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Thermophilic aerobic treatment of a synthetic wastewater in a membrane-coupled bioreactor

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Synthetic wastewater containing α-lactose and gelatin was treated in a thermophilic membrane-coupled bioreactor (MBR). Thermophilic (>45°C) treatment represents a potentially advantageous process for high-temperature as well as high-strength industrial wastewaters susceptible to reactor autoheating. Thermophilic systems, however, generally support a nonflocculating biomass that resists conventional methods of cell separation from the treated wastewater. MBRs were applied to thermophilic treatment systems because bacterial cells can be retained regardless of cell aggregation. Thermophilic aerobic MBRs were successfully operated at high levels of biocatalyst and produced a better effluent quality than analogous thermophilic bioreactors without cell recycle. At a hydraulic residence time (HRT) of 13.1 h, the chemical oxygen demand (COD) of the membrane eluate improved from 760 mg l^{-1} (without cell recycle) to 160 mg l^{-1} (with cell recycle). Bacterial community shifts were detected by denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR)-amplified 16S rRNA gene fragments — 6 of 13 bands disappeared within 2 days of MBR operation. A concomitant 40–50% reduction in physiological indicators of cell reactivity (RNA:protein; ATP:protein) was also observed. The specific activity of β-galactosidase and amino-peptidase, however, increased by 10–25%, indicating that there is a definite advantage to MBR operation at the highest biomass level possible. Nucleotide sequence analysis of 16S rDNA clones identified phylotypes from the low-G+C Gram-positive division and the β- and γ-subdivisions of Proteobacteria. *Journal of Industrial Microbiology & Biotechnology* (2001) **26,** 203–209.

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Introduction

Thermophilic aerobic biological treatment processes are potentially advantageous for high-temperature (>45°C) industrial wastewaters and for waste streams with a high organic content susceptible to reactor autoheating. However, thermophilic systems support a persistent, nonflocculating bacterial community [22] that prevents biomass separation from the treated effluent by conventional gravitational sedimentation/clarification. Failure to achieve biomass clarification prevents cell recycle to the aeration basin — a necessary step to maintain elevated levels of biocatalyst and produce a high-quality effluent. Application of thermophilic aerobic biological treatment, therefore, has been limited to pretreatment applications, typically involving further downstream treatment by more conventional mesophilic processes (e.g., see Ref. [23]).

Membrane-coupled bioreactors (MBRs) can achieve 100% cell recycle regardless of cell flocculation or lack thereof. Historically, MBRs have been used as model systems for studying the physiology of pure bacterial cultures [7,30,40] and for biotechnological purposes [6,39]. MBRs support extremely high levels of biocatalyst and therefore can metabolize substrate and generate metabolic products at much faster rates than conventional batch, fed-batch, or continuous-flow systems. In recent years, a reduction in membrane cost has stimulated interest in the application of MBRs as an alternative operational strategy for biological wastewater treatment [8,9,17,18,31].

MBRs impose fundamentally different physiological constraints than conventional bioreactor systems. Pure culture work with MBRs has demonstrated that the bacterial cells proceed through discrete phases in which growth yield incrementally declines due to increasing maintenance demands [30,40]. Mixed culture MBRs, however, have more complex growth patterns, where linear biomass increases are interrupted by plateaus and declines [18]. The physiological state of bacteria in MBRs appears to be an intermediate of nutrient-limited cells grown in chemostats and of starved cells [19,37].

In this paper, we demonstrate that thermophilic aerobic MBRs can successfully maintain high biomass levels, thereby improving pollutant removal efficiency compared to thermophilic processes without cell recycle. The associated bacterial communities adapt to the severe nutrient limitation by shifts in bacterial community composition and by physiological changes that maintain substrate-specific enzyme activity but reduce overall biochemical reactivity. Nucleotide sequence analysis of clones containing polymerase chain reaction (PCR)-amplified nearly complete 16S rRNA genes was used to further characterize the dominant phylotypes of the bacterial community.

Materials and methods

Membrane-coupled bioreactors (MBRs)

MBRs were initially operated as continuous-flow bioreactors without cell recycle. These bioreactors were inoculated with 1 ml of

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a suspension of microbial cells collected from the West Lafayette municipal wastewater treatment plant. Sterile feed medium was pumped into the 580-ml laboratory reactor (CYTOLIFT glass airlift bioreactor; Kontes, Vineland, NJ, USA) at the specified rate using a Masterflex variable-speed console drive pump (Cole Parmer, Vernon Hills, IL, USA). Cultures were aerated and mixed at a rate of 1.0–1.5 l min^{-1}. Previous studies have demonstrated that such aeration rates were sufficient to maintain dissolved oxygen concentrations >80% of saturation in mesophilic MBRs [18]. Reactor temperatures were maintained at 55°C (±0.2°C) by circulating water through the jacket of the reactor. Bioreactors were assumed to be suitable for transition to cell recycle mode after steady state had been reached with respect to biomass level (<5% change per day in OD_{600}) plus an additional four hydraulic residence times (HRTs).

MBR operation was initiated by pumping culture fluid from the reactor through a 0.2-μm pore size microfilter membrane cartridge (A/G Technology, Needham, MA, USA) and then returning it to the reactor. The MBR system was operated in fed-batch mode until the reactor volume reached a predetermined level (700 ml). Cell-free treated water was then continuously pumped from the microfilter at a manually set rate to maintain a constant level in the reactor. Reactor contents were rapidly pumped through the membrane cartridge (residence time <10 s) to approximate a well-mixed reactor system.

The synthetic feed medium was designed to be representative of a high-strength, readily biodegradable industrial wastewater, and contained (per liter of deionized water): 1.5 g gelatin (Aldrich Chemical, Milwaukee, WI, USA), 1.5 g α-lactose, 50 mg yeast extract (Difco Laboratories, Detroit, MI, USA), 830 mg $NaH_2PO_4 \cdot H_2O$, 570 mg Na_2HPO_4, 500 mg NH_4Cl, 60 mg $MgSO_4 \cdot 7H_2O$, 2 mg KCl, 600 μg $CaCl_2$, and 1 ml of SL7 trace mineral solution [5]. This medium had a chemical oxygen demand (COD) of 3100 mg l^{-1}.

Community analysis

Bacterial community fingerprints were generated by denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments as described previously [24]. Briefly, total DNA was purified from well-mixed reactor samples using the FastDNA Spin Kit per manufacturer's instructions (BIO 101, Vista, CA, USA). The variable V3 region of the 16S rRNA gene was amplified from the extracted genomic DNA by PCR using the PRBA338F [21] and PRUN518R primers [32] with a GC clamp attached to the forward primer [32]. DGGE was performed on a D-Gene apparatus (BioRad, Hercules, CA, USA) using a denaturing gradient ranging from 30% to 55% denaturant (100% denaturant contains 7 M urea and 40% vol/vol formamide). Following electrophoresis, the gel was stained with SYBR Green I (Molecular Probes, Eugene, OR, USA; diluted 1:5000 in 0.5× TAE), then visualized and photographed on a UV transilluminator.

Nearly complete 16S rRNA genes were PCR-amplified from genomic extracts (described above) using the pA and pH primers [11]. The final 50-μl reaction mixture contained: 1× PCR buffer, 75 μmol $MgCl_2$, 4 nmol deoxynucleoside triphosphates, 100 pmol of forward and reverse primers, and 50–100 ng of template DNA. PCR amplicons were ligated into the pGEM-T Easy cloning vector (Promega, Madison, WI, USA) and introduced into competent *Escherichia coli* DH5α cells *via* transformation [36]. Plasmids were extracted by the alkaline lysis method [36]. Different 16S rDNA clones were putatively identified by agarose gel electrophoresis of *Rsa*I-digested plasmid extracts and then confirmed by PCR–DGGE. Nucleotide sequences were determined using the Thermo Sequenase cycle sequencing kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and an ALFexpress automated sequencer (Amersham Pharmacia Biotech).

Nucleotide sequences were compared with sequences in the GenBank database [4] with the BLASTn program [1] and in the Ribosomal Database Project (RDP) database [28] using the SEQUENCE_MATCH program. Nucleotide sequences were also checked for possible chimeric sequences using the CHECK_CHIMERA program at the RDP website. Phylogenetic trees were constructed by the neighbor-joining method [35] using DNAMAN (version 4.1; Lynnon Biosoft, Vaudreuil-Dorion, Quebec, Canada) and TREEVIEW [33] software. Different nucleotide sequences were arbitrarily clustered into groups with similarities of >99% to reduce the size of the dendrograms. Reference nucleotide sequences used in tree construction were obtained from the GenBank

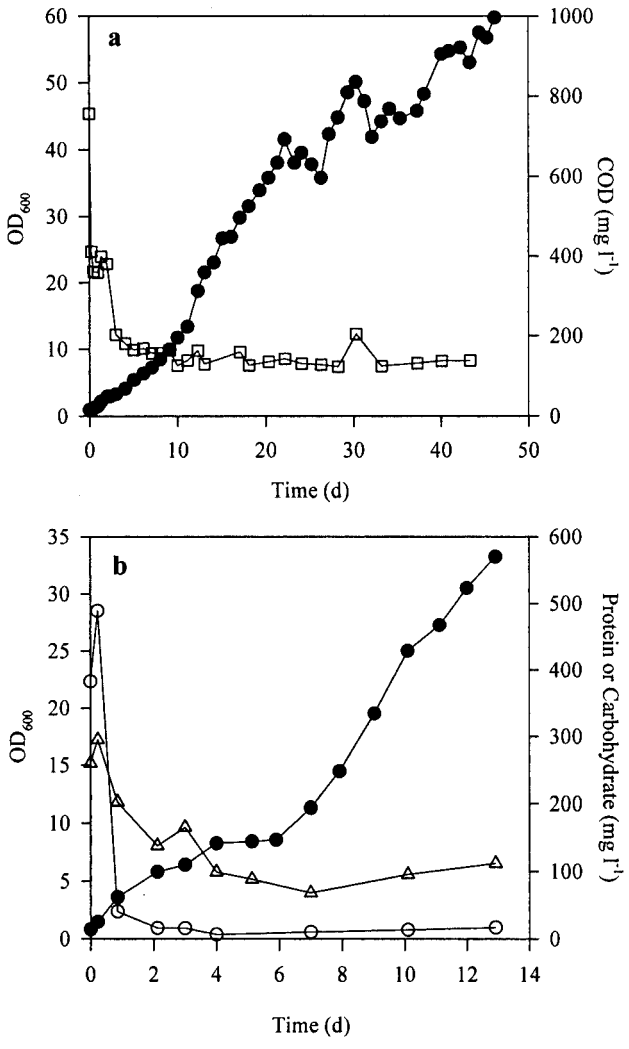

Figure 1 Response of two thermophilic bioreactors following transition from continuous-flow bioreactor to MBR: (a) increase in biomass levels coupled to COD removal at an HRT of 13.1 h, and (b) increase in biomass levels coupled to protein and carbohydrate removal at an HRT of 7.9 h. (●) OD_{600}, (□) COD, (△) protein, (○) carbohydrate.

Table 1 Summary of MBR operating characteristics (biomass accumulation rates, maximum biomass levels, and substrate removal efficiency) compared to chemostats

COD loading rate (g COD l^{-1} day^{-1})	HRT (h)	Biomass accumulation rate (OD_{600} day^{-1})	Maximum biomass level (OD_{600})	Chemostat mode			MBR mode		
				COD (mg l^{-1})	Carbohydrate (mg l^{-1})	Protein (mg l^{-1})	COD (mg l^{-1})	Carbohydrate (mg l^{-1})	Protein (mg l^{-1})
2.4	37.0	0.94	20	60	10	30	60	10	30
3.6	24.6	1.36	21	150	nd	nd	60	nd	nd
5.7	13.1	1.69	60	760	nd	nd	160	nd	nd
9.4	7.9	2.21	45	1300	400	300	200	15	100

COD, protein, and carbohydrate concentrations were measured in the effluent stream. Influent concentrations were: COD=3100 mg l^{-1}, protein=950 mg l^{-1}, and carbohydrate=1350 mg l^{-1}. nd, not determined.

database. Nearly complete 16S rDNA sequences (>1500 bp) from this study as well as reference sequences were optimally aligned [38] prior to tree construction. Nucleotide sequences determined in this study have been deposited in the GenBank database under accession nos. AF309808 to AF309818.

Analytical methods

Cell concentration was measured as optical density (OD_{600}), particulate protein, and/or dry cell mass. OD_{600} was measured at 600 nm with a 1-cm path length on a Shimadzu UV–visible recording spectrophotometer (Model UV 160U; Shimadzu, Columbia, MD, USA). OD_{600} samples were diluted to a value between 0.1 and 0.4; the reported OD_{600} values were then multiplied by the dilution factor to produce an estimated biomass level. Cellular protein was extracted [15] and measured according to the Lowry method [27] using bovine serum albumin (BSA) as a protein standard. Dry cell mass was measured by centrifuging (16,000×*g*; 5 min) a known sample volume in a preweighed, dry centrifuge tube. Centrifuge tubes were then dried at 103°C to a constant weight. One OD_{600} unit corresponded to 220 mg l^{-1} cell protein and 440 mg l^{-1} dry cell mass. OD_{600} was used as the primary measure of cell concentration to limit the sample volume (typically <50 μl) taken from the reactor. Cellular RNA was measured according to the orcinol method [15] using Type III RNA from Bakers Yeast (Sigma, St. Louis, MO, USA) as an RNA standard. Cellular ATP was analyzed by the luciferin/luciferase method [10].

COD analysis was performed on the microfiltration eluate according to the closed reflux, colorimetric method [3]. Soluble protein and soluble carbohydrate levels were also determined on this eluate according to the Hartee [14] modification of the Lowry method [27] and the anthrone method [15], respectively. BSA and glucose were used as protein and carbohydrate standards, respectively. Experimental analysis revealed that 1 g of gelatin corresponded to 0.6 g of protein (as BSA) and 1.08 g of COD; 1 g of α-lactose was equal to 0.9 g of carbohydrate (as glucose) and 1.04 g of COD.

Enzymatic activity was determined corresponding to the principal organic constituents of the feed medium. β-galactosidase activity was measured by the *o*-nitrophenyl-β-D-galactoside method [29]. Aminopeptidase activity was determined by the L-leucine-*p*-nitroanilide method [34]. One unit of enzyme activity

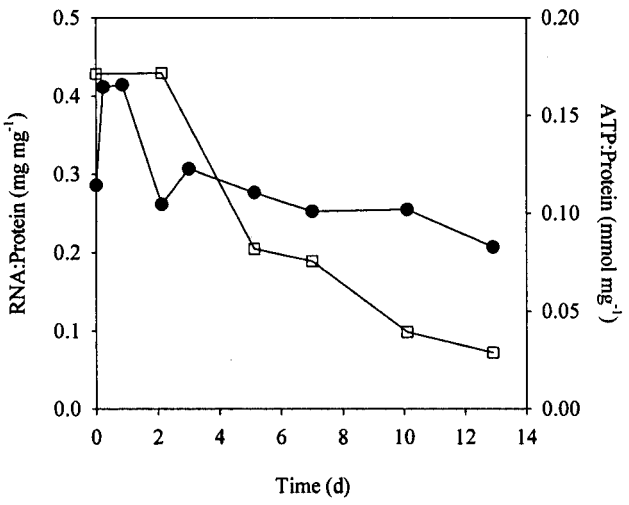

Figure 2 Physiological indicators of biomass reactivity (RNA:cell protein and ATP:cell protein) in a thermophilic MBR operated at an HRT of 7.9 h. (●) RNA:protein, (□) ATP:protein.

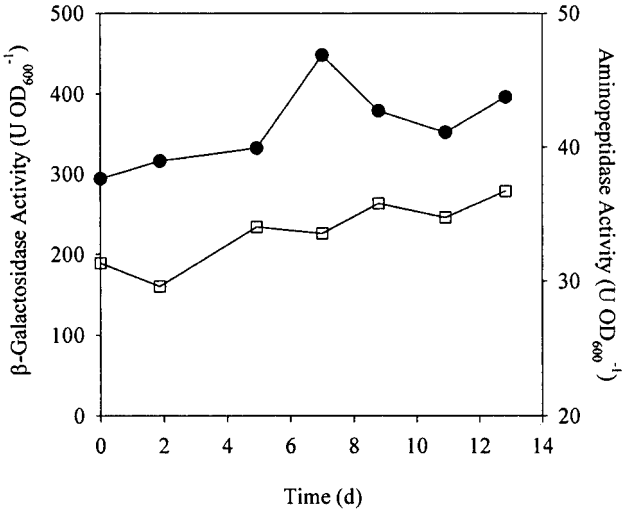

Figure 3 Biomass catabolic enzyme activities (specific β-galactosidase and aminopeptidase) in a thermophilic MBR operated at an HRT of 37 h. (●) β-galactosidase, (□) aminopeptidase.

corresponded to the production of 1 μmol of either *o*-nitrophenol or *p*-nitroaniline per minute (relative to controls).

Results

MBRs were first operated as continuous-flow bioreactors without cell recycle until steady state was reached with respect to biomass concentration. Following a minimum of three additional HRTs to ensure that a definitive physiological state had been established, a 0.2-μm pore size microfilter membrane system was connected to initiate operation of these bioreactors in 100% cell recycle mode. During this transition, the reactor volume increased from 580 ml (without membrane) to 700 ml (with membrane) by operating the MBR system in fed-batch mode.

The MBR performed in a consistent pattern following transition to cell recycle mode. Biomass increases were coupled with a concomitant decrease of substrate concentrations in the membrane eluate (Figure 1). At an HRT of 13.1 h, the COD of the membrane eluate declined from 760 to 160 mg l^{-1} after 5 days. COD levels then remained within 10% of this value for the next 40 days (Figure 1a). As with the COD levels, protein and carbohydrate levels in the membrane eluate declined asymptotically from their initial values within 2–5 days and then remained relatively constant throughout the remainder of the experiment (Figure 1b).

Biomass levels increased in complex patterns that could be approximately modeled as linear ($r^2 \geq 0.93$). The characteristics of this biomass increase became particularly complex for the run shown in Figure 1a once OD_{600} exceeded 40, with occasional plateaus and declines in biomass density. In the MBR experiment at an HRT of 7.9 h (Figure 1b), biomass accumulated without plateaus or declines even as OD_{600} exceeded 30.

Four different runs were performed with HRTs ranging from 7.9 to 37.0 h (Table 1). Comparing these four runs, the approximately linear rate of biomass accumulation increased as the organic loading was increased. The final concentrations of COD, protein, and carbohydrate in the treated water were lower at the higher HRTs. Except for the MBR with an HRT of 37 h, each of the runs demonstrated a reduction in the levels of COD, protein, and carbohydrate compared to operation without cell recycle; at the HRT of 37 h, the quality of the treated water was the same with and without cell recycle.

By assuming linear biomass increases, the bacterial growth rate can be calculated as this accumulation rate (see Table 1) divided by the time-specific biomass concentration. For example, the slowest growth rate in the MBR with an HRT of 13.1 h occurred when the $OD_{600}=60$, and corresponded to a bacterial growth rate (μ) of 0.0011 h^{-1}. This μ was 0.3% of the maximum growth rate ($\mu_{max}=0.32\ h^{-1}$) observed at 55°C on this synthetic waste stream [24] and 1% of μ in the continuous-flow bioreactor prior to initiation of MBR mode. Each of the four MBRs was operated until $\mu<1\%\ \mu_{max}$.

Bacterial physiological state was investigated with respect to the ability of the microbial cells to: (1) respond to environmental transients, such as increases in substrate levels (i.e., reactivity), and (2) catabolize the principal organics of the waste stream (i.e., activity). Reactivity was investigated by measuring the fraction of RNA and ATP per cell mass (measured as particulate protein) (Figure 2). In the MBR operated at an HRT of 7.9 h, RNA and ATP levels dropped by approximately 40% and 50%, respectively. The corresponding μ values declined from 0.15 h^{-1} (without cell recycle) to 0.003 h^{-1} over this time interval. Bacterial activities were determined in the MBR with an HRT of 37 h by measuring β-galactosidase and aminopeptidase activity — the initial enzymes used in the biodegradation of α-lactose and gelatin, respectively. Specific activities of β-galactosidase and aminopeptidase slightly increased by 25% and 10%, respectively (Figure 3), as μ declined from 0.8 h^{-1} (without recycle) to 0.002 h^{-1}.

Another potential factor that could influence MBR operation is bacterial community shifts as biomass accumulates. Such bacterial community shifts were verified in the MBR operated at an HRT of 7.9 h by DGGE of PCR-amplified 16S rRNA gene fragments (Figure 4). Prior to initiation of cell recycle mode (lane 2, Figure

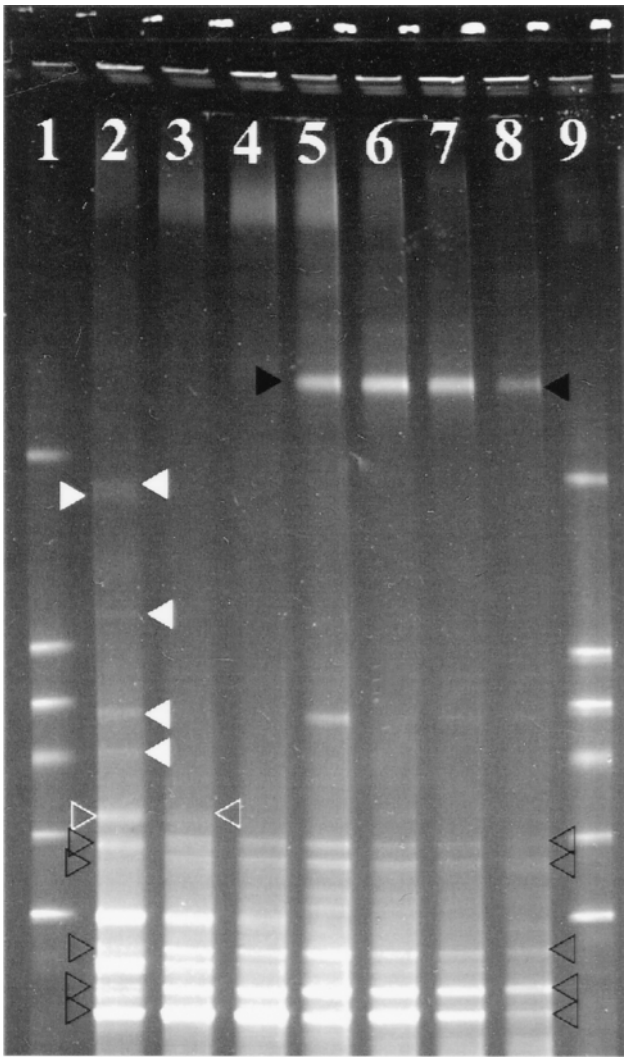

Figure 4 Denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA gene fragments of the consortia supported by a thermophilic MBR at an HRT of 7.9 h. Individual lanes contain community fingerprints from genomic DNA extracts taken at different times following initiation of the thermophilic MBR. Lane 1, marker [24]; lane 2, time 0; lane 3, 1 day; lane 4, 3 days; lane 5, 5 days; lane 6, 7 days; lane 7, 10 days; lane 8, 13 days; lane 9, marker. Solid white arrows identify bands (lane 2) that disappear within 1 day of MBR operation; open white arrows identify a band that disappears after 2 days of operation; solid black arrows identify a band that appears after 5 days of MBR operation; and open black arrows identify bands that are dominant throughout the MBR operation.

Table 2 Sequence length and closest phylogenetic affiliation of the clones analyzed in this study

Clone number	Sequence length (bases)	Phylogenetic relationship		
		Species	Accession number	Percent similarity
tmbr11-3	1539	*Pseudoxanthomonas* sp. M1-3	AB039330	99.8
tmbr11-6	1545	*Bacillus chitinolyticus* (strain IFO15660)	AB021183	94.4
tmbr11-7	1546	*Brevibacillus thermoruber*	Z26921	96.3
tmbr11-28	1540	*Pseudoxanthomonas* sp. M1-3	AB039330	99.7
tmbr11-29	1544	*B. chitinolyticus* (strain IFO15660)	AB021183	94.1
tmbr15-3	1542	*Pseudoxanthomonas* sp. M1-3	AB039330	99.9
tmbr15-4	1539	*Pseudoxanthomonas* sp. M1-3	AB039330	99.9
tmbr15-12	1534	*B. thermoaerophilus* (strain L420-91)	X94196	99.7
tmbr15-20	1519	*Paenibacillus* sp. WN9	AF164345	92.1
tmbr15-22	1521	Unidentified β-proteobacterium	Z93971	93.5
tmbr15-26	1531	*B. thermoaerophilus* (strain L420-91)	X94196	99.9

4), 13 bands were distinguished by PCR–DGGE. Five faint bands (solid white arrows, Figure 4) disappeared within 1 day of MBR operation; another band disappeared within 2 days of operation (open white arrows, Figure 4). However, after 5 days of MBR operation, one additional band appeared and remained for the duration of this MBR run (solid black arrows, Figure 4). A number of faint bands also appeared and disappeared throughout the duration of this MBR run. Several of the bands present at the initiation of cell recycle mode were common to each of the DGGE lanes (open black arrows, Figure 4).

Bacterial community structure was further investigated by cloning and nucleotide sequence determination of PCR-amplified, nearly complete 16S rRNA genes from days 1 and 5 of the MBR operated at an HRT of 7.9 h (lanes 3 and 5, respectively, in Figure 4). By screening 30 clones from each of these samples putatively containing 16S rDNA inserts, five and six different clones were obtained from the days 1 and 5 communities, respectively. Using the BLASTn algorithms, all of these clones had 16S rDNA inserts that were highly similar (>92%) to reference sequences found in the GenBank database (Table 2). None of the clones was identified as chimeric (data not shown). A dendrogram was generated to help visualize the phylogenetic relationships between these nucleotide sequences and the previously established bacterial lineages (Figure 5). This approach identified the 16S rDNA clones as being related to the low-G+C Gram-positive division (i.e., *Bacillus*, *Brevibacillus*, and *Paenibacillus* spp.) and the β- (*Ralstonia*, *Acidovorax*, and *Leptothrix* spp.) and γ-subdivisions (*Xanthamonas* and *Stenotrophomonas* spp.) of Proteobacteria.

Discussion

The goal of this research was to demonstrate the effectiveness of thermophilic aerobic MBRs for wastewater treatment. Thermophilic systems typically support a nonflocculating biomass [22] and an alternative approach is needed to retain cells in the bioreactor. Our results show that thermophilic MBRs support elevated biomass levels that improve the quality of treated

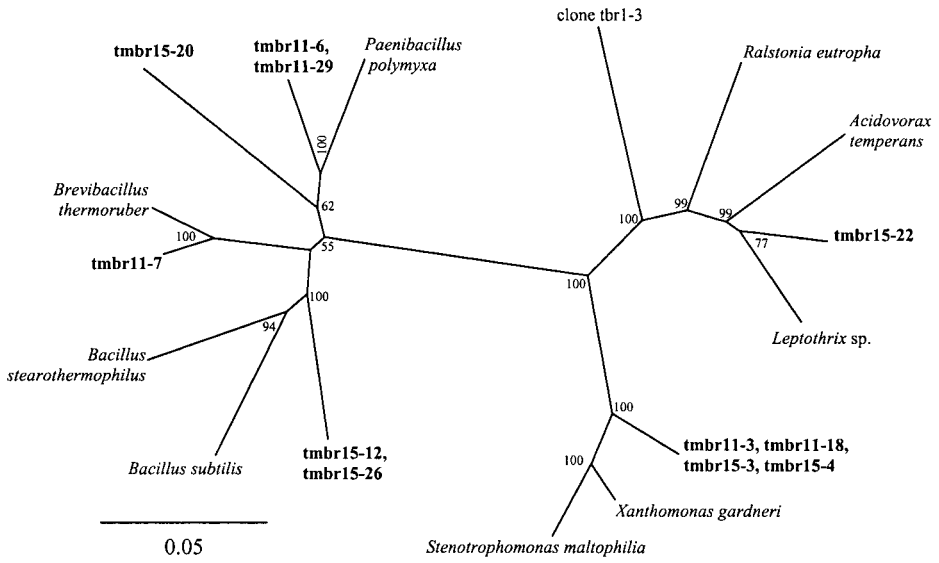

Figure 5 Unrooted phylogenetic tree revealing relationships between the clones obtained from a thermophilic MBR (HRT = 7.9 h) and reference strains from the domain bacteria based on 16S rDNA sequences. Bootstrap values are shown for nodes that had > 50% support of 1000 replicates. Clones sequenced in this study are presented in bold. Clone tbr1-3 is a thermophilic clone found in a previous study [26]. Clones with > 99% similarity were clustered and are listed on the same tree branch. The scale bar indicates an estimated change of 5%.

wastewater compared to thermophilic bioreactors without cell recycle. This work is of practical significance because a number of industrial wastewaters either are produced at high temperature or have an elevated organic content sufficient to promote reactor autoheating. Thermophilic treatment could be advantageous compared to other biological processes because cooling costs could be avoided.

From the perspective of bacterial physiology, MBRs present an excellent model system to study bacteria growing at very low rates. In this study, biomass accumulated in an approximately linear fashion in each of the four runs even as μ dropped to less then 1% of μ_{max} (e.g., Figure 1b). This result differs from previous work on both pure culture [30,40] and mixed culture [18,19,31] systems that demonstrated multiphasic growth kinetics in which biomass initially accumulated in a linear fashion but then slowed and eventually reached a plateau. Short-term plateaus and declines in biomass level were observed in one run at very high biomass densities ($OD_{600} > 40$) (Figure 1a); however, these were unstable and were followed by further biomass increases.

A consequence of slow microbial growth rates was that the cells regulated their physiological state by reducing their RNA (primarily ribosomal RNA) and ATP levels relative to the amount of cell protein (Figure 2). Similar results were observed previously during the treatment of graywater at mesophilic temperatures and are indicative of a reduction in culture reactivity in response to environmental transients [19]. Previous researchers have described this physiological state as an intermediate between nutrient-limited growth in continuous-flow bioreactors without cell recycle and that of starvation [19,37].

A pertinent issue to MBR operation at high biomass densities is whether the biomass can sustain catabolic activity despite the associated "reduced" physiological state (Figure 2). In the present study, the bacterial community increased the specific activities of catabolic enzymes by 10–25% from the levels expressed in a chemostat (Figure 3). These enzyme activities were approximately threefold higher than the activities expressed during nutrient-sufficient growth [25]. These results are consistent with previous work on *E. coli* grown in an MBR on a minimal growth medium [7]. From a practical perspective, these results imply that the capacity of MBRs to catabolize substrate is, at worst, proportional to the biomass level, and that there is an advantage to operating MBRs with as much biocatalyst as is technically feasible.

Microbial community composition shifted under the conditions imposed by the MBR, as demonstrated by DGGE of PCR-amplified 16S rRNA gene fragments (Figure 4). Previous researchers have demonstrated that PCR–DGGE generates a fingerprint of the dominant phylotypes in a bacterial community in natural environments [13] and wastewater treatment bioreactors [12,20,41]. Molecular-based methods of microbial community analysis are generally assumed to provide a considerably less-biased measure of bacterial community structure than traditional, cultivation-based techniques [2]. The culture shift that occurred as growth rates declined to <1% of their original value was significant, as only 5 of 13 bands initially present remained throughout the duration of the MBR run. The band that appeared after 5 days of MBR operation (solid black arrows, Figure 4) was likely present in the bioreactor prior to initiation of MBR mode, but not a numerically significant fraction of the total bacterial population. The observed shifts in bacterial physiology (Figures 2 and 3) and community structure also suggest that the consortia adapted to the conditions imposed by the MBR in a manner that sustains reactor function from the applied perspective (i.e., pollutant removal).

While PCR–DGGE provides an excellent method to track changes in bacterial community composition, it provides no phylogenetic information on the bacteria detected. Cultivation-independent community analysis by PCR–DGGE was therefore complemented by cloning and nucleotide sequence determination of PCR-amplified, nearly complete 16S rRNA genes. The advantage of PCR–DGGE is its simplicity and sensitivity (single base differences can be detected [32]), whereas PCR cloning and nucleotide sequence determination can be expensive and laborious. Although individual PCR–DGGE bands can be excised and sequenced, the short length of PCR-amplified DNA (~200 bp) is insufficient for phylogenetic identification unless the sequence is highly similar (>90%) to that of reference strains [16]. These two PCR-based approaches for cultivation-independent community analysis provide qualitatively comparable (i.e., the same phylotypes are detected) but quantitatively different results (i.e., the relative distribution of phylotypes varies) [26].

In this study, the determination of 16S rRNA gene sequences was used to identify bacteria from the low-G+C Gram-positives and the β- and γ-subdivisions of Proteobacteria. Previous investigators studying thermophilic wastewater treatment have found low-G+C Gram-positives by cultivation-based [22] and β-proteobacteria by cultivation-independent [26] approaches. The identification of moderately thermophilic aerobic heterotrophs among the γ-proteobacteria represents a novel discovery. In particular, there was very high similarity (>99%) between the γ-proteobacteria sequences identified and the 16S rDNA sequence of a mesophilic *Pseudoxanthomonas* spp. (see Table 2). This suggests that phylogenetically related bacteria can have markedly different temperature ranges for growth.

In summary, MBRs were successfully demonstrated as a viable technology alternative to retain biomass in a thermophilic wastewater treatment process. Retention of thermophilic bacteria resulted in reduced levels of substrate (COD, carbohydrate, and protein) in the treated reactor effluent. Analysis of bacterial community structure and function demonstrated that the bacterial cells adapted to the conditions imposed by the MBR by shifting to phylotypes that reduced indicators of cell reactivity (i.e., RNA:protein and ATP:protein) while slightly increasing catabolic enzyme activity.

Acknowledgements

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