Biotrickling Filters for Municipal Odor Control – The Next Step!

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Abstract

Biotrickling filter (BTF) technology has become a widely accepted and preferred technology for the elimination of odors originating from municipal wastewater treatment plants and collection systems. It is capable of reliable and efficient odor removal, and can be more cost effective and environmentally friendly compared to conventional air pollution control technologies such as biofilters, physical-chemical, or adsorptive technologies. Despite the widespread use of BTFs for municipal odor control, there is still ample room to improve the technology. In particular, BTFs are considered to be very effective in removing inorganic odors, but its ability to remove organic odors is sometimes questioned. This paper reviews the deficiencies in the current BTF science and provides suggestions for further research on how it could be improved to effectively and reliably remove most, if not all, municipal odors to regulated levels.

BTF technology is most often treated as a "black box" and little is known about the complex combination of different physical-chemical and biological phenomena comprising the process. To improve the technology, it is essential that the fundamental mechanisms involved in odor removal be well understood. Key issues such as microbial biofilm architecture and ecology, media characteristics, mechanisms of mass transfer, and control of process conditions warrant further research in order to optimize the technology.

Molecular fingerprinting techniques such as terminal-restriction fragment length polymorphism (T-RFLP) could be used to characterize microbial populations in bioreactors, thus leading to a better understanding of which bacteria are present and their role in the oxidation of inorganic and organic odorous compounds. The use of scanning electron microscopy could provide insight into the biofilm architecture and bacterial morphology. The microbial ecology of a full scale BTF was evaluated using T-RFLP. The results show that T-RFLP can be an effective tool to further the understanding of the complex microbial ecology inside BTFs. Ultimately, such understanding can result in smaller, more efficient biotrickling reactors that effectively remove both inorganic and organic odors.

Keywords: BTFs, biological odor control, organic odor removal

Introduction

Control of volatile organic compounds (VOCs) and odor emissions from wastewater treatment facilities is a high priority for municipalities across North America (Lee et al., 2008). The use of odor degrading microorganisms for municipal waste air treatment is an important and developing application of cell immobilization technology (Deshusses, 2005). Biotrickling filter (BTF) technology utilizes immobilized microbial cells that are attached to a medium inside the reactor, which then oxidize the odorous constituents to odorless compounds. Since the process relies completely on biological means, it is environmentally friendly and has a much lower operating cost compared to physical/chemical alternatives (Gabriel and Deshusses, 2003).

Inspite of the widespread use of BTFs for municipal odor control, there is still ample room to improve the technology. In particular, BTFs are considered to be very effective in removing inorganic odors, such as hydrogen sulfide (H_2S), but its ability to remove organic odors is sometimes questioned. To improve the technology, it is essential that the fundamental mechanisms involved with odor removal in BTFs be better understood. Most often BTF technology is treated as a "black box" and little is known about the complex combination of different physical-chemical and biological phenomena comprising the process (Maestre et al., 2008). There are a number of key steps involved during odor removal in a BTF system, which could be summarized as: i) mass transfer of the odorous constituents from the air to the microorganisms, ii) oxidation of the odorous compounds to odorless by-products, and iii) removal of oxidative by-products and dead microorganisms from the reactor. Key issues such as microbial biofilm architecture and ecology, media characteristics, mechanisms of mass transfer, and control of process conditions warrant further research in order to optimize the technology.

Background

There are two main groups of organisms that are active in BTFs: autotrophic bacteria that are responsible for oxidizing inorganic odors (mainly H_2S), and heterotrophic bacteria that are responsible for oxidizing organic odors, such as methyl mercaptan (MM), dimethyl sulfide (DMS), and dimethyl disulfide (DMDS). Autotrophic H_2S-degrading microorganisms use carbon dioxide (CO_2) as their carbon source for growth, while H_2S provides energy for cell growth. On the other hand, heterotrophic microorganisms use organic odorous compounds as both their carbon and energy source (Brock et al., 1994). Co-treatment of both inorganic and organic odors in one BTF reactor therefore requires the existence of a consortium of both autotrophic and heterotrophic microorganisms containing sub-populations with different requirements for energy and growth.

Studies of the microbiology of BTFs have been limited. Most have been focused on the organisms used for BTF inoculation, and there are only a few that have looked at the *in situ* microbial ecology of BTFs. Microorganisms that have been used for inoculation include mainly *Thiobacillus*, *Acidithiobacillus*, and *Hyphomicrobium* (Sercu et al., 2005) or the pseudomonads (Chung et al., 1996; Zhang et al., 1992). For example, *Acidithiobacillus thiooxidans* has been shown to be suited for inoculation because of its ability to grow using H_2S as energy source at low pH. The important reactions involved in chemolithotrophic oxidation of sulfide, sulfur and thiosulfate under aerobic conditions can be summarized as (Tang et al., 2009):

$H_2S + 12\ O_2 \rightarrow S^\circ + H_2O$ $\quad \Delta G^{\circ\prime} = -209.4$ kJ/reaction
$S^\circ + 32\ O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$ $\quad \Delta G^\circ = -587.1$ kJ/reaction

$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+$ $\quad \Delta G^\circ = -798.2$ kJ/reaction
$S_2O_3^{2-} + H_2O + 2O_2 \rightarrow 2SO_4^{2-} + 2H^+$ $\quad \Delta G^\circ = -818.3$ kJ/reaction

Compared to H_2S removal, the removal of a mixture of reduced inorganic and organic sulfur compounds is challenging for several reasons. First, H_2S is preferentially degraded over dimethyl sulfide or other organic sulfur compounds (Cho et al., 1992; Wani et al., 1999; Zhang et al., 1991) because H_2S oxidation is the energy-yielding process (Smet et al., 1998). Second, the degradation of MM, DMS, and DMDS is realized with high efficiency at neutral pH, but decreases at low pH (Smet et al., 1998). Third, the degradation rates decrease in the order H_2S > MM > DMDS > DMS (Cho et al., 1991; Smet et al., 1998). These challenges are borne out by the few studies available. For example, pilot scale studies at the Hyperion Treatment Plant in

the city of Los Angeles showed that biotrickling filters were very effective in removing H_2S, but VOC removal was limited (Cox et al., 2002).

A key factor is pH. The autotrophic sulfide oxidizing bacteria typically grow best under low pH, acidic conditions, whereas heterotrophic bacteria grow best under neutral pH conditions. Co-treatment of both H_2S and organic odors would require that different process conditions be established inside the biotrickling filter to allow these groups of bacteria to co-exist. Several researchers have proposed two-stage systems (Kasakura and Tatsukawa, 1995; Ruokojarvi et al., 2001) consisting of two BTFs in series, with different pH conditions. Typically, the first BTF has a low pH (~3 to 4) and is operated to remove H_2S, while the second BTF is operated to remove the organic sulfides at a more neutral pH. However, ongoing pilot work has shown that it is possible to develop a pH gradient in media within the same biotrickling filter. Optimizing this single BTF reactor will allow a more cost-effective technology.

Objectives

In this study, the microbial ecology of a full scale, synthetic media BTF system treating odors from a municipal primary sludge treatment building was determined using polymerase chain reaction (PCR) of the Bacterial 16S rRNA gene and subsequent terminal-restriction fragment length polymorphism (T-RFLP) of PCR products. The microbial communities at different depths, expressed as groups of terminal restriction fragments (T-RFs), were analyzed to determine the possible existence of a community shift associated with depth within the BTF.

Materials and Methods

Full Scale BTF System: A full-scale BTF (BioAir Solutions, EcoFilter™ EF51) was installed at the Jacksonville Electric Authority Buckman Water Reclamation Facility in Jacksonville, Florida. The BTF contained structured synthetic EcoBase™ media and treated air from the primary sludge treatment building. The untreated air H_2S concentration varied between 100 and 200 ppmv, and the H_2S removal efficiency was greater than 99.5% at all times. The volumetric loading rates varied between 50 and 100 g H_2S/m^3.hr.

Media Sample Collection: Three (3) EcoBase™ media samples were collected at different depths of the BTF reactor and labeled as “top”, “middle” and “bottom”. The water pH at each of the media sample locations were 8.19, 4.56, and 2.90, respectively. The samples were shipped overnight on ice to NCSU Environmental Engineering Laboratories for analysis.

Sample Treatment: Upon receipt at the NCSU laboratories, the samples were immediately frozen in a -80 ^oC freezer, homogenized using sterile mortar and pestle, and hand-mixed using a sterile spatula in preparation for genomic DNA extraction. Remaining intact samples were stored at -80^oC for future scanning electron microscopy analysis.

DNA Extraction: A new laboratory-based DNA extraction method (Hicks et al., in preparation) that incorporates the removal of PCR-inhibiting humic acids via aluminum sulfate was utilized in obtaining the community genomic DNA. The addition of aluminum sulfate solution (100mM $Al_2(SO_4)_3$ + 100mM NaH_2PO_4; pH6.0) and 15-minute incubation to each of 0.25g sample precipitates the humic acid present in the homogenate (Dong et al., 2005). After incubation and pH adjustment to 9.0 - 9.4, cell disruption in the samples were accomplished with the addition of a lysis solution (100mM NaCl, 500mM Tris, 10% w/v sodium dodecyl sulfate, 1% sodium pyrophosphate; pH 9.0) in conjunction with bead-beating at maximum speed using 0.25g 100um sterile zirconium beads. After removal of the cellular debris by centrifugation, protein

contaminants were removed by the addition of 7.5M ammonium acetate and 10-minute incubation on ice. The DNA was later precipitated by adding 100% isoporanol, washed with 70% ethanol and resuspended in TE buffer (pH 8.0) after air-drying. DNA was quantified at 260 nm using a Nanodrop 1000 Spectrophotometer.

DNA amplification and Restriction Digestion: 16S rRNA genes were amplified using 6-carboxyfluoroscein (FAM)- labeled primer Bac-8F (AGAGTTTGATCCTGGCTCAG) forward and unlabeled Bac-1492R (GGTTACCTTGTTACGACT) reverse for *Bacteria*. The PCR reaction was carried out in 50 μl volumes consisting of 2× FailSafe PCR PreMix D (Epicentre Technologies, Madison, Wis.), 0.875 unit of FailSafe PCR enzyme mix, and 10ng of extracted DNA with the following Thermocycler conditions: an initial 94°C denaturation (12 min); 30 cycles of 94°C (60 s), 56°C (45 s), and 72°C (120 s); and a final extension step at 72° C (12 min).

PCR products from sample replicates were pooled and visualized on a 1.0% agarose gel with ethidium bromide staining, purified using Wizard PCR Preps DNA Purification System (Promega, Madison, Wis.) and quantified on a Nanodrop 1000 Spectrophotometer. The purified PCR products (350ng) were digested for 4 hours at 37° C with 5 units of *Hha*I, *Rsa*I and *Msp*I (New England Biolabs, Ipswich, Mass.) and 2 μl of the appropriate buffer.

TRFLP Analysis: After digestion, unwanted nucleotides were removed using the Qiagen Nucleotide removal kit (Qiagen, Valencia, Calif.). Purified digested samples were then sent to the NCSU Genomics Sciences Laboratory (GSL) where the terminal restriction fragments (T-RFs) were separated via capillary electrophoresis with a 3730xl DNA Analyzer (Applied Biosystems) using MapMarker size standards labeled with X-Rhodamine (BioVentures, Murfreesboro, Tenn.). T-RFLP electropherograms were examined using Peak Scanner software (version 1, Applied Biosystems) and T-RF peaks were analyzed based on the methods described by Abdo et al. (2005). Shannon-Weiner diversity and Species Richness were calculated using Species Diversity and Richness Software (version 4.1.2, PISCES Conservation Ltd).

Inferred Phylogeny: The identity of the dominant microorganisms contributing to the T-RFs was obtained using the web-based phylogenetic assignment tool (PAT) (http://trflp.limnology.wisc.edu/index.jsp) (Kent et al., 2003). T-RFLP-PAT allows researchers to determine possible phylogenetic assignments based on a database of predicted T-RFs. Predicted T-RFs are obtained from *in silico* restriction enzyme digests (e.g., *Hha*I, *Msp*I, and *Rsa*I) of known 16S rDNA sequences amplified using the bacterial 16S rDNA forward primer 8F (AGAGTTTGATCCTGGCTCAG). The output from PAT is a list of species/group matches with their corresponding T-RFs (*Hha*I, *Msp*I, or *Rsa*I).

Results and Discussion

Terminal restriction fragment length polymorphism relies on the sequence-specific digestion action of restriction enzymes to detect the presence of different sequences. These sequences are then used to infer phylogeny based on existing T-RFLP databases. T-RFLP is useful for characterizing microbial communities and determining community changes in structure and diversity (Liu et al., 1997). It is a relatively fast fingerprinting method compared to other techniques, such as Denaturing Gradient Gel Electrophoresis (DGGE), and allows frequent monitoring of microbial communities in environmental or bioreactor samples. However, T-RFLP, like most traditional PCR-based methods, is not quantitative. Biases in DNA extraction, PCR amplification, and enzyme digestion may affect the results and lead to non-accurate

quantification of species/group levels. Nevertheless, T-RFLP can be used to assess shifts in microbial community structure, particularly when used on the same sample matrix from the same bioreactor. Estimates of the abundance of specific T-RFs can be made using normalized peak areas that appear in the electropherogram. These T-RFs are expressed as OTUs (operational taxonomic units).

In this study, T-RFLP was used on samples from the same bioreactor. Table 1 shows the results for the *Hha*1 digestion. The results show the highest richness (number of T-RFs) for the bottom sample, and the lowest richness for the top sample. This indicates a higher number of OTUs in the inlet portion of the BTF, where there is a higher sulfide to oxygen ratio. Presumably, these are sulfide oxidizers that occur at lower pH. The smaller diversity at the top of the reactor is most likely represented by organisms that oxidize the organics present.

Table 1. Community indices of the BioAir samples based on *Hha*I digestion profiles of 16S rRNA gene.

Sample	Richness	Shannon Diversity (H)
Bottom	14	1.373
Middle	12	1.349
Top	8	1.281

The T-RFs from the bottom, middle, and top of the reactor can be arranged by relative abundance (as measured by normalized peak areas). Figure 1 shows a clear shift in the microbial community structure as inlet enters the bottom and exits at the top of the reactor. The dominant T-RFs at the bottom decrease in relative abundance in the middle and top of the reactor. For example, a T-RF of 200 bp is the most dominant (56%), but is only less than 5% at the top. Similarly, T-RF of 53 bp is 56% and 59% of the top and middle of the reactor, respectively, but is only less than 5% at the bottom of the reactor. Clearly, the microbial populations favored at the top are not as dominant at the bottom, and vice versa.

The inferred phylogeny from the PAT database (using the three enzymes) shows that a majority (71% to 83%) of the T-RFs corresponds to unidentified organisms (Figure 2). This shows the limitations of T-RFLP as a stand-alone technique for identifying organisms present in different environmental samples. In this instance, preparation of clone libraries and sequencing of the 16S rRNA gene would be most helpful; these studies are ongoing. However, it is interesting to note that sulfide oxidizing bacteria are still indicated by T-RFLP in the bottom and middle of the reactor, but not at the top. Other organisms indicated include iron oxidizers (*Gallionella*), heterotrophs, and ammonia oxidizers. An interesting OTU, which PAT assigns as *Rhizobium*, *Ensifer*, or *Cellvibrio*, is present in the middle and top of the reactor, but is absent at the bottom of the reactor. Since identification by T-RFLP is simply inferred, it is highly possible that this OTU is composed of different organisms that are currently unrepresented in the PAT database. Cloning-sequencing, and subsequent inclusion into the PAT database will allow more definitive identification of these organisms. Note also that with the exception of T-RF 173 bp, none of these T-RFs are closely related to those found by Maestre et al. (2008) in lab-scale reactors, also using Hha1. In that study, they reported T-RF 173 bp as *Thiothrix lacustris*, which they

showed as diminishing in the inlet part of their reactors. This is consistent with the results of this study, which shows T-RF bp 173 as present at the top of the reactor, but not in the middle or the top.

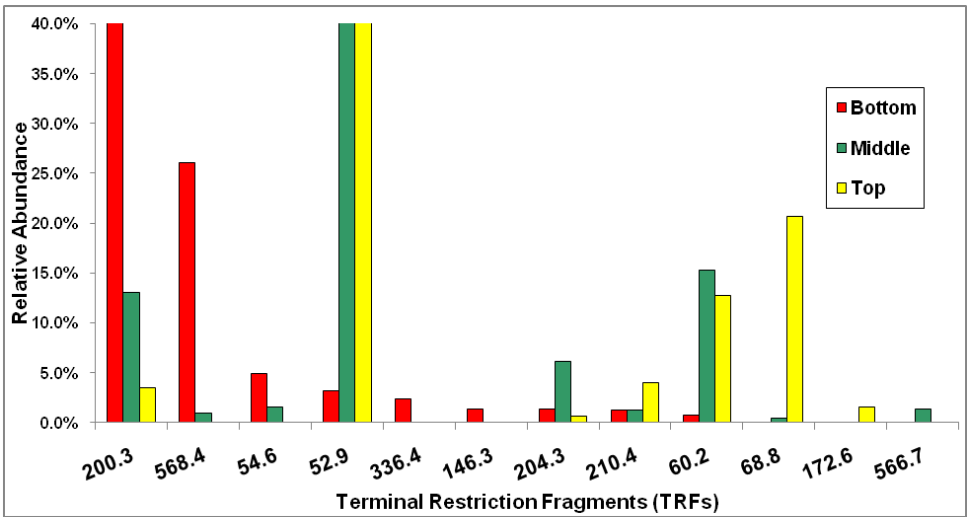
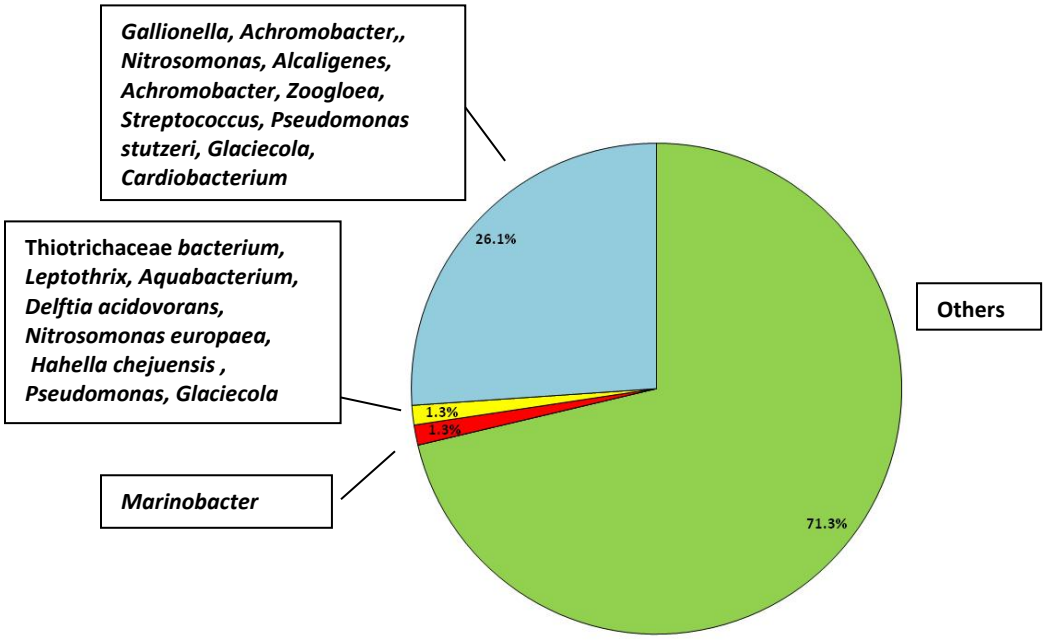
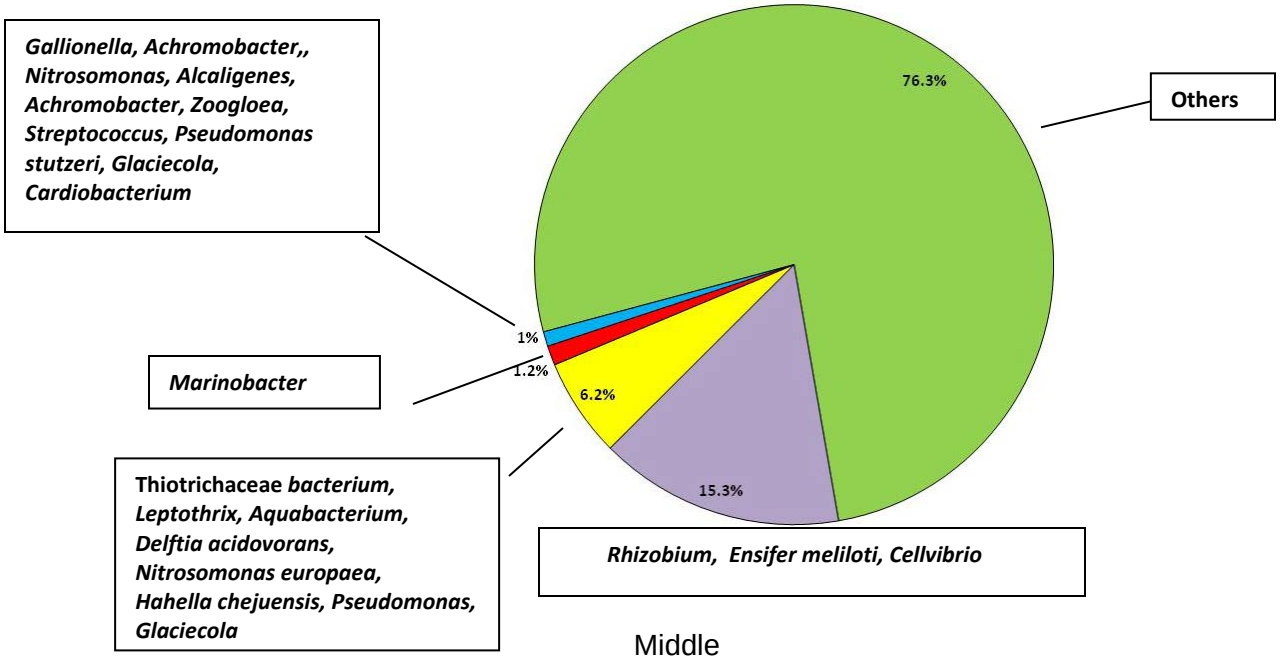


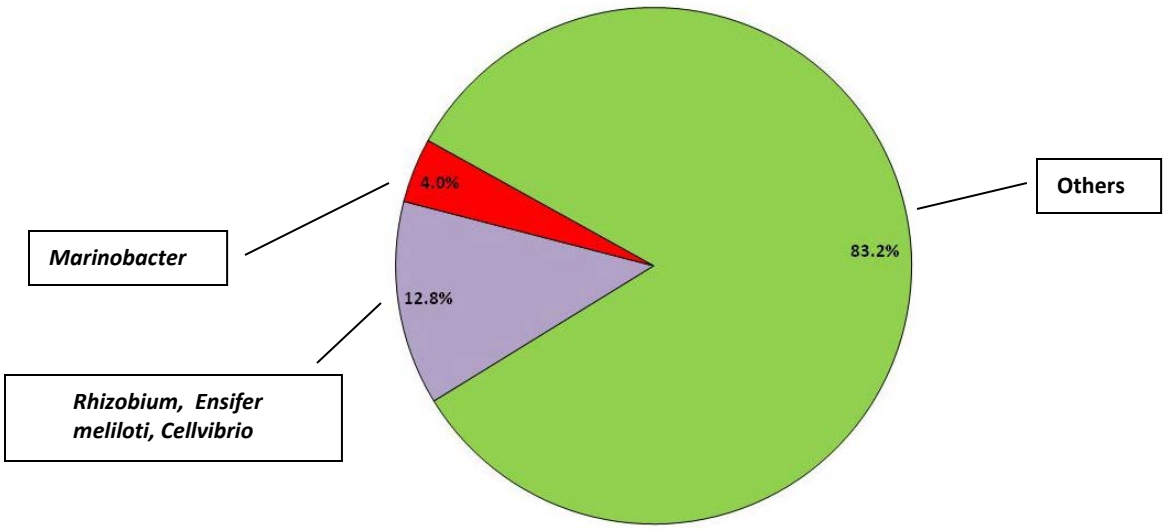
Figure 1. Dominant (>1%) Bacterial TRF profile based on HhaI digestion of 16S rRNA gene.



Bottom



Middle



Top

Figure 2. Inferred phylogeny of various T-RFs are obtained from *in silico* restriction enzyme digests (e.g. *Hha*I, *Msp*I, and *Rsa*I) of known 16S rDNA sequences amplified using the bacterial 16S rDNA.

Conclusions

T-RFLP analysis of the samples from a full-scale BTF shows a clear shift in the microbial community structure at different layers of the media. This shift is most likely due to the changing pH and sulfide to oxygen ratio at different depths. Dominant OTUs at the top are not as prevalent at the bottom, and vice versa. However, the correlation of organism identity to function is not clear, given that a majority of the OTUs cannot be definitively identified by T-RFLP. Sequencing of clone libraries is needed to help identify these organisms. This can be achieved by including clone sequences into the T-RFLP phylogenetic assignment tool (PAT) database. This work is currently underway.

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