24 h and then switched to continuous feed with 100% biomass recycle. On average, greater than 96% of THF fed to the reactor was removed during the 8-month study. THF loading rates ranged from 0.62 to 9.07 g l^{-1} day^{-1} with a hydraulic retention time of 24 h. THF concentrations as high as 800 mg/l were tolerated by the culture. Biomass production averaged 0.28 kg total suspended solids/kg chemical oxygen demand removed, i.e., comparable to a conventional wastewater treatment process. Periodic batch wasting resulted in a solids retention time of 7–14 days. Reactor biomass typically ranged from 4 to 10 g/l volatile suspended solids and the effluent contained no solids. Pure THF-degrading cultures were isolated from the mixed culture based on morphological characteristics, Gram-staining and THF degradation. Based on 16S rDNA analysis the isolates were identified as *Pseudonocardia* sp. M1 and *Rhodococcus ruber* M2.

Keywords Tetrahydrofuran · Membrane bioreactor · *Pseudonocardia* sp. · *Rhodococcus ruber*

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Introduction

The membrane bioreactor (MBR) process is a modification of the conventional activated sludge process where the clarifier is replaced by a membrane system for separating the biomass solids from the treated effluent stream [34]. Retention of all microorganisms results in high biomass concentrations in the bioreactor, which allow the system to treat high-strength wastewater resulting in a system with a relatively small footprint. MBR technology has been successfully applied to treat a variety of high-strength wastes including high molecular weight compounds [8, 34], organic wastes containing surfactants [19], fermentation wastewater [21], phenol [20], contaminated gas streams [11, 27, 28] and industrial waste [29]. Retention of soluble high molecular weight compounds keeps slowly degradable compounds in the bioreactor while non-degradable compounds are discharged with the sludge [16]. The system is capable of handling fluctuations in waste influent concentrations due to the high biomass concentration in the reactor [21]. High biomass concentrations can lead to decreased oxygen transfer rates and a decline in permeate flow, both of which can adversely affect system performance. Energy consumption costs have been typically higher with the MBR compared to a traditional activated sludge system [6]. However, recent advances such as transfer flow membranes [32] and submerged membrane bioreactors [7] make energy requirements comparable to those of conventional wastewater systems. The small size, coupled with the ability to treat high-strength wastes, make the MBR an attractive option for pretreating waste prior to conventional treatment.

Tetrahydrofuran (THF) is a common cyclic aliphatic ether used in the bulk chemical and pharmaceutical industries. THF is an inhibitor of cytochrome P450-dependent enzymes and may cause human health problems [10, 23]. Degradation assessment studies using activated sludge from different sources have concluded that THF is not readily biodegradable [25]. However,

a number of results from within the larger data set show that THF was degradable, and degradation appeared to be dependent on inoculum source, temperature and incubation time [25]. Differences in the biodegradability of THF between conventional wastewater treatment plants can be attributed to a number of factors, including wastestream composition, substrate competition, plant operations and the relative number of THF-degrading organisms present. As a result, maintaining high specific THF degradation rates in conventional wastewater treatment processes can be a challenge. It may be possible to achieve high THF degradation rates in a MBR by selecting organisms capable of using THF as a sole carbon and energy source. Pure microbial cultures capable of using THF as a sole carbon and energy source have been isolated from municipal and industrial wastewater treatment plants [4, 5, 18] and contaminated sludge [26]. High specific THF degradation rates have been observed previously with a pure culture [5], and the degradation pathway has been elucidated [4, 5]. However, there have been few published reports on the fate and biodegradation of THF in the environment.

The overall objective of this study was to assess the effectiveness of a MBR in treating a synthetic THF wastestream. The first objective was to isolate both mixed and pure microbial cultures capable of using THF as a sole carbon and energy source. The second objective was to construct and operate a bench-scale MBR using a mixed culture capable of degrading a synthetic THF wastestream. THF removal efficiency was monitored throughout the 8-month study.

Materials and methods

Sources of chemicals

All chemicals were purchased from Fisher Scientific (Pittsburgh, Pa.).

Growth and isolation of THF-degrading cultures

Samples of mixed liquor were collected from an industrial wastewater treatment plant (WWTP) and used as the inoculum. A 1 ml aliquot of mixed liquor was inoculated aseptically into 500 ml sterile single carbon source medium (pH 7.0) containing, per liter: 380 mg Na_2HPO_4, 68 mg KH_2PO_4, 55 mg NH_4Cl, 12 mg $NaNO_3$, 5 mg $CaCl_2{\cdot}2H_2O$, 50 mg $MgSO_4$, 100 mg MOPS, 10 ml Wolfe's mineral solution [2] and 1 ml Wolfe's vitamin solution [2]. THF was added to a final concentration of 250 mg/l and allowed to incubate at 32°C and 150 rpm. Growth of mixed and pure cultures was monitored as an increase in optical density (OD) in a 1 cm cell at 578 nm on a DR/3000 spectrophotometer (Hach Company, Loveland, Co.) and degradation of THF was monitored by gas chromatography (GC) as described below. A control flask containing a single-carbon-source medium was used to measure THF volatilization loss. When an increase in OD was observed, samples were assayed by GC and transferred to fresh medium. After several transfers in liquid medium, the culture was used to inoculate the MBR. After repeated dilution streaks on THF-agar plates, two pure THF-degrading organisms were isolated based on morphological characteristics and Gram staining. The isolates were streaked onto additional agar plates and incubated under air at 32°C with and without addition of THF. The isolates were also used to inoculate a series of 125 ml flasks containing 50 ml single-carbon-source medium with and without addition of 250 mg/l THF to confirm THF degradation. The flasks were incubated at 32°C and 200 rpm. THF-agar plates consisted of 15 g/l agar in single-carbon-source medium. THF was supplied via the gas phase in glass Petri plates by injecting 25 µl THF into a moistened sterile cotton ball. The plates were sealed and incubated under air at 32°C.

16S rDNA and phylogenetic analysis

Genus and species-level identification came from analysis of 1,500 bp of the 16S rDNA from each isolate (Accugenix, Newark, Del.). The 16S rRNA gene was PCR amplified from genomic DNA isolated from bacterial colonies. Primers used for the amplification correspond to *Escherichia coli* positions 005 and 1,540 for the full sequence package. The amplification products were purified from excess primers and dNTPs using Microcon 100 (Amicon) molecular weight cut-off membranes and checked for quality and quantity by running a portion of the products on an agarose gel. Cycle sequencing of the 16S rRNA amplification products was carried out using AmpliTaq FS DNA polymerase and dRhodamine dye terminators. Excess dye-labeled terminators were removed from the sequencing reactions using a Sephadex G-50 spin column. The products were collected by centrifugation, dried under vacuum and frozen at −20°C until ready to load. Samples were resuspended in a solution of formamide/blue dextrans/EDTA and denatured prior to loading. The samples were electrophoresed on a ABI Prism 377 DNA Sequencer using a 5% Long Ranger (RMC) polyacrylamide/urea gel for 6 h.

The 16S rRNA sequences from organisms related to the isolated strains were retrieved from the NCBI nucleotide database by using NCBI BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The computer program CLUSTALW [15, 31] with default gap-change cost was used for multiple sequence alignment. Some ambiguous alignments were realigned by eye in MacClade [22]. Maximum parsimony (MP) phylogenetic analyses and distance analyses [17] (Jukes-Cantor model of nucleotide substitution) of the aligned sequences were performed using the computer program PAUP* version 4.0b2 [30]. Heuristic searches were performed using random stepwise addition (1,000 replicates) of taxa followed by tree bisection-reconnection branch swapping. Branches with a maximum length of zero were collapsed. Branch support for the MP analysis was evaluated using the nonparametric bootstrap procedure [13].

Analytical methods

MBR influent, effluent and reactor samples were centrifuged at 10,000 *g* for 10 min at 4°C prior to GC and chemical oxygen demand (COD) analysis. The resulting supernatant was analyzed for THF on a Hewlett-Packard (Palo Alto, Calif.) Series II gas chromatograph with tandem PID-FID detectors (OI Analytical, College Station, Tex.). The limit of quantitation for THF was 1.0 mg/l. All GC analyses followed USEPA Method 1671, and all quality assurance and quality control (QA/QC) objectives were met. An Agilent Technologies (Wilmington, Del.) model G2891A micro-GC with thermal conductivity detection was used for reactor THF off-gas analysis. Off-gas was routed through a heated (120°C) sample line to the micro-GC, and the internal pump sampled the contents of the line approximately every 4 min by pulling in a slipstream for 30 s. USEPA Method 18C was followed and all QA/QC objectives were met. The limit of quantitation for THF was 2 ppmv (parts per million by volume).

Analyses of COD, total suspended solids (TSS) and volatile suspended solids (VSS) were performed according to standard methods [1]. Effluent samples were analyzed by ion

chromatography within 48 h of collection. Analysis was performed using a DX-500 ion chromatograph (Dionex, Sunnyvale, Calif.) with suppressed conductivity detection. On-line measurements of dissolved oxygen (DO), oxidation-reduction potential (redox), temperature, pH, and carbon dioxide were also monitored.

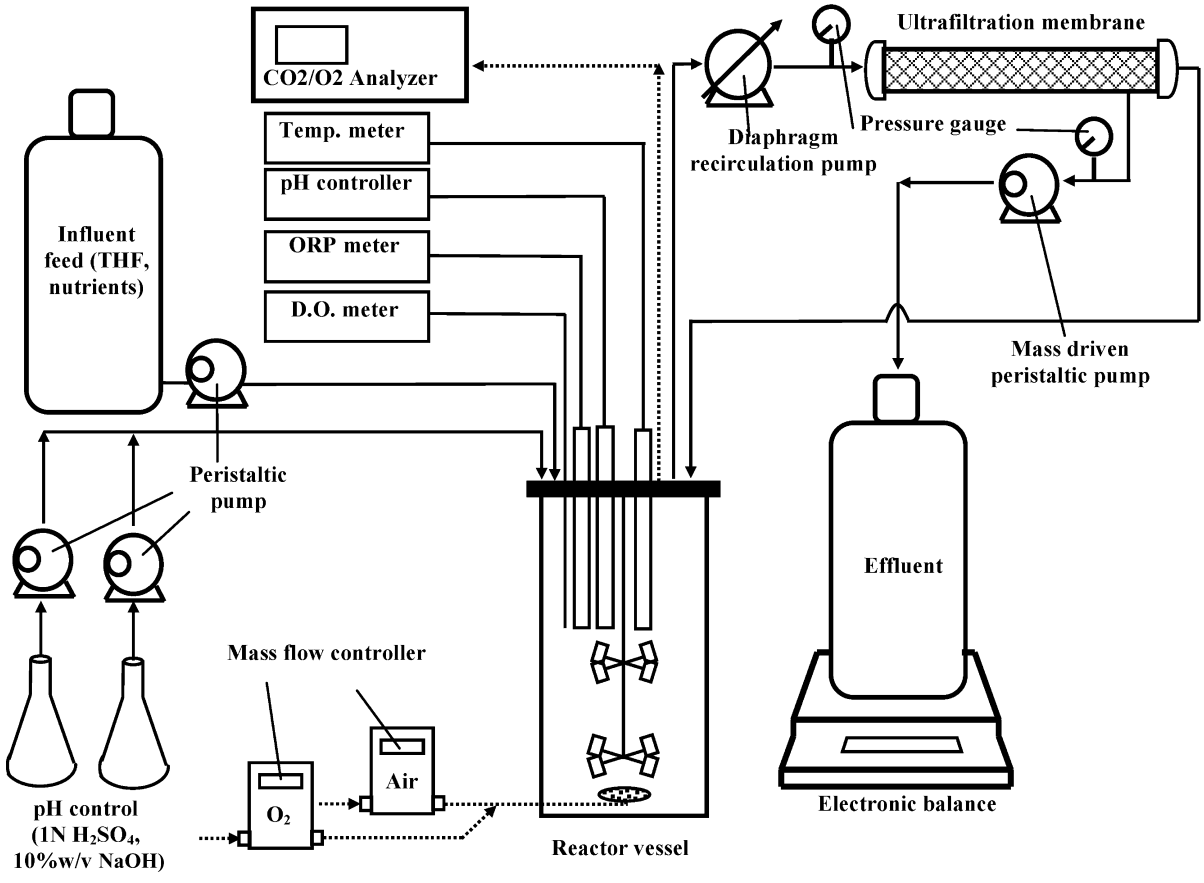
Reactor design

The MBR system (Fig. 1) was a continuously stirred 14 l fermentor (New Brunswick Scientific, New Brunswick, N.J.) coupled with an ultrafiltration membrane (UFP-500-E-9A; A/G Technology, Needham, Mass.) having an average porosity of 500 kDa and an effective filtration surface of 0.84 m^2. A variable speed diaphragm pump (Cole-Parmer, Vernon Hills, Ill.) continuously recycled biomass through the filtration membrane at 600 ml/min. The total working volume of the MBR system was 10 l. The reactor volume was kept constant by feeding influent at 7 ml/min, while pumping filtrate from the membrane at the same rate. This was accomplished by using a peristaltic pump (Cole-Parmer) as the feed pump, and a mass driven peristaltic pump (SciLog, Madison, Wis.) coupled to an EOM210 electronic balance (Ohaus, Florham Park, N.J.) to remove the effluent from the membrane. Effluent was collected in a 50-l carboy. Air and oxygen mass flow controllers (Cole-Parmer) supplied aeration through a 2-inch (50.8 mm) stainless steel diffuser disc in the bottom of the reactor. The culture was kept at a constant temperature of 32–34°C through a heat exchange in the fermentor. Temperature was monitored using a temperature probe inserted into the thermometer well of the head plate. Cooling water (4–6°C) was circulated through the air exhaust condenser in order to dry the reactor exit gas prior to analysis on an Ultamat-23 CO_2/O_2 analyzer (Siemens, Haguenau, France). A pH controller and probe (Cole-Parmer) monitored and adjusted reactor pH through on/off control of a pair of peristaltic pumps (Cole-Parmer). The pumps delivered acid (1 N H_2SO_4) or base (10% w/v NaOH) at 0.6 ml/min as needed to keep pH within 7.0 ± 0.2. DO was monitored using a polarographic DO probe and meter (Cole-Parmer), and was maintained at >1.0 mg/l by adjusting aeration rate, oxygen rate, mixing speed, loading rate and biomass concentration. Reactor agitation was controlled by an external LabMaster SI mixer (Lightnin, Rochester, N.Y.) attached to the fermentor impeller shaft. Additional mixing came from a 4-inch (102 mm) stirbar at the bottom of the reactor, which was driven by a magnetic stir plate (Fisher Scientific) at medium speed. Redox was monitored using a redox probe and meter (Cole-Parmer).

Fig. 1 Schematic drawing of membrane bioreactor (MBR) system. *Solid arrows* Liquid flow, *dashed arrows* gas flow

Reactor startup and operation

The THF-degrading culture was grown as described above and 10 l was transferred to the MBR. The culture was allowed to mix in batch mode for 24 h with periodic addition of THF to a final concentration of 100–200 mg/l. On-line reactor parameters were monitored and periodic THF analysis was performed. When on-line measurements stabilized, and the THF concentration dropped below 10 mg/l, the reactor was switched to continuous feed with 100% biomass recycle. Synthetic wastewater containing THF was used as the feed source and contained the following components per gram of THF in potable water: 52.2 mg/l $(NH_4)_2HPO_4$, 126.6 mg/l NH_4Cl, 71.0 mg/l urea, 9.0 mg/l Na_2HPO_4, 5 ml/l Wolfe's mineral solution [2] and 1 ml/l Wolfe's vitamin solution [2]. On 10 November 2000, the nutrient composition of the synthetic wastewater containing THF was changed to the following components per gram of THF in potable water: 26.1 mg/l $(NH_4)_2HPO_4$, 63.3 mg/l NH_4Cl, 35.5 mg/l urea, 4.5 mg/l Na_2HPO_4, 5 ml/l Wolfe's mineral solution [2]and 1 ml/l



Wolfe's vitamin solution [2]. Once the reactor had been in continuous operation for 2 weeks, DO was maintained at >1.0 mg/l by adjusting biomass concentration. The following reactor parameters were set for the duration of the study: mixing speed 250 rpm, oxygen addition 80 ml/min and aeration 2.5 l/min. Approximately 2–4 l biomass was wasted when the DO dropped below 1.0 mg/l and replaced with an equivalent volume of potable water.

The membrane was backflushed with potable water whenever membrane inlet pressure exceeded 20–25 psi ($1.38–1.72\times10^{-5}$ Pa), and was performed on a weekly basis. During the backflush, effluent and recycle pumps were turned off and membrane inlet and outlet lines were disconnected. Potable water was pumped through the permeate port and into the membrane at <10 psi (6.89×10^{-4} Pa) with a total volume of approximately 4 l. Backflush water was collected and discarded. Membrane inlet and outlet lines were reconnected and the effluent and recycle pumps turned on. Membrane regeneration was performed when backflushing did not sufficiently lower membrane inlet pressure, and was performed on a monthly basis. During regeneration, effluent and recycle pumps were turned off and membrane inlet and outlet lines disconnected. Clean tap water was flushed through the membrane for 10 min followed by recirculation of 0.5 *N* NaOH at 50°C for 1 h. Finally, the membrane was flushed a second time with clean tap water for 10 min. Membrane inlet and outlet lines were reconnected and effluent and recycle pumps were started. The reactor was placed in batch mode with influent feed at 7 ml/min during membrane backflush and regeneration.

Reactor biomass samples (15 ml) were collected for TSS, VSS, COD and THF analyses. The effluent pump was turned off, and a 24-, 48- or 72-h composite effluent sample (50 ml) was collected from the effluent carboy for ion, COD and THF analysis. The remaining effluent was discarded, and the carboy was placed on the balance and the effluent pump restarted.

Results

Characterization of mixed culture

Inoculation of single-carbon-source medium containing THF with sludge samples from an industrial wastewater treatment plant yielded a THF-degrading mixed culture. Repeated dilution streaking on THF-agar plates resulted in separation of the mixed culture and isolation of two distinct cultures. Single colonies were picked and further transferred to THF-agar plates until homogeneous-looking colonies were obtained. Purity of the THF-degrading cultures was initially judged by visual examination followed by Gram-staining. Each purified culture grew on both solid and liquid medium amended with THF; however, the cultures failed to grow on either liquid or solid medium with no added THF. Culture purity was confirmed by obtaining the same PCR product from different colonies during analysis of 1,500 bp of 16S rDNA. Phylogenetic relationships between known actinomycetes and isolates M2 and M1 are shown in Figs 2 and 3, respectively. Results of the 16S rDNA analysis are reported as a percent similarity between the sample sequence and the closest sequence match in the NCBI GenBank database. Isolates M1 and M2 had a similarity of 98% and 100%, respectively, and were identified as *Pseudonocardia* sp. and *Rhodococcus ruber*, respectively. The sequences obtained from 16S rDNA analysis from *Pseudonocardia* sp. strain M1 and *R. ruber* M2 were deposited at the NCBI GenBank and assigned accession number AY247276 and AY247275, respectively.

Operation of MBR

Following a switch from batch to continuous feed with biomass recycle, the MBR was fed a synthetic THF wastestream at varied loading rates (Fig. 4) with a hydraulic retention time of 24 h. On average, the THF removal efficiency was greater than 96% during the 8-month study and THF mass removal ranged from 0.62 to 9.07 g l^{-1} day^{-1} (Fig. 4). COD removal efficiency mirrored that of THF and is not shown. No significant decrease in removal efficiency was seen when the THF mass load was increased or decreased. The maximum THF loading rate of 9.07 g l^{-1} day^{-1} caused a decrease in DO concentrations below 1.0 mg/l and, as a result, the MBR was operated at decreased THF loading rates for the duration of the study. At maximum loading, the MBR showed a greater-than-100-times increase in THF mass removal (g l^{-1} day^{-1}) when compared to a full-scale WWTP (data not shown). Composite effluent THF concentrations were used to estimate average reactor THF concentrations, which were used to calculate stripping loss. The reactor was assumed to be at equilibrium. Stripping rates (g l^{-1} day^{-1}) at 33°C and an airflow of 158.7 l/h were calculated using a temperature-adjusted Henry's Law constant of 2.77×10^{-3} l_{gas}/l_{liquid}, and a temperature-adjusted ideal gas constant of 2.51×10^{-2} m^3/mol. Removal efficiency represents the percent difference between the daily influent loading rate and composite effluent loading rate plus any stripping loss (Fig. 4). Figure 5 shows the daily THF loading rates, along with daily combined THF effluent and stripping loss rates. Calculated stripping losses accounted for $<2\%$ of total THF removed during the study. Effluent THF concentration was typically <1.0 mg/l and no measurable THF was detected in the reactor off-gas during analysis performed 12–16 June 2000. THF accumulation of 100–650 mg/l occurred in the reactor and effluent on several occasions, and was caused by decreased DO at increased loading rates coupled with high biomass concentrations. Wasting of solids followed by a period in batch mode raised DO concentrations and quickly reduced the THF concentration to below 1.0 mg/l. The unit was then returned to continuous feed.

Periodic batch wasting of the biomass resulted in a biomass retention time of 7–14 days and typical reactor concentrations were 4–10 g/l VSS (Fig. 6). Biomass concentrations as high as 18 g/l VSS were achieved but were not sustainable due to oxygen limitation. Oxygen limitation was encountered when VSS increased beyond 5 g/l. The MBR was supplemented with oxygen because an increase of impeller speed or aeration rate was not possible. DO concentrations decreased significantly when the VSS increased beyond 8–10 g/l, even with

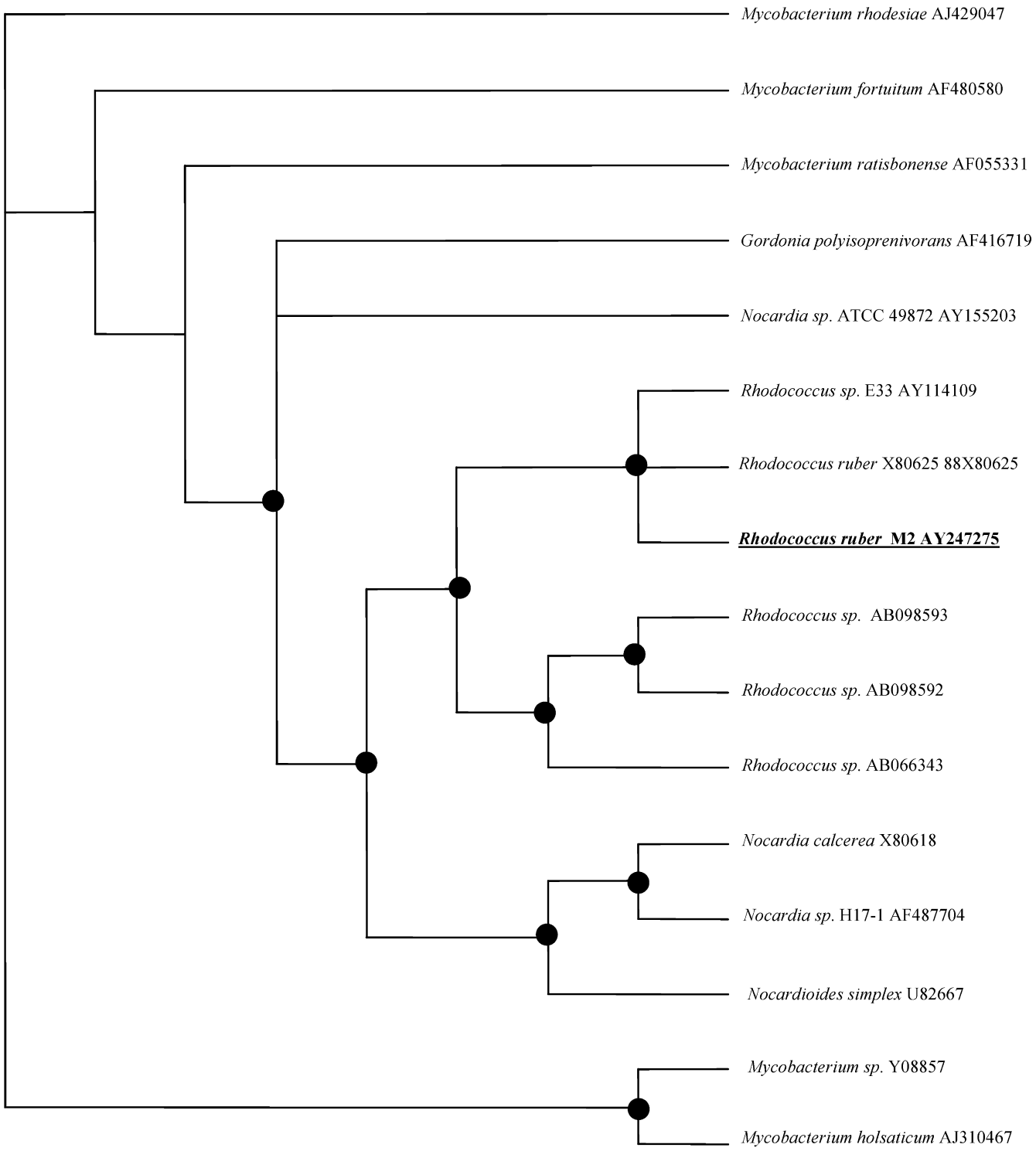


Fig. 2 Strict consensus of all equally parsimonious trees based on 1,503 bp of 16S rRNA alignment. *Circles* Statistically supported nodes (>70% bootstrap support). *Rhodococcus ruber* isolate M2 shares a common ancestor with *R. ruber* X80625 and *Rhodococcus* sp. E33 AY114109, and is most similar to *R. ruber* X80625 (100% similarity)

oxygen addition. At this point the solids were wasted and this proved effective in controlling reactor DO concentrations. However, when wasting was not carried out on a timely basis, THF began to accumulate in the reactor due to high biomass concentrations and low DO. Biomass production averaged 0.28 ± 0.06 kg TSS/kg $COD_{removed}$ during the study and was calculated by using reactor TSS and COD data from successive days between batch-wasting episodes. Reactor VSS represented at least 90% of TSS and the effluent contained no measurable solids.

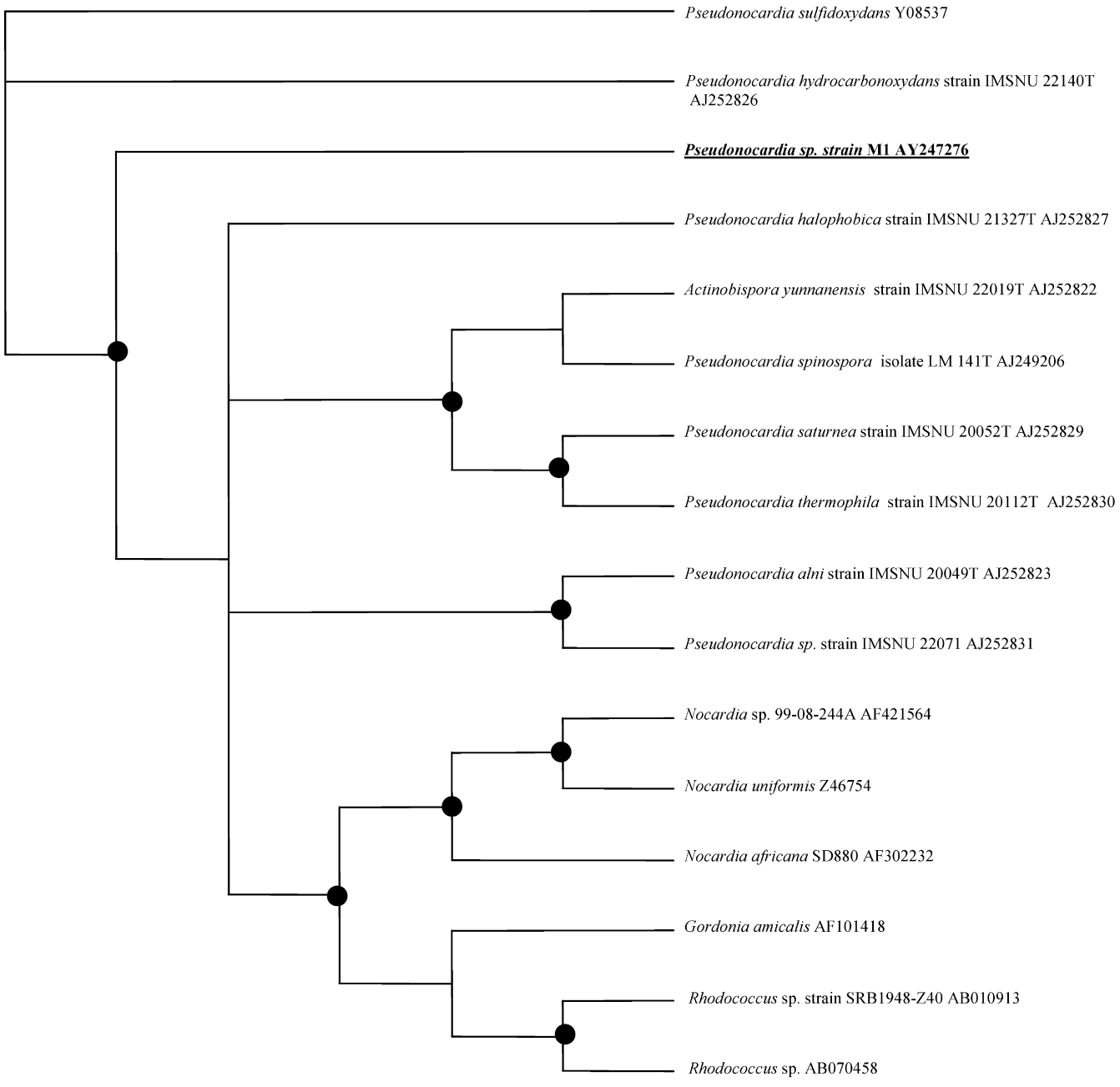


Fig. 3 Strict consensus of all equally parsimonious trees based on 1,499 bp of 16S rRNA alignment. *Circles* Statistically supported nodes (>70% bootstrap support). *Pseudonocardia* sp. strain M1 shares a common ancestor with *Pseudonocardia hydrocarbonoxydans* strain IMSNU 22140T AJ252826 and *Pseudonocardia sulfidoxydans* Y08537, and is most similar to *P. hydrocarbonoxydans* strain IMSNU 22140T AJ252826 (98% similarity)

A nutrient limitation study was conducted during November. During this time no additional nutrients were added to the synthetic wastestream and THF loading was kept constant at between 2.41 and 2.55 g l^{-1} day^{-1} (Fig. 5). THF removal efficiency was reduced to as low as 36% (Fig. 4) and the effluent THF concentration increased to as high as 800 mg/l. THF removal during this period was not used to calculate overall THF removal efficiency. THF removal efficiency was >90% within 7 days of returning nutrients to the feed (Fig. 4). THF removal efficiency was significantly decreased under nutrient-limiting conditions even for short periods of time.

Ion analysis was performed to assess overall effluent quality by monitoring ions including phosphate, ammonium and nitrate. Effluent phosphate ranged from 0.08 to 250 mg/l, ammonium ranged from 0.04 to 236 mg/l and nitrate ranged from non-detectable to 25.1 mg/l (Fig. 7). Effluent concentrations of both ammonium and phosphate were highly variable during the first 5 months of operation (Fig. 7), and nutrient loading to the MBR was reduced to a COD:N:P ratio of

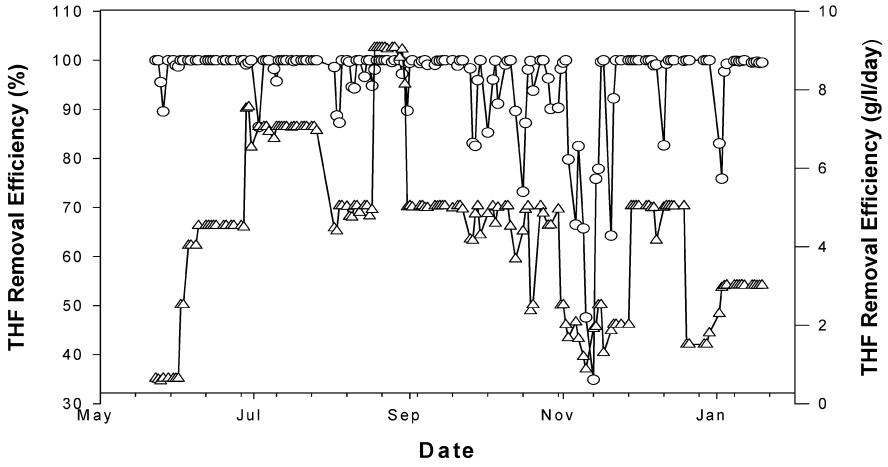


Fig. 4 Data represent the tetrahydrofuran (THF) removal per day based on composite influent and effluent samples and shown as a percentage (○) and grams per liter (△). Flow rate was 7.0 ml/min. THF removed during the nutrient limitation period (1–9 November 2000) was not used in calculating overall THF removal efficiency

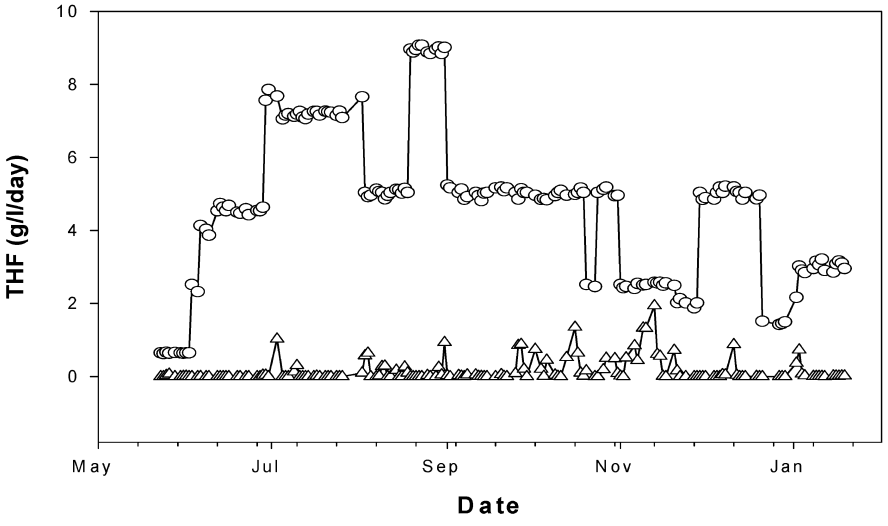


Fig. 5 Daily loading rate of THF in the influent (○) and total daily THF removal from effluent + stripping (△). THF was analyzed from composite influent and effluent samples by gas chromatography. Flow rate was 7.0 ml/min. No nutrients were added from 1–9 November 2000

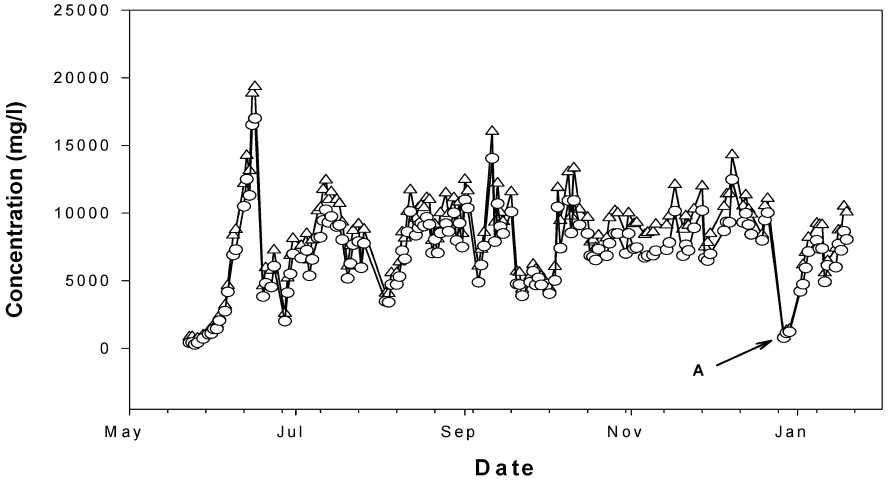


Fig. 6 MBR biomass measured as total suspended solids (TSS) (△) and volatile suspended solids (VSS) (○). Biomass was reduced to allow for unattended operation during the Christmas vacation (*A*)

100:1.6:0.6 following the nutrient limitation study. After the nutrient reduction, the effluent concentrations of ammonium and phosphate were at much lower concentrations while maintaining good THF removal performance.

Discussion

Isolate M1 and another *Pseudonocardia* sp. [18] are capable of growth on THF as the sole carbon and energy

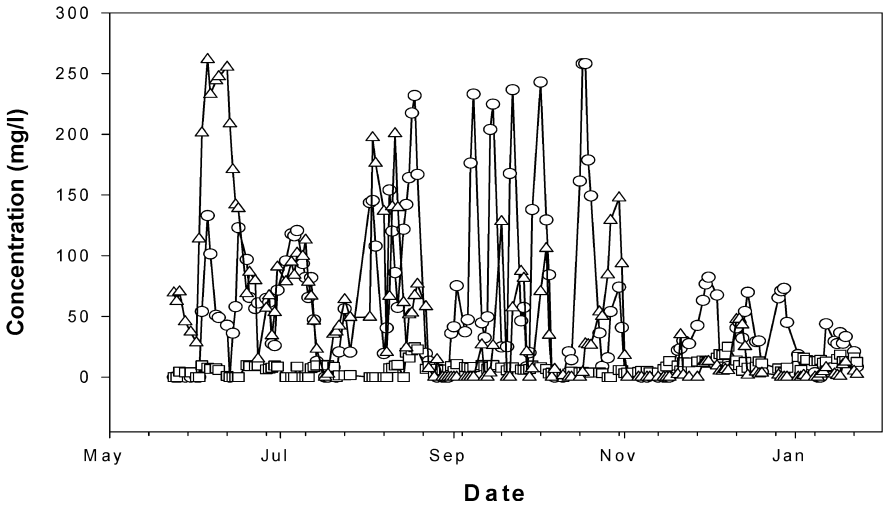


Fig. 7 Effluent samples were analyzed for ammonium (○), nitrate (□) and phosphate (△) by ion chromatography. No nutrients were added from 1–9 November 2000. Reduced nutrient dosing began 10 November 2000 and continued for the duration of the study

source. Isolate M2, along with another *R. ruber* strain [5], also have the ability to grow on THF as a sole growth substrate. Phylogenetic analysis indicates that isolate M1 may be a new *Pseudonocardia* species. However, whole genomic DNA/DNA hybridization experiments are necessary to prove this assumption. Previous attempts at isolating pure THF-degraders have led to the identification of several organisms in pure culture. The isolates have been identified as *R. ruber* [5], *Pseudonocardia* sp. [18] and an actinomycete designated as strain 1190 belonging to the family *Pseudonocardiaceae* [26]. All THF-degrading cultures identified thus far [4, 5, 18, 26], including those presented here, are Gram-positive Actinomycetes. Resistance to solvent attack by the cell wall of these isolates may allow the use of THF and other cyclic aliphatic ethers as a sole carbon and energy source [33]. A key aspect of the mixed culture was the ability to withstand THF concentrations as high as 800 mg/l without system upset. Other Gram-positive THF-degrading cultures have shown resistance to THF at concentrations in excess of 4,000 mg/l [18].

THF was readily degraded by the mixed culture at varied loading rates, achieving an average removal efficiency of at least 96% for the entire 8-month study. THF was quickly degraded in the MBR under normal operating conditions as evidenced by non-detectable effluent and reactor THF concentrations and no detectable THF stripping losses. Off-gas measurements confirmed that THF stripping losses were minimal at an influent feed rate of 4.32 g l^{-1} day^{-1}. Assuming the off-gas concentration was at the limit of quantitation of 2 ppmv, the calculated mass removal rate due to stripping would be 2.13×10^{-3} g l^{-1} day^{-1}. This represents a loss of THF due to stripping of 0.05% with an influent feed rate of 4.32 g l^{-1} day^{-1}. Therefore, stripping of THF to the air phase was assumed to be negligible when effluent and reactor THF concentrations were less than the limit of quantitation of 1.0 mg/l. However, under oxygen limiting conditions the THF stripping rate increased as THF began to accumulate in the reactor. High feed concentrations, high biomass concentrations and limited agitator speed caused oxygen limitation to occur, and this caused a decrease in THF removal efficiency. Reliance on biomass wasting to maintain adequate DO concentrations was a limitation of this system. Future scale-up will include a more efficient aeration system and increased agitator speed.

In comparison to the performance of other MBR systems reported in the literature, the maximum THF loading rate in this study was higher than that for a highly degradable COD waste stream [8, 34], but was significantly lower than that reported for phenol degradation using two-phase flow [20]. Loading rates of different MBR systems are affected by the degradability of the waste stream, biomass concentration, temperature and system configuration, and may not be readily comparable to one another. One possible reason for failure to maintain higher THF loading rates was DO limitation caused by the inability to stir the reactor at speeds greater than 250 rpm.

Reactor biomass concentrations were within the range reported for other MBR systems [8, 9, 21, 34]; however, concentrations as high as 60 g/l cell dry weight have been reported [20]. The high biomass concentrations in the MBR caused difficulties in maintaining reactor DO, in part because increasing the VSS increases the biomass viscosity [3] while the oxygen transfer coefficient decreases [24]. A continuous biomass-wasting system was not implemented because of the low hydraulic flows involved. Biomass production of 0.28 kg TSS/kg $COD_{removed}$ was very close to the lower part of the range of 0.3 to 0.6 kg TSS/kg $COD_{removed}$ for a conventional activated sludge treatment process [12]. Biomass production was similar to other MBR processes [8, 9, 29, 34], but was much higher than those observed by another MBR system [14]. One of the advantages of the MBR compared to conventional systems has been thought to be a very low solids yield. The results of this MBR system indicate that sludge yields are similar to conventional processes. However, the observed sludge yield, which is a combined result of growth and decay processes, is a function of system sludge retention time

(SRT). Because the SRT was constrained by the oxygen transfer capacity in this system, higher SRT values could not be evaluated. It is likely that a higher SRT would have produced lower sludge yields, although the membrane characteristics would at some point limit the maximum achievable SRT. This MBR system is non-optimized with respect to nutrient addition. It may be possible to further reduce the effluent nitrogen and phosphate concentrations by conducting batch nutrient addition studies. Reductions in nutrient loading may in turn help reduce overall biomass production.

Results of this pilot scale study suggest that the MBR may be useful in the pretreatment of wastestreams containing THF. The effects of pretreatment on the larger WWTP will need to be investigated; however, because of the low hydraulic flow it can be argued that the overall negative impact on WWTP performance would be small. For example, excess nitrogen and phosphate in the MBR effluent would be significantly diluted upon entering a WWTP and would not be expected to cause a significant increase in nutrient loading to a full-scale system. Likewise, biomass wasted from the MBR system would be diluted in a WWTP and would not be expected to negatively impact loading or sludge settling.

THF degradation in a full-scale WWTP is limited by a number of factors such as the recalcitrant nature of THF, the apparent small proportion of THF-degraders to total microbial biomass and substrate competition. By selecting for THF-degrading cultures, this MBR system achieved high biomass concentrations and high THF removal rates when THF was used as a sole carbon and energy source. Because of compact size and relatively low flow rates, this MBR system could be used in an industrial application for at-source THF pre-treatment, and thereby reduce or eliminate the THF load entering a full-scale WWTP. Plans are underway to pilot this system at 100 l using a process wastestream containing THF as the feed source.

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