Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2013.792139

51 (2013) 5909–5917
September

Taylor & Francis
Taylor & Francis Group

Application of a unique miniature MBR for screening the biodegradation of brominated flame retardants

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Received 2 April 2012; Accepted 1 January 2013

ABSTRACT

A unique miniature membrane bioreactor (mMBR) was designed and applied to examine the biodegradability of two complex organic compounds belonging to a family of brominated flame retardants (BFR) under continuous culture conditions using a bacterial consortium. BFRs are a widely used group of anthropogenic environmental contaminants. Many of these compounds are toxic, persistent, have limited biodegradability, and tend to bioaccumulate in the environment. Their widespread production and use combined with the inappropriate treatment and disposal of industrial wastewater have caused myriad global health and environmental concerns. Dibromoneopentyl glycol (DBNPG) and tribromoneopentyl alcohol (TBNPA) are aliphatic BFRs, classified as recalcitrant compounds, having half-lives of more than 100 years. Following successful debromination and complete biodegradation of the two target compounds in the mMBR, we used molecular and bioinformatic techniques to track changes in bacterial community composition during the biodegradation process.

Keywords: Membrane bioreactor; Miniature reactor; Brominated flame retardants; Dibromoneopentyl glycol; Tribromoneopentyl alcohol; Bacterial debromination

1. Introduction

Brominated flame retardants (BFR) are a diverse group of anthropogenic chemicals of varying structures, and chemical and physical properties. BFRs are widely used in many applications, including the manufacture of electronic equipment, textiles and plastic polymers, and in the car industry. They are used primarily to protect materials against ignition and to prevent fire-related damage [1]. Due to chemical characteristics of the bromide substituent(s), such as an electron-withdrawing effect, physical size and shape, chemical reactivity, increased compound lipid solubility, and reduced water solubility, these compounds are characterized by their high toxicities

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(acute and chronic), persistence, and bioaccumulation in the environment [2,3]. Despite these properties, only limited information is available on many of the BFRs and their fates in the environment.

The BFRs find their way into the environment via several routes: in wastewaters discharged from industrial facilities producing BFRs, manufacturing facilities that incorporate BFRs into products, and through volatilization and leaching from products during manufacturing or use or breakdown of foam products. Post-usage stages, including the disposal of BFR-containing products (e.g. electronic equipments), leaching from landfills, combustion, and recycling of waste products or adsorption onto dust particles also contribute to environmental pollution [4].

Recent reports have demonstrated the ubiquitous presence of BFRs at various concentrations in air, water, soil, wastewater, and sediments far from their production locations. Moreover, BFRs have been detected in plants and wildlife throughout the food web as well as in human tissues, blood serum, and breast milk [5–11]. Consequently, their widespread production and use combined with the inappropriate treatment and disposal of industrial wastewater and solid waste have generated massive global concern about their effects on the environment.

Physicochemical processes such as oxidation or adsorption on activated carbon are often used to remove BFRs and other halogenated organic compounds from wastewater. Nevertheless when applicable, biological treatment technology is generally preferred alternative.

Dibromoneopentyl glycol (DBNPG) and tribromoneopentyl alcohol (TBNPA) are aliphatic BFRs (Fig. 1) used as additives during the manufacture of plastic polymers and as chemical intermediates for the synthesis of high molecular weight flame retardants. The halogen groups of these two compounds make them highly resistant to biodegradation and, indeed, both are classified as not readily biodegradable having half-lives of more than 100 years [12]. Based on evidence of their carcinogenicity from experimental studies in animals, DBNPG is believed to be a human carcinogen, while TBNPA is thought to cause aquatic environmental damage [13,14].

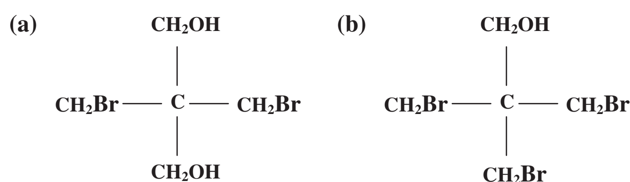

Fig. 1. DBNPG (a) and TBNPA (b) chemical structures.

In previous studies, we have demonstrated the biodegradation of DBNPG and TBNPA under aerobic conditions by a bacterial consortium enriched in a batch growth culture from soil sediments from a contaminated site. Biodegradation was accompanied by the release of bromide into the medium as a result of a bacterial debromination reaction [15,16] in which a hydrogen or hydroxyl group often replaces the bromide. The debromination reaction is considered the key reaction during the biodegradation of brominated organic compounds [17,18].

While complete biodegradation of both DBNPG and TBNPA was repeatedly attained in batch experiments [15,16], bacterial growth during these experiments was slow with low biomass yields and little or no flocculation. This precluded the use of chemostats or of gravitation-based biomass separation as a continuous flow system as the microbial community would have been washed out of the system [19]. Membrane-based solids separation was chosen as it allows complete biomass retention, including that of suspended bacterial cells. Even with slow growing, non-flocculating microorganisms, membrane bioreactor (MBR) systems enable the accumulation and maintenance of high biomass concentrations [19] and are increasing in popularity in various industrial applications, including wastewater treatment processes [20]. The high solids retention time attainable with these systems allows micro-organisms to adapt to recalcitrant compounds [21]. Indeed, MBR's were previously used in the successful removal of trace concentrations of halogenated organic compounds such as pharmaceuticals and pesticides [20]. However, the operation of laboratory-scale MBR is often a cumbersome and expensive procedure due to the need to incorporate a large membrane module into the system.

Here, we designed and applied a unique design miniature membrane bioreactor (mMBR) with small operating volume to examine the biodegradability of DBNPG and TBNPA under continuous culture conditions. Following successful biodegradation, we applied molecular and bioinformatic techniques to track changes in the microbial community during biodegradation.

2. Materials and methods

2.1. mMBR design

General mMBR design is similar to that of a previously described system designated a retentostat [19], but with smaller working volume (100 mL) and less complex structure and assembly (Fig. 2). The mMBR has three main parts: reactor body, stirrer house, and

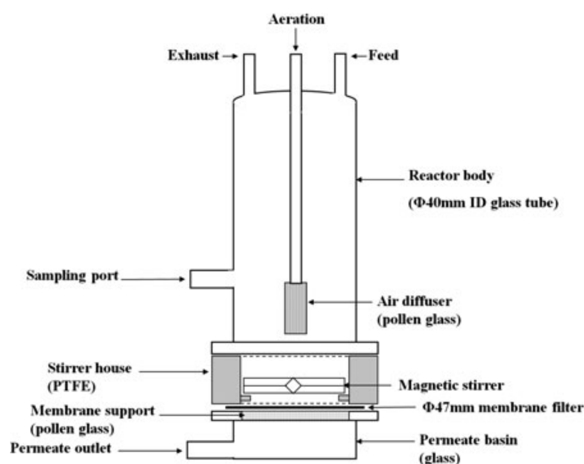

Fig. 2. Schematic drawing of the mMBR.

permeate basin. The reactor body, where the biomass grows and the biodegradation occurs, is a glass cylinder (internal diameter 40 mm, wall thickness 2 mm) with four pores used for feed, aeration, exhaust, and sampling. Aeration was performed by airflow through a pollen glass diffuser controlled by a rotameter. The stirrer house is made of polytetraflouroethylene and holds a magnetic 38×38 mm cross-shaped stirring bar suspended 1 mm above the membrane. The stirrer is used to mix the bioreactor contents, to improve oxygen mass transfer, and to prevent the precipitation of solids and biomass on the membrane. The permeate basin is a glass structure with an exit pore to collect the filtered permeate. The upper side of the permeate basin is made of pollen glass and supports the membrane. The membrane used here was a simple 0.2 μ 47 mm polyethersulfone (supor®) membrane filter (PALL Life Sciences, Mexico), but may be replaced with any other 47 mm membrane filter. The three parts of the mMBR are held together by aluminum clamps.

The mMBR was placed on an F13 magnetic stirrer driver (Fried electric, Haifa; Israel). A peristaltic pump (Manostat Carter 12/8 cassette pump with Manostat 72-560-000 cassette) was used to pump the feed from a glass bottle with GL45 cups (DURAN, Manz; Germany) to the feed pore of the mMBR and to collect the effluent. Santoprene tubing (Manostat Peristaltic Pump Tubing Links 0.25 mm model No. 72-470-025) linked the systems parts. The whole system was sterilized in an autoclave (120℃, 20 min).

2.2. mMBR operation

A system of two mMBRs operated in parallel was constructed to simulate continuous flow treatment of a synthetic mixture containing BFRs. The two BFRs, DBNPG and TBNPA, were tested for continuous biodegradation in the mMBRs. In each mMBR, a different compound was tested: mMBR-1: DBNPG and mMBR-2: TBNPA.

The results presented here represent the first 14 days of reactor operation. The feed to the mMBRs was a sterile (autoclave; 20 min at 121℃) mineral salt medium, following the Zahn-Wellens/EMPA test [22] with an addition of 1,000 mgL^{-1} yeast extract (Sigma Aldrich) and 100 mgL^{-1} DBNPG (mMBR-1) or TBNPA (mMBR-2) as a carbon source.

The operational conditions were as follows: hydraulic retention time: 10 days, flow rate: 6.9 $\mu LMin^{-1}$, aeration: 0.5 L air h^{-1} and mixing intensity: 400 rpm. The mMBR was operated at room temperature (20–22℃). To control foaming events, 50 μL of silicon-based polymer antifoam (Sigma) was added to the mMBR.

The mMBR's permeates were collected daily, and DBNPG, TBNPA, and bromide concentrations were measured to track biodegradation. Biomass samples were also collected to characterize the bacterial population.

2.3. Analytical methods

DBNPG and TBNPA concentrations were determined with an Agilent 19091S-433 Gas Chromatography-Mass Spectrometer (GC-MS) with a 5,973 Network mass selective detector and an HP-5MS ($0.25\,mm \times 30\,m \times 0.25\,\mu m$) column. GC-MS samples were prepared using Starta™–X 33 μ polymeric sorb ent (Phenomenex®, USA).

Bromide concentration was determined by an ISE25Br-9 ion selective electrode (Radiometer Analytical, USA). Samples for bromide analysis were prepared by diluting effluent sample with distilled water. The bromide concentrations in the samples were calculated using a bromide calibration curve. Based on the previous studies, an increase in the bromide concentration in the medium indicates the presence of a bacterial debromination reaction [15,16]. The initial concentrations were measured 3 h after inoculation of the bacterial consortium.

2.4. mMBR biomass

The inoculum (1:10 v/v) for mMBR biomass was obtained from a batch culture that previously demonstrated complete biodegradation of DBNPG. (Segev et al. [15,16], referred to this inoculum as the "bacterial consortium"). The batch culture was the 38th batch transfer of the bacterial consortium. The bacterial consortium was originally enriched from a soil sample

obtained from a site contaminated with the target compounds [15].

Biomass samples (1.5 mL) from the mMBR were collected through the sampling port using a sterile syringe and stored at −20˚C for subsequent analysis. Samples collected at three time points during mMBR operation (Day 0-Inoculum, Day 3-Acclimation, and Day 12-biodegradation) were eventually used for sequence-based analyses.

2.5. DNA extraction and PCR amplification

Total genomic DNA was extracted from mMBR biomass samples using a MoBio Power soil DNA isolation kit (MoBio Laboratories, Solana Beach, CA) according to the manufacturer's protocol with one modification: purified DNA was eluted in 40 μl of C6 solution (MoBio Laboratories) and stored at −20˚C. The DNA concentration was determined by an ND-1000 UV–vis spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Total DNA was amplified by polymerase chain reaction (PCR) with a Mastercycler gradient thermocycler (Eppendorf, Westbury, NY) using general 16s-rRNA primers for bacteria (Sigma-Genosys); forward primer, 8F (GGATCCAGA CTTTGAT(C/T)(A/C) TGGCTCAG), and reverse primer, 1512R (GTGAAGCTTACGG(C/T)TAGCTTGTTA CGACTT) [23]. The PCR reaction mixtures included 12.5 μl Reddy-Mix (PCR Master mix containing 1.5 mM $MgCl_2$ and 0.2 mM of each deoxynucleoside triphosphate (ABgene, Surrey, UK), 1 pmol each of the forward and reverse primers, and 2 μl of the DNA sample. Double-distilled water was added for a total final volume of 25 μl. The PCR protocol included an initial step of 5 min at 95˚C followed by 34 cycles of the following incubation pattern: 94˚C for 40 s, 52˚C for 40 s, and 72˚C for 100 s. A final extension at 72˚C for 20 min concluded the reaction. The PCR samples were stored at −20˚C until further processed.

2.6. Clone library construction and sequencing

The PCR products were purified by electrophoresis on a 0.8% agarose gel (Sigma), stained with ethidium bromide and visualized with a UV transilluminator. The approximately 1.5 kbp heterologous 16s-rRNA products were excised from the gel, and the DNA products were purified from the gel slice using a Wizard PCR prep kit (Promega, Madison, Wisconsin). The gel-purified PCR products were cloned into the pCRII-TOPO-TA cloning vector, as specified by Invitrogen (Carlsbad, CA), and transformed into calcium chloride-competent *Escherichia coli* DH5? cells according to the manufacturer's protocol and standard techniques. The bacterial colonies were scanned and picked (up to 48 colonies for each library), and the plasmid DNA was PCR-amplified with plasmid primers, M13-F and M13-R. The PCR products were purified and sequenced with M13-F primer using ABI PRISM dye terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase FS and an ABI model 373A DNA sequencer (Perkin–Elmer). Five clone libraries were constructed (inoculum and two time points for each mMBR).

2.7. Sequence analyses

The sequences were screened for chimeras using the Bellerophon server [24]. The classifier program at the RDP site [25], and the database of the National Center for Biotechnology Information and GeneBank (BlustN) [26] were used to assign sequences in taxonomical hierarchy. The sequences returning less than 95% homology were manually checked to further eliminate corrupt sequences. The sequences from all clone libraries were aligned using MUSCLE [27], and a distance matrix was generated using the MEGA 4.0 software pack [28]. The sequences were assigned to operational taxonomic units (OTU) using MOTHUR [29]. Thetayc (θ_{YC}), Bray–Curtis, and Jclass indices [30,31] were applied to compare the $OTU_{0.03}$ sequences (97% similarity) based on the composition and abundance of each, and a dendrogram was constructed using MOTHUR [29].

3. Results and discussion

3.1. mMBR operation and biodegradation analyses

The present study is the first published demonstration of the usefulness of the novel design mMBR (Fig. 2). The small operating volume combined with simple structure and ease of operation allows several mMBR systems to be operated in parallel making this a practical system for demonstrating continuous biodegradation processes in the laboratory under controlled operating conditions. In the present study, two mMBRs were operated in parallel under aerobic conditions for a period of 14 days as described. After that period, the mMBR-1 fed with DBNPG apparently suffered a collapse of the microbial community and the biodegradation was no longer observed. Complete biodegradation was observed in the mMBR-2 fed with TBNPA for 7 more days before the experiment was terminated (data not shown).

Both systems were inoculated with a bacterial consortium, that was shown previously, to biodegrade

the aliphatic BFRs, DBNPG, and TBNPA when grown in batch culture [15,16]. The DBNPG and TBNPA concentrations increased and then decreased significantly following 7 days of mMBR operation, while bromide concentrations increased in both systems. After 12 days of mMBR operation, DBNPG and TBNPA were no longer detectable in the systems (Fig. 3(a) and (b)). This result was further verified using adsorbable organic halides analysis where no halogenated organic compounds were detected by Day 14 (data not shown) indicating complete dehalogenation of the target compounds. In spite of the short testing period, these results are the first demonstration of the complete biodegradation of DBNPG and TBNPA under continuous culture conditions using a unique and simple laboratory MBR. This is a necessary step for further development of this process for future applications. Furthermore, since the removal of the halogen substituent usually makes the compound more easily biodegradable and susceptible to complete mineralization [32], it was previously suggested that the debromination reaction is the first step in DBNPG and TBNPA biodegradation and that the resulting intermediates then undergo complete mineralization [16]. The results presented here further support this assumption. The increases in DBNPG and TBNPA in the first days of the MBRs operation may be explained by the adsorption of BFRs on the membrane. However, this assumption needs to be further examined.

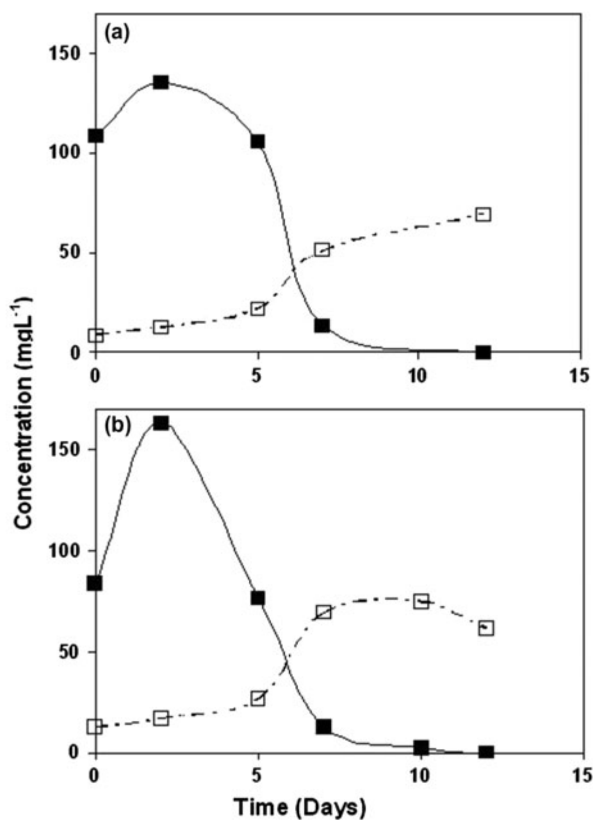

Fig. 3. Biodegradation and debromination of the BFR in the mMBR: DBNPG (■) and bromide (□) concentrations (mgL^{-1}) in mMBR-1 (a); TBNPA (■) and bromide (□) concentrations (mgL^{-1}) in mMBR-1 (b).

The time to complete biomineralization attained here was considerably shorter than the time reported for batch culture experiments, where DBNPG and TBNPA were biodegraded after 20 and 50 days, respectively [15,16] indicating that the continuous biodegradation of these compounds in an MBR system is a realistic target. However, further experiments are needed to establish the operation parameters required for the efficient biodegradation of these BFRs in MBR systems for prolonged periods.

There is a growing body of research addressing the biodegradation of different BFRs in laboratory cultures (for review see [4]). However, only a limited number of studies have demonstrated BFR biodegradation under conditions that are relevant to industrial treatment processes. Tetrabromobisphenol-A, the most widely used BFR, was successfully biodegraded under anaerobic conditions in a semi-continuous batch reactor [33]. Brenner et al. [34] showed aerobic biodegradation of the BFR 2,4,6–tribromophenol (TBP) in a laboratory sequencing batch reactor simulating an activated sludge process. Rayne et al. [35] demonstrated anaerobic reductive microbial debromination and photochemical degradation of the BFR 4,4´–dibromodiphenyl ether (BDE15) within a fixed-film plug-flow bioreactor. Since biodegradation under laboratory conditions does not guarantee successful biodegradation in practical biological treatment processes, it is important to test this biodegradation method under similar conditions. We believe that understanding the bromoneopentyl biodegradation process will be an important milestone in developing practical biological treatment processes for wastewater and sediments contaminated with these substances and possibly, other halogenated organic compounds.

3.2. mMBR biomass molecular analyses

To improve the biodegradation process, it is important to identify the microorganism involved and to understand the biochemical pathways that lead to biodegradation. To track changes in the microbial community during the biodegradation process, 16s-rRNA gene sequences were amplified from total DNA of the inoculum (Day 0) and from two additional time points during mMBR operation (Day 3-acclimation and Day 12-biodegradation).

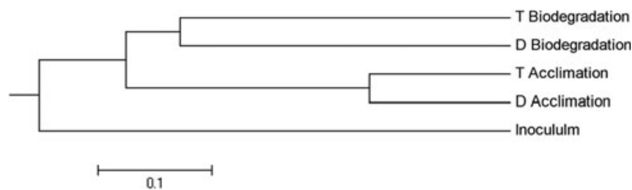

Fig. 4. $OTU_{0.03}$ based dendrograms describing differences between the clone libraries based on Thetayc (θ_{YC}): D = DBNPG; T = TBNPA. Scale represents distance between nodes—calculated as (1 - similarity).

The composition of the bacterial consortium community as represented by the generated clone libraries was analyzed based on the composition and relative abundance of OTU defined using farthest neighbor analysis at 3% sequence divergence ($OTU_{0.03}$) using the θ_{YC} community similarity index (Fig. 4) [31]. The results indicate that the bacterial communities in the two mMBRs rapidly diverged from the inoculum and from each other during 14 days of mMBR operation. A similar pattern was obtained using additional similarity indices (e.g. Bray–Curtis, Jclass) (Data not shown).

The communities in the two systems appeared to evolve similarly over the first three days of operation, but they markedly diverged by Day-12, the time point corresponding to the complete biodegradation of the target compounds. The inoculum for the current experiment was obtained from a batch culture biodegrading DBNPG and grown in the same medium as used in the current setting. Assuming that the community composition is determined only by the BFR fed to it, we should expect the community from mMBR-1 (fed with DBNPG) during biodegradation to resemble that of the inoculum. As seen from Fig. 4, this is clearly not the case. In fact, the clone libraries from both mMBRs during biodegradation are more closely related to each other than to the inoculum. We believe that this result indicates a strong and somewhat unexpected influence of the culture type (batch vs. continuous) on the microbial community.

The increasing divergence between the two communities as the biodegradation progressed indicates that the carbon source had a strong influence on microbial community composition. The higher similarity in community composition on Day-3 suggests that initial changes in community composition were driven mostly by the shared properties of the two systems–i.e. medium composition, like yeast extract–and reactor operating conditions, while the higher divergence on Day-12 likely reflected the different target BFRs. The grouping of clone libraries according to the time of sampling indicates that the microbial communities have yet to stabilize during the sampling period, as is to be expected, due to the short duration of the experiment.

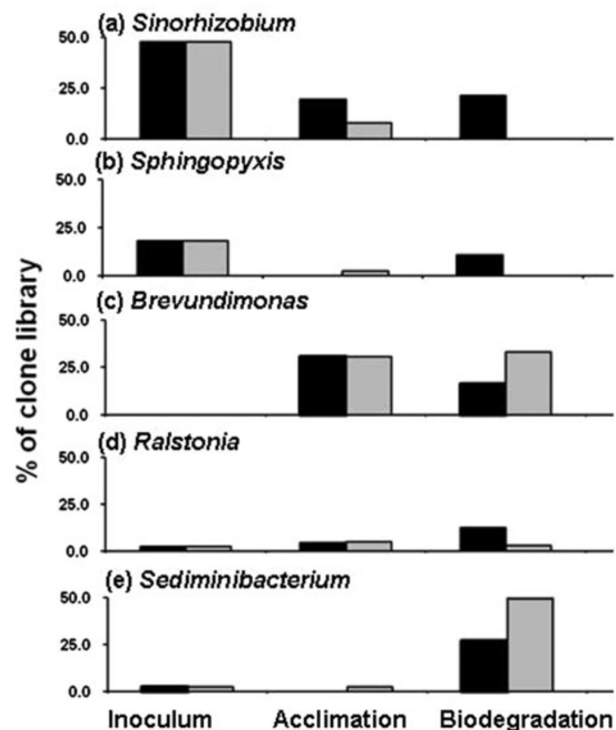

Fig. 5. Changes in relative abundance of six dominant bacterial groups from both mMBRs during the different stages of the operation (inoculum, acclimation, and biodegradation): (■) mMBR-1; (■) mMBR-2. The represented group of clones comprise more than 4% of the clone library. Identification of the groups by RDP and BlustN.

Changes in relative abundance of dominant bacterial groups from both mMBRs during the different operation stages (inoculum, acclimation, and biodegradation) are shown in Fig. 5. The five groups presented in Fig. 5 comprise close to 73% of the sequences in the clone libraries.

The clone library derived from the inoculum (identical for both systems) appears to be dominated by sequences related to the microbial genera *Sinorhizobium* (Fig. 5(a)) and *Sphingopyxis* (Fig. 5(b)) (48 and 18% of the clone library, respectively). The same groups were found to dominate clone libraries derived from three batch cultures fed with DBNPG in two separate but technically identical experiments (48–65% and 8–17% for *Sphingopyxis* and *Sinorhizobium*, respectively) [16 unpublished results]. A third group of sequences related to *Ralstonia* found in these clone libraries at 10–42% was represented in the mMBRs inoculum by a single sequence.

During the acclimation period (Day 3), both microbial communities (DBNPG and TBNPA) were dominated by *Brevundimonas* (30 and 31% of clone

library, respectively (Fig. 5(c)). Sequences related to *Sinorhizobium* were found at lower concentrations (20 and 8% for DBNPG and TBNPA, respectively; Fig. 5(a)), while sequences related to *Sphingopyxis* were either missing or negligible in both libraries (Fig. 5(b)).

By Day-12 (maximal biodegradation), there was a marked difference between the dominant sequences found in the two systems. The microbial community in mMBR-1 was dominated by five different microbial groups, each comprising between 11 and 27% of the clone library (Fig. 5(a-e)). Clones related to the genera *Sinorhizobium* and *Sphingopyxis* accounted for 21 and 11%, respectively, of the library (Fig. 5(a) and (b)). The relative abundance of the sequences associated with *Brevundimonas* and *Ralstonia* accounted for over 16 and 13%, respectively, of the clone library (Fig. 5(c) and (d)). A group of sequences related to *Sediminibacterium* that was found at low numbers in the previous stages rose to over 25% of the clone library (Fig. 5(e)). In contrast, the microbial community in mMBR-2 from the same day was dominated by only two groups, *Brevundimonas* and *Sediminibacterium* (33 and 52%, respectively, of clone library; Fig. 5(c) and (e)), with *Ralstonia* at lower abundance, and neither *Sinorhizobium* nor *Sphingopyxis* were detected.

Clone libraries from the inoculum and acclimation periods were also dominated by the sequences related to the order GP4 within the *Acidobacteria* (11% in the inoculum; 11 and 10% during acclimation for DBNPG and TBNPA, respectively) and various genera within the order *Bacillales* (together amounting to 14% in the inoculum, 26 and 14% during acclimation for DBNPG and TBNPA, respectively). Sequences from these groups were not found in the clone libraries representing biodegradation and were thus omitted from Fig. 5.

The results in Fig. 5 clearly demonstrate the dominance of different bacterial groups during different stages of the biodegradation process thus emphasizing the need to sample such processes at multiple time points.

The removal of the bromide substituent(s) in the debromination reaction during biodegradation usually reduces the compounds resistance to biodegradation, its toxicity, and the risk of forming toxic intermediates during biodegradation. Moreover, the physiological properties and substrate range of dehalogenating microorganisms will determine the process conditions and the range of transformations that can be obtained in practical treatment systems [17,18]. Thus, it is important to identify and characterize the microorganisms involved in this reaction and to understand the conditions favoring it.

Although it was suggested that specific populations involved in the dehalogenation reactions do not always dominate their consortia [36], the unique conditions of the MBR system (i.e. continuous flow of the target compound at relatively high concentrations) are expected to favor the growth of these populations. Accordingly, the dominant bacterial groups at the time of biodegradation (Day 12) are likely to play a key role in DBNPG and TBNPA debromination.

Three bacterial groups (*Brevundimonas*, *Sediminibacterium*, and *Ralstonia*) were represented in clone libraries from both systems during the maximal biodegradation stage. As the abundance of *Brevundimonas* increased in both systems during the acclimation stage (Day 3), its growth may be based on alternative carbon sources in the media rather than on the biodegradation of the BFRs.

Ralstonia strains were previously reported to possess dehalogenating abilities [37]. Sequences related to this genus were detected at low numbers during all the stages of mMBR operation and were repeatedly found in clone libraries from previous batch experiments [16 unpublished results]. This indicates the possible involvement of *Ralstonia* strains in the biodegradation and possible debromination of both BFRs.

The only group to increase in abundance during the biodegradation stage of both mMBRs was *Sediminibacterium*. This group was not found in previous batch clone libraries and may demonstrate the influence of the culture type on the microbial community.

As was previously suggested, the debromination reaction is the first step in DBNPG and TBNPA biodegradation and then the resulting intermediates undergo mineralization [16]. In bacterial consortia, different bacterial groups may carry out these reactions. A major difference between the batch and continuous experiments is in the concentrations of target compounds at the time of sampling. As batch cultures are usually sampled for genomic analysis following complete biodegradation [16 unpublished results], the target compound is no longer available at the time of sampling. As shown here, the microbial community composition in such cultures may be rapidly shifting so that the abundance of bacterial groups directly involved in the biodegradation may be below the detection limit at the time of sampling. In continuous cultures, the target compounds are constantly replenished thus favoring the growth of these bacterial groups. Hence, despite the lack of previous reports attributing dehalogenation abilities to *Sediminibacterium* strains, it is possible that this group plays a key role in DBNPG and TBNPA biodegradation and debromination.

Two bacterial groups (*Sinorhizobium* and *Sphingopyxis*) were found during the biodegradation of DBNPG but not TBNPA. These groups were repeatedly detected in the batch culture with DBNPG as the target compound [16,unpublished results], and both were previously shown to have dehalogenating capabilities [38,39]. Combined with the results present in Fig. 4, these results demonstrate the influence of the BFR on microbial community composition. Hence, it is possible that different bacterial groups are responsible for the debromination of DBNPG and TBNPA.

The microbial communities associated with DBNPG and TBNPA in the biodegradation stage appear to be different, although the dominant TBNPA bacterial groups are a subset of DBNPG bacterial groups. Further research is needed to determine whether different bacterial groups are involved in DBNPG and TBNPA debromination and biodegradation.

4. Conclusions

A unique mMBR with a very small operating volume was designed and applied to examine the biodegradability of two BFR, DBNPG and TBNPA. It enabled to demonstrate for the first time, the complete debromination and biodegradation of these target compounds under continuous culture conditions. Since the operation of laboratory scale MBRs is often a cumbersome and expensive procedure, the proposed mMBR may become a simple and efficient tool for laboratory screening of biodegradability. Even with its small-scale, it enabled to apply molecular and bioinformatic techniques to track changes in bacterial community composition during the biodegradation process. It was found that three dominant bacterial groups (*Brevundimonas*, *Sediminibacterium*, and *Ralstonia*) were represented in DBNPG and TBNPA clone libraries, and two additional groups (*Sinorhizobium* and *Sphingopyxis*) were found only in DBNPG.

Acknowledgments

The work was supported by a grant from BMBF-MOST cooperation in Water Technologies Grants WT-501, WT-901 and a grant from the Ramat Hovav Council, Israel. We also thank the Rieger Foundation and the Israel Commercial Industrial club for O. Segev's generous fellowship and The Israeli Ministry of Science for O. Shapiro's Eshkol scholarship. Special thanks to Mrs. Moran Zangi for all her help.

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