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Structure of microbial communities in ethanol biofilters

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Abstract

Despite its central role in biofiltration, the ecology of the microbial community in biofilters remains largely unknown, primarily because of the difficulty of making detailed observations. Recently developed molecular techniques now allow determination of community composition from DNA extracted from the biofilter, without the need to culture the organisms. This study examined the structure of the microbial communities in ethanol biofilters through the application of the molecular fingerprinting technique, automated ribosomal RNA intergenic spacer analysis (ARISA). Three communities were tracked through acclimation to ethanol in liquid culture, and two of these were followed through growth within two biofilters packed with sand or lava rock. One of these created a successful set of biofilters while the other failed because of acidification. Analysis of the fingerprints revealed a strong decrease in species diversity towards the end of the acclimation period. Diversity indices show a rise in diversity for the biofilter with inconsistent removal of ethanol and low diversities in the successful biofilters, which removed ethanol with an efficiency near 80% for both packing materials. The results indicate that community fingerprinting shows promise as a means of assessing biofilter microbial communities.

Keywords: Bacterial community; Biofilters; ARISA

1. Introduction

Biofilms play an integral role in waste management applications such as groundwater treatment, wastewater treatment, and degradation of airborne chemicals in biofilters. Biofilters remove contaminants by exploiting the degradative abilities of biofilm communities, which are presumed to experience a strong selection pressure in favor of those members that can metabolize the contaminant or its metabolic products. In spite of the central role of biodegradation, investigation of the microbial communities in gas treatment biofilters has only just begun. Previous studies of the community structure in biofilters, most of them in wastewater treatment biofilters, have relied on the following methods to elucidate community structure: (a) culture methods (e.g. BIOLOG plates in [1]), (b) cloning and sequencing of conserved housekeeping genes (e.g. 16S ribosomal RNA in [2,3]) or functional genes (e.g. AmoA in [4]), (c) chemical fingerprints (e.g. respiratory quinones in [5] or phospholipid fatty acid analysis [6–8]), (d) nucleotide probes (e.g. 16S oligonucleotide probes in [6,9,10]) and (e) polymerase chain reaction (PCR)-based fingerprint techniques (e.g. denaturing gradient gel electrophoresis (DGGE) in [11–13]). Each of these methods provides information on the structure of the microbial community being studied; however, each method has limitations. Many of these methods have been reviewed in the overall context of biofilms [14], but not specifically biofilters.

The main limitation of culture methods is that they exclude any organisms that do not grow on the culture media. Clone libraries, sequencing, and, more recently, metagenome shotgun sequencing provide a large amount of specific data about the organisms in the sample and avoid the culturability limitations. These methods are powerful tools for species

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identification. However, to determine the species distribution in different communities many clones must be sequenced and analyzed; therefore, it is difficult to use these methods to compare communities [15]. Recent PCR-based molecular techniques have allowed generation of a community fingerprint from an environmental DNA sample, providing information on the number of different taxa present and an approximate abundance of each. Because of the PCR basis for the techniques, a slight overestimation of lesser members and a slight underestimation of greater members of the community may be expected. This has the benefit of not swamping the analysis with the dominant members of the community while still maintaining a semi-quantitative measure of their relative abundance [16,17].

Fingerprinting methods are the most time- and cost-effective molecular techniques for observing community changes [18,19]. A whole community can be characterized in less time than it takes to clone five members of that community. Fingerprints provide high-resolution data and include unculturable organisms. These techniques include denaturing gradient gel electrophoresis (DGGE) [20], terminal restriction fragment length polymorphism (TRFLP) on conserved genes, and automated ribosomal RNA (rRNA) intergenic spacer analysis (ARISA). DGGE relies on sequence heterogeneity to separate amplified DNA markers along a denaturing gradient [20]. TRFLP relies on heterogeneity in restriction digest sites on conserved genes between taxa in order to determine community structure. ARISA uses the 16S-23S intergenic spacer (internally transcribed spacer, ITS) length heterogeneity between taxa in order to distinguish members of the community [21].

TRFLP and ARISA are potentially more useful than DGGE for comparing communities because they can be standardized between different runs, compared between different laboratories, and cross-referenced by other researchers. In addition, ARISA and TRFLP profiles can be used to screen a clone library and attain a putative identification of the members of the community [14]. ARISA amplifies the variable spacer region between the highly conserved rRNA genes 16S and 23S in bacteria. One of the primers contains a fluorescent tag and attaches to the amplified DNA. This DNA is run on a sequencing gel and an electropherogram is produced, separating the amplified sequences according to their lengths. The intergenic spacer is variable in both sequence and length among different taxa, which may be able to provide a distinctive fingerprint-associated identification when included with even a partial 16S rRNA gene sequence [17]. Based on data available so far, the different lengths of amplified fragments distinguish bacteria near the species level [16]. ARISA was chosen for this study because of its higher resolution compared with TRFLP, its low cost, and its ease of comparison between different samples.

In this study, the bacterial community was investigated during typical acclimation and operation of an ethanol biofilter. Ethanol-consuming microbial communities can be upset when initial metabolic steps produce acids more rapidly than the acids can be oxidized in subsequent steps. Falling pH further depresses acid oxidation, and the system is trapped in a low pH state that does not support efficient ethanol removal. The biofilters of this study were operated primarily to study clogging, and so were treating high loads of ethanol, creating conditions under which acidification could occur. Comparisons between failed ($\ll$80% ethanol removal efficiency) and successful ($\sim$80% removal efficiency) biofilters and between communities on different packing materials were also made in order to determine the microbial community's response to the different conditions. The decrease in diversity observed during the acclimation and operation of biofilters, with only a few detectable kinds of bacteria, indicates that high diversity of the community is not important in successful biofiltration. Moreover, diversity seemed to suppress biofiltration in these lab biofilters.

2. Materials and methods

2.1. Inoculum acclimation

Preparation of the inoculum began with mixing 10 mL raw municipal wastewater treatment sludge (pH = 6.2) and 0.3 mL ethanol with 1 L of nutrient solution including 1.0 g/L KH_2PO_4, 1.0 g/L K_2HPO_4, 1.0 g/L KNO_3, 1.0 g/L NaCl, 0.2 g/L $MgSO_4$ and 0.02 g/L $CaCl_2$ and 0.5 mL of trace elements solution. The trace elements solution contained 12.2 g/L $FeCl_2{\cdot}4H_2O$, 4.09 g/L $MnCl_2{\cdot}4H_20$, 0.927 g/L $CoCl_2{\cdot}6H_20$, 2.37 g/L ZnCl, 0.616 g $CuCl_2{\cdot}2H_2O$, 0.579 g/L $NaMoO_4{\cdot}2H_2O$, 0.16 g/L H_3BO_3, 0.148 g/L KI, 0.067 g/L $NiCl_2{\cdot}6H_2O$ and 6.5 g/L EDTA $Na_2{\cdot}4H_2O$ [22]. The mixed community was kept in semi-continuous culture. Every 4th day, 1/20 of the volume of the culture was removed and replaced with new media. Ethanol addition was increased to 1 mL in two consecutive steps. The mixture was kept for 8 weeks and aerated continuously. The pH gradually increased to 7.1 (from $\sim$pH 4) just before the acclimated inoculum was transferred to the biofilters. The packing grains were first flooded with the nutrient solution and then with inoculum and then drained. The biofilter was flooded with fresh nutrient solution once a week during the experiments.

2.2. Experimental biofilter setup

The biofilters were acrylic plastic columns 25 cm long with 7 cm inner diameter. The airflow rate was 1.5 L/min (LPM), generating 20 s of empty bed residence time (EBRT) and the average inlet concentration was 100 ppm ethanol. The lava rock biofilter was operated in parallel with a sand biofilter, which has a smaller grain size. The contaminated vapor was generated by passing air through a reactor where liquid ethanol was injected by a syringe pump. Air was moisturized by passing it through a flask in which distilled water was nebulized by a fogger (Artistic Delights, Milpitas, CA).

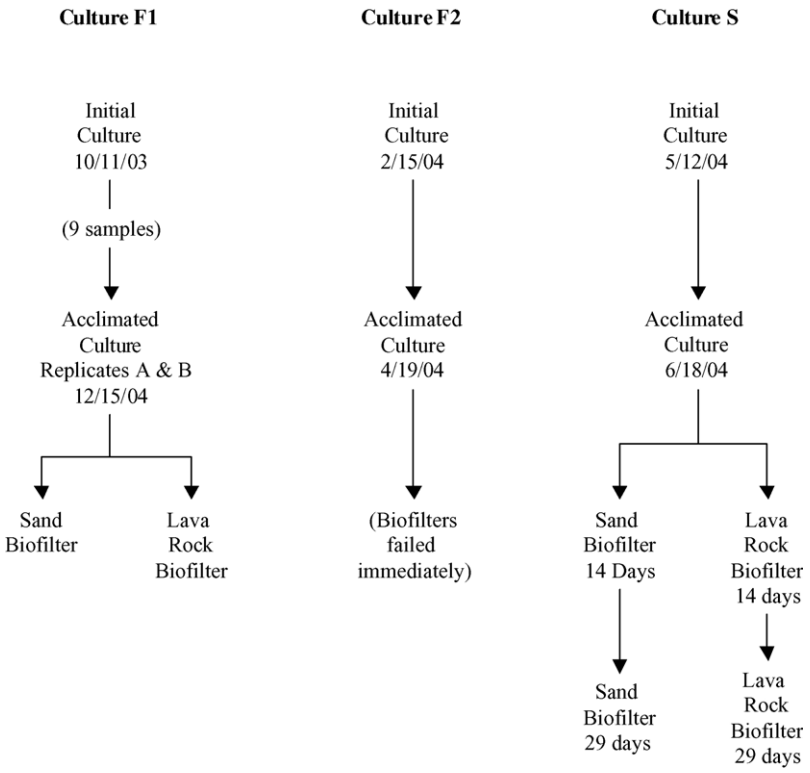

Fig. 1. Flow chart describing sampling scheme.

These two airflows were metered separately, and then mixed to produce the desired concentration in the influent to each biofilter. The head loss across the biofilter bed was measured by a U-tube water manometer.

2.3. Sampling scheme

Three evolving microbial communities were followed. The first, Failed 1, was sampled throughout acclimation and biofiltration of ethanol (Fig. 1). To track the community change during the 68-day acclimation period, the culture was sampled regularly. A second culture, Failed 2, was sampled at the beginning and end of the acclimation period, but no further work was done because the biofilters were failing immediately. The third culture, which led to successful biofilters (~80% removal efficiency), was sampled at the beginning and the end of a 1-month acclimation period and on two further occasions in the biofilters. A 10 mL aliquot was drawn from the inoculum using a sterile plastic pipette and placed in a sterile Fisher 15 mL centrifuge tube and frozen at −20 °C until the DNA was extracted. Samples were taken from the packing material (sand and lava rock) of a set of two "Successful" and two "Failed" biofilters near the ethanol inlet every 2 weeks for 1 month and stored in sterile Fisher 15 mL centrifuge tubes and frozen at −20 °C until the DNA was extracted. Sampling at the inlet was due to limitations on budget and time, and by constraints imposed by the design of the biofilters.

2.4. DNA extraction

DNA was extracted from the biofilter sample following the protocol in the Bio 101 Soil DNA Kit, quantified by Pico Green fluorescence (Molecular Probes Inc.), and diluted to 2.5 ng/μL.

2.5. Automated rRNA intergenic spacer analysis (ARISA)

ARISA was conducted on 5 ng of extracted DNA. PCR was carried out in 50 μL reactions containing 1 X PCR buffer, 2.5 mM $MgCl_2$, 1.25 mM dNTPs (Promega PCR Nucleotide Mix), 0.5 μM each of universal primer 1392 F and eubacterial-specific 23S-115R (labeled with a 5′-TET tag) [21], bovine serum albumin (40 ng/μL final concentration), and 2.5 U Taq DNA polymerase (Promega). Amplified samples for which negative controls had no amplification were purified, quantified, and diluted to a concentration of 5 ng/μL. These samples were run on an ABI 377XL automated sequencer with ROX-labeled Bioventures 1500 bp standards.

2.6. Analysis of ARISA fingerprints

Amplified DNA segments included an ITS length of more than 200 bp and approximately 215 bp of adjacent 16 and 23S ribosomal DNA; thus, the few fragments shorter than 400 bp were ignored. Fragments providing signals less than

five times baseline fluorescence in height were also ignored because they could not be distinguished from instrument noise. The area under each peak was then expressed as a percentage of the total integrated area. Simpson's reciprocal index (1/*D*) measures the number of equally common categories (e.g. major taxa or operational taxonomic units, (OTUs)) that will produce the observed Simpson's index. The Shannon–Weaver index (*H*) reflects the amount of disorder in the species distribution of the observed community. Evenness is an index that provides a sense of how evenly the different categories contribute to the Shannon–Weaver index. These indices were used to display the changes in the initial sludge communities during acclimation, and to compare between the successful and failed biofilters. Simpson's reciprocal index (1/*D*), the Shannon–Weaver index (*H*), and Evenness (*E*) [23] were calculated according to the following equations:

$$\frac{1}{D} = \frac{1}{\sum_{i=1}^{n} P_i^2} \tag{1}$$

$$H = \sum_{i=1}^{n} P_i \log(P_i) \tag{2}$$

$$E = \frac{H}{\log S} \tag{3}$$

where P_i is the fraction of each peak of total integrated area and S is the number of OTUs (>0.1% of total amplified DNA) present. Communities (i.e. all OTUs, again >0.1% of total amplified DNA) were analyzed by calculating Whittaker's index of association (S_w) [23] using the following equation:

$$S_w = 1 - \sum_{i=1}^{n} \frac{|b_{i1} - b_{i2}|}{2} \tag{4}$$

where b_1 and b_2 are the percentage contributions to amplified DNA of the *i*th OTU in samples 1 and 2, respectively. S_w is the similarity between assemblages, with values from 0 to 1. This index was used to compare initial and acclimated liquid culture communities and the communities in the variously packed biofilters.

3. Results and discussion

3.1. Acclimation period community diversity

The Simpson's reciprocal index and the Shannon–Weaver index show an oscillation between the starting value and an increase of 200 and 150%, respectively, with the evenness approaching 1 (i.e. similar abundance of OTUs) over the first 7 days (Fig. 2). This is likely due to the response of the community to the introduction of relatively rich nutrient solution that is added to the culture resulting in the creation of new niches for the sludge sample. Over the next 38 days, the community shifted to favor fewer taxa and the evenness declined. A decrease in all three indices after 45 days shows a further change in the acclimating community. The decrease in community disorder and in the number of dominant community members is likely due to competitive exclusion among ethanol users. The OTUs best adapted to metabolizing ethanol and its products presumably gained dominance towards the end of the 68-day period in semi-continuous culture. It is important, however, not to read too much into this decrease, since it is based on the final time point. The decrease in diversity is likely real because of similar index values (not shown) determined from the acclimated community used to inoculate the biofilter.

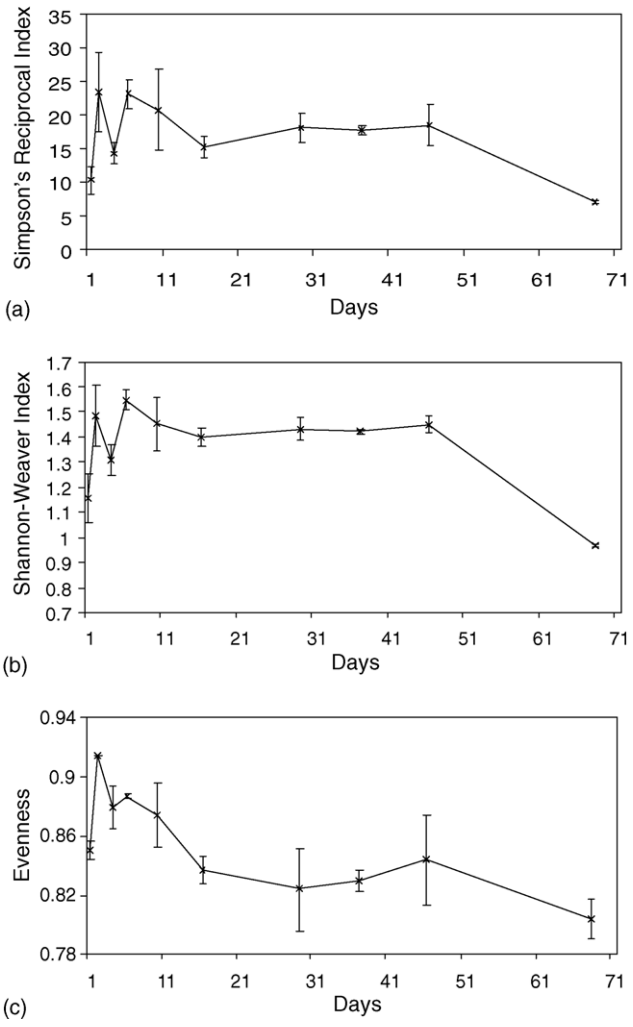

Fig. 2. Community change in the Failed 1 sample over a 68-day acclimation process. The diversity indices used are: (a) Simpson's reciprocal index (1/*D*), (b) Shannon–Weaver index (*H*), (c) evenness (*E*). Error bars denote one standard error confidence interval.

The Shannon–Weaver and Simpson's reciprocal indices of the microbial community after 14 days of acclimation were similar in value to those at the beginning (Fig. 2). While the observation might suggest that the community was returning to its original composition, the more likely explanation is that a new stable community was adapting to the ethanol carbon source. Examination of the electropherograms (not shown) showed no strong similarity between the 14-day acclimation

Table 1
Diversity indices of the initial and acclimated communities calculated from percent area of the ARISA fingerprint

Sample	Date	Diversity indices		
		Simpson's reciprocal index (1/*D*)	Shannon–Weaver index (*H*)	Evenness (*E*)
Initial Failed 1	10/11/2003	10.40 ± 2.06	1.16 ± 0.09	0.85 ± 0.01
Initial Failed 2	2/15/2004	4.25 ± 0.62	0.73 ± 0.03	0.72 ± 0.04
Initial Successful	5/12/2004	24.62 ± 0.89	1.55 ± 0.02	0.85 ± 0.01
Acclimated Failed 1A	12/15/2003	7.08 ± 0.12	0.97 ± 0.01	0.80 ± 0.02
Acclimated Failed 1B	12/15/2003	6.36 ± 0.04	0.92 ± 0.04	0.78 ± 0.07
Acclimated Failed 2	4/19/2004	27.98 ± 1.07	1.57 ± 0.03	0.91 ± 0.01
Acclimated Successful	6/18/2004	8.68 ± 0.42	1.12 ± 0.02	0.77 ± 0.01

Indices used are Simpson's reciprocal index (1/*D*), Shannon–Weaver index (H), and evenness (E). Confidence intervals are one standard error.

point and the initial point, further suggesting that a new community structure arose. The value of an acclimation period for the process to provide a community that can maximize the degradation of ethanol seems apparent.

3.2. Comparison of initial and acclimated communities

The "Initial" and "Acclimated" communities that led to the successful and failed biofilter communities show different trends in the Shannon–Weaver, Simpson's reciprocal, and evenness indices (Table 1). While there are only small decreases in the Shannon–Weaver and evenness indices, there is a three-fold decrease in the Simpson's reciprocal indices between the Initial Successful and Acclimated Successful communities, and nearly a seven-fold increase in the Simpson's reciprocal index (and also the largest change in the other indices), between the Initial Failed 2 and Acclimated Failed 2 communities (Table 1). These strong trends in diversity suggest a major restructuring of the community, and the other indices suggest, although not as strongly, a similar trend towards development of a different community.

The convergent trend between the Acclimated Successful and the Acclimated Failed 1 Simpson's reciprocal index (Table 1) suggests that the lower diversity is correlated with the removal of ethanol. The Failed 1 communities produced a biofilter that initially removed ethanol, but failed (removal efficiency oscillating between −96% and +93%) after 4 weeks; this may have been due to high ethanol loading in

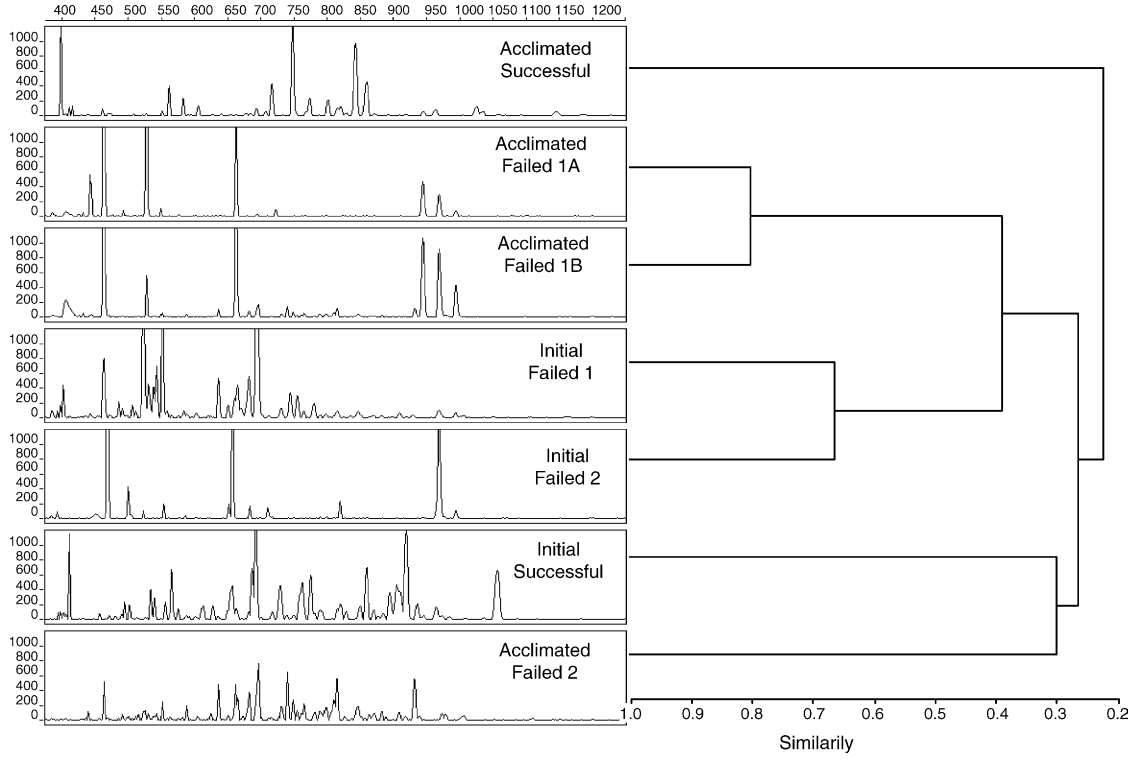

Fig. 3. Dendrogram of percent similarity of the initial and acclimated liquid culture communities as measured by Whittaker's index. The branches are labeled by the state of the community Initial or Acclimated and whether the community produced successful or failed biofilters. Left of each branch are the corresponding ARISA fingerprint electropherograms. Electropherograms are shown on the same scale (in bp) but on relative peak height scales. Acclimated Failed 1A and 1B represent replicate samples of one community.

the biofilters (discussed in detail in Section 3.3 below). The Failed 2 communities were unable to attain and maintain a high (80%) removal efficiency of ethanol for 2 weeks. These two communities were from different sludge communities but underwent replicate acclimation processes. The differing responses of Failed 1 and Failed 2 indicate that the sludge sample used may have affected the ability of the biofilter community to remove ethanol.

The Whittaker's index differences between the initial and acclimated communities that led to successful and failed biofilters are also striking (Fig. 3). The low similarity (<30%) between the Successful Initial and Acclimated communities reflects a substantial difference in their community structures. In fact, the electropherograms show that they do not share many OTUs. The moderately higher similarity (68%) between the two Initial Failed communities seems to stem from the major peaks in the electropherograms, i.e. the OTUs with the greatest peak area are of similar length in these sludge communities. The low similarity between both of the Successful communities and the rest of the communities could reflect the ability of bacteria in these communities to form a successful ethanol-removing biofilter system. The different sludge samples in this study did not produce an equally effective biofilter community.

As a side note, the inoculum for biofilters Acclimated Failed 1A and 1B represent replicates from the same community. Use of inoculum from the same community may provide an upper limit on the degree of similarity assessed by this index (82%). Whittaker's index considers the relative peak area in the comparison of the communities that allows for the differences in relative abundance of species to contribute to the measured similarity between communities.

3.3. Comparison of successful and failed biofilter communities

The community fingerprints of the initial communities, and acclimated communities at 0, 14, and 29 days from two pairs of successful and failed biofilters revealed distinct differences in the diversity of the communities (Fig. 4). During the first few days after the start of the biofilters, the successful and failed biofilters increased their removal efficiency from baseline levels to 80%. After that point, the successful biofilters maintained removal efficiencies at 80% while removal efficiencies of the failed biofilters dropped strongly and oscillated for the duration of the experiment. The diversity of the communities appears to correlate with differences in removal efficiency. Simpson's reciprocal and

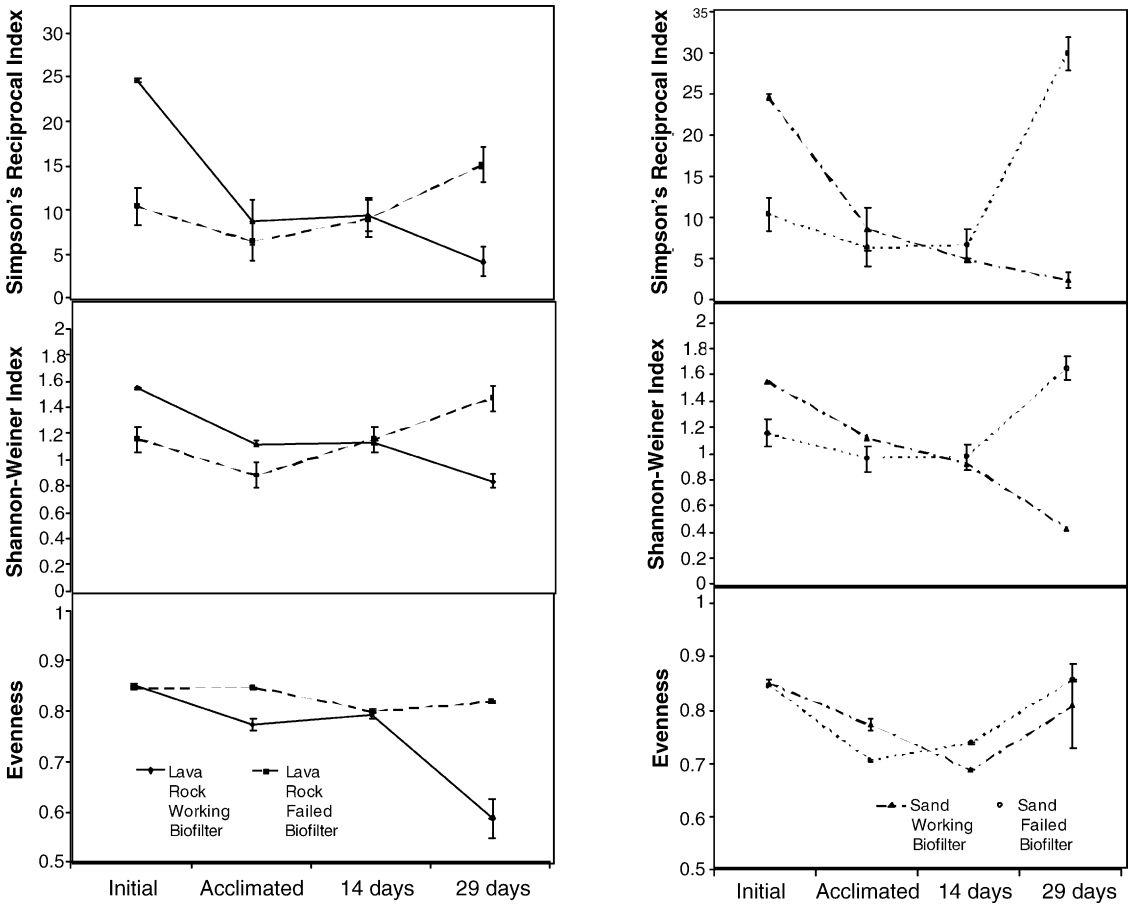

Fig. 4. Diversity indices of the successful and failed lava rock biofilters (left) and sand biofilters (right). Indices used are Simpson's reciprocal index (1/*D*), Shannon–Weaver index (*H*), and evenness (*E*). Error bars denote one standard error.

Shannon–Weaver indices for the successful and failed communities converge at 14 days but show a substantial drop after 29 days for the successful biofilters and increase sharply after 29 days in the failed biofilters (Fig. 4).

The high diversity in the communities also appears to be correlated with the acidity of the media. The relatively high diversity in the Acclimated communities compared to the 14 day biofilter communities correlates with the acidic (pH 4) conditions which occurred during the acclimation process. The pH was brought up to 7.1 before addition to the biofilters and the successful biofilter communities, which had a low diversity, maintained the near neutral pH. Acidity measurements of leachate (i.e. liquid that drained from the biofilters during normal operation) from the failed biofilters showed a pH of 4.5 in lava rock and pH of 5 in sand biofilter after 29 days. The acidity indicates increased production of acidic intermediates by these highly diverse communities. This is likely due to high ethanol loading in the biofilters as suggested by Devinny and Hodge [24]. Webster et al., [7] showed that microbial stress indicators measured through PLFA correlated with a low pH in biofilters. We may speculate that high ethanol loading in the biofilters could create new niches for bacteria (e.g. local anaerobic zones). The increase in diversity could result in greater competition within the biofilters could disrupt the normal ethanol metabolism by the community and produce a greater amount of acidic intermediates.

The evenness index drops sharply as expected in the Successful Lava Rock community, suggesting the emergence of dominant OTUs, but increases in the Successful Sand community and in both of the Failed communities, which indicates a community where the competition is more evenly distributed (Fig. 4). This does not indicate that the Successful Sand community is getting more diverse; in fact, the electropherograms show the emergence of a few overwhelmingly dominant OTUs in the sand biofilter 29-day sample, suggesting that the best-adapted organisms had become dominant. In each of the successful biofilters there were strong, dominant peaks at 985, 1000, 1007, and 975 bp; in the Sand 29-day sample, these peaks were responsible for nearly 90% of the total area. In the failed biofilters, none of these OTUs were dominant. These OTUs are likely key members of the biofilter system, possibly the minimum members in the Initial community necessary for ethanol degradation.

This differs from the high diversity found in an animal-rendering plant biofilter in Friedrich et al. [2] and in a wastewater bioreactor by Hu et al. [5]. Some caution should be

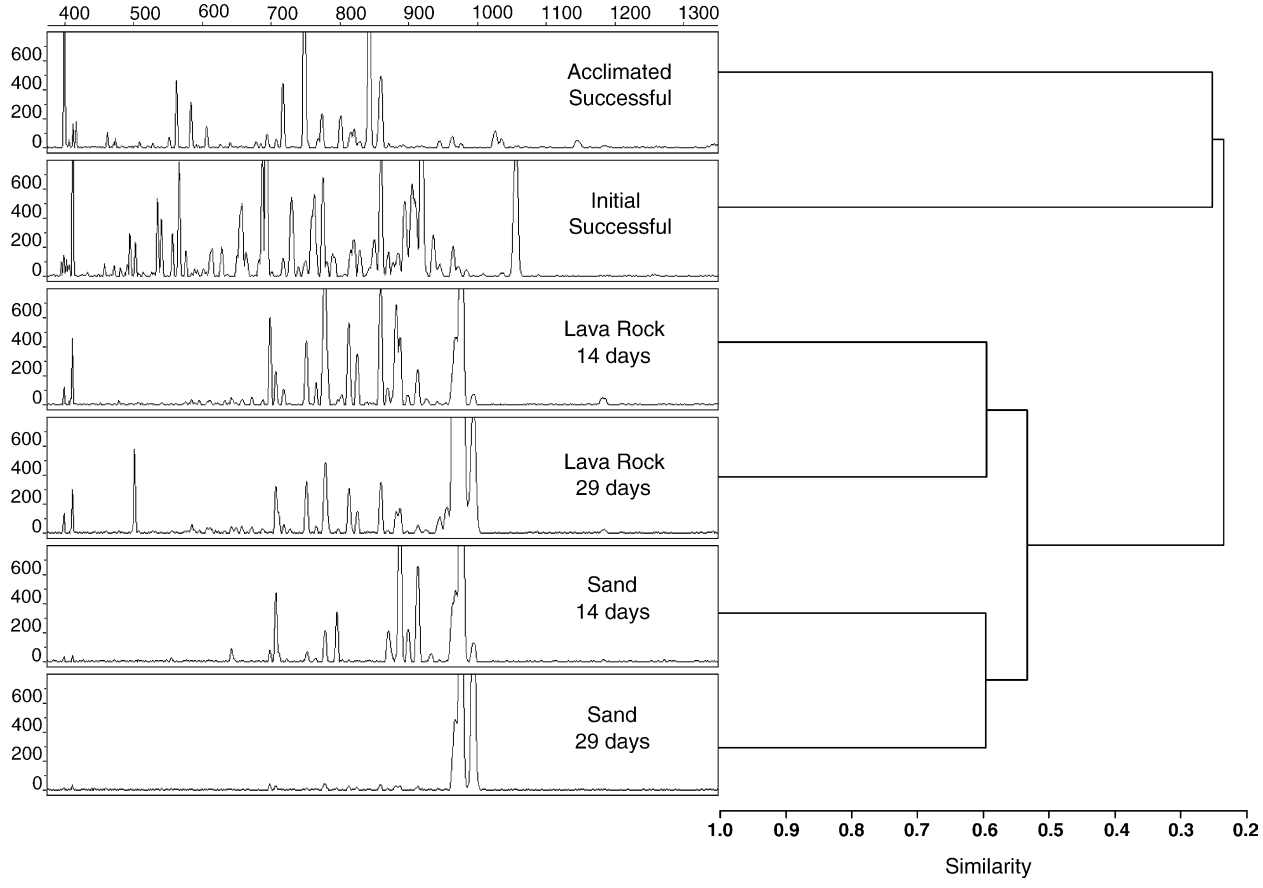

Fig. 5. Dendrogram of percent similarity based on Whittaker's index comparing communities in sand and lava rock-packing materials in biofilters which successfully removed ethanol. The branches are labeled with the origin of the community sampled and the day where appropriate. Left of each branch are the corresponding ARISA fingerprint electropherograms. Electropherograms are shown on the same scale (in bp) but on relative peak height scales.

observed in these comparisons, however, since the animal-rendering plant and the wastewater biofilters treated a wide variety of compounds, were much larger in scale, and contained far different packing materials compared to the lab-scale ethanol biofilters observed in this study. The effect of a single carbon source of the ethanol biofilter are similar to the results seen in the study done by Sakano and Kerkhoff [4] in an ammonia biofilter: biofiltration of a single compound requires relatively few OTUs.

3.4. Biofilter packing material comparison

The communities in the lava rock and sand packing in the successful biofilters show a clear divergence from the Initial Successful and Acclimated Successful liquid culture communities from which they came (Fig. 5). These fingerprints are included in the cluster to illustrate that the transition from a suspended culture to an immobile biofilm is accompanied by substantial changes in community composition. It is also worth re-emphasizing that the Initial Successful and Acclimated Successful are as different from each other by the Whittaker's index as from the biofilter communities, i.e. substantial changes in community composition have taken place during acclimation and during the transition to the biofilter.

The Lava Rock communities and the Sand communities were clustered separately showing a role of the packing material in selecting the community, but the within-packing differences were only moderately smaller than the between packing differences. The difference may be unimportant in a practical sense because both communities were able to remove ethanol with 80% efficiency, i.e. containing a few important members in the community may be the most important factor. The differences in packing material may have affected the community as a whole, but the major OTUs were the same.

There was also significant change within these communities over time. The communities at 14 days are only 58% similar to the communities at 29 days in each of the materials. While it may not be obvious from the electropherograms that these communities do not share similar patterns of peaks (Fig. 5), Whittaker's index gives much greater emphasis to major peaks and minimizes the effect of minor peaks. The sand biofilter at 14 and 29 days has only 3–4 major peaks; the minor peaks in the sand biofilter at 29 days are difficult to see above the baseline but are detectable by the analysis software. The major peaks account for the similarity values in the index the other large peaks in the electropherogram at 14 days are present in the 29 days biofilter, but they are a much smaller proportion of the index compared to the major peaks. The change in Whittaker's index and the Shannon–Weaver and Simpson's reciprocal indices (Fig. 4), show a community where there is decreasing diversity over time. It may show the major members of the community outcompeting others for ethanol metabolism. These changes in community population were not, however, reflected in the removal efficiencies of the biofilters.

4. Conclusions

A sharp distinction observed between the Initial communities and the Acclimated communities reflected a restructuring of the community during the acclimation process. A substantial decrease in microbial diversity at the end of successful acclimation and during successful biofiltration was observed, i.e. the biofilter communities with the least diversity outperformed the biofilter communities with greater diversity. It follows that either the failed biofilter communities contained extra organisms which produced the acidic intermediates or the successful biofilter communities contained the correct few organisms available in the inoculum. Dominant OTUs tentatively identified by ITS length were present in all of the initial communities studied, although they were proportionally greater contributors in the successful biofilters, and could represent the key organisms capable of ethanol degradation.

Community fingerprinting methods allow for a holistic approach to understand the complex microbial community in biofilters. The information community fingerprints can provide for comparison between communities or as a diagnostic or predictive tool for biofilter operation, similar to the suggestion to use DGGE fingerprinting as a diagnostic tool in Li et al. [11]. If the community fingerprint could be compared to a set of fingerprints known to come from working biofilters, it could be used to determine what may be missing from the failing community. This technique, in combination with sequencing to assign identification to the ARISA peaks, might guide efforts to adjust the system in order to make it successful.

It is of interest to note that the electropherograms of the three initial communities (Fig. 3) differ substantially. These are three samples of wastewater treatment sludge, which is typically assumed to be a constant and virtually universal inoculant. It is possible that the difference among them, and particularly the greater diversity of OTUs in the Initial Successful sample, may have had some effect on subsequent results. While this is speculative at this time, it illustrates questions that can be asked and answered using the ARISA fingerprinting technique: are sludge inoculants not all equal, and should we be taking steps to determine which are best? It also raises the question where to get the best inocula for new biofilters. Would use successful biofilter samples as inoculants be a more practical solution? The community fingerprints, in concert with other measurements of the biofilms such as nutrient gradients, metabolism measurements, or removal efficiency will allow further understanding of the interactions between the community and successful biofiltration.

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