Assessment of changes in the microorganism community in a biofilter

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Abstract
Community level physiological profiling (CLPP) with BIOLOG ECO-plates was used to assess the changes in functional diversity of the microbial community in a compost biofilter over time. The biofilter was treating a humidified airstream contaminated with hexane and later with ethanol. The results are consistent with spatial and temporal variation in the community when analysed using principal component (PC) analysis. BIOLOG ECO-plates provide a simple and rapid way of assessing changes in community structure of biofiltration systems. Here, the technique is demonstrated and possibilities for its application in biofilter analysis and monitoring are explored.

Keywords: Biofilter; BIOLOG; Microbial community; Community structure; Hexane; Ethanol

1. Introduction
Biofiltration is a biological technology for air pollution control. Contaminated air flows through a biologically active porous medium [1]. A mixed microbial community in the biofilm coating the medium degrades the pollutants [1]. Classical microbiological techniques indicate the predominance of bacteria and fungi in biofilters, and also the presence of yeast, actinomycetes and higher organisms [2]. Community level physiological profiling (CLPP) is an adaptation of a technique developed for the identification of pure bacterial cultures [3]. The technique was first applied to a microbial consortium by Garland and Mills [4] and there have been a number of subsequent studies to characterise different environmental communities [3]. In the technique, a microtitre plate with multiple sole carbon sources is inoculated with a mixed microbial sample. Each well also contains a redox indicator dye (tetrazolium violet) which changes colour in response to microbial growth. The amount of colour development in each well, assessed spectrophotometrically, may be used as an indicator of the utilisation of that carbon source. These community level physiological profiles may be used to characterise the functional diversity of microbial communities using multivariate statistical analysis [5]. A variety of multivariate techniques for analysing the data have been employed by different researchers and these are discussed by Glimm et al. [6], whose methodology is adopted here.

BIOLOG ECO-plates are more appropriate for this type of analysis than GN or GP plates, since there is evidence that the selection of the carbon substrates allows greater discrimination between communities [5]; they are also more convenient since replication of sub-samples is achieved without the need for multiple plates. Fungal functional diversity is not assessed, since fungi cannot utilise the Tetrazolium indicator dye; BIOLOG FF plates do not contain the dye and are intended for use with fungi [5]. The use of MT plates, in which the carbon substrate is selected and added by the experimenter, would allow the use of single or mixed sources tailored to a particular system and might give greater resolving power if some basis for selecting those carbon sources can be found [5].

The technique has advantages over traditional cell culture techniques which are time-consuming and biased due to the small number of species that may be cultured; and, molecular-level techniques such as RNA amplification, which is time-consuming and requires some expertise [7]. The BIOLOG plate technique is easy and rapid; however, it is still unclear to what extent it is dissimilar to traditional culture techniques [8].
2. Methods and materials

2.1. Biofilter set-up and operation

A biofilter was constructed from 11.5 cm internal diameter glass tubing and packed with peat compost. The packed bed height was 67 cm. A mixed microbial inoculum was prepared by incubating a stock culture for 24 h (150 rpm, 23 °C) and then for 27 h with aeration at room temperature. Seven litres of inoculum (pH 7.2) were added to the top of the column, of which 21 (pH 5.4) were recovered as leachate.

Compressed air was bubbled through the VOC in a bottle containing an approximately constant volume of liquid; a separate stream was bubbled through a column packed with ceramic saddles and filled with water to humidify it. The two streams were combined to create a humidified VOC–air mixture. Air flow commenced 4 days after inoculation of the biofilter using hexane as the VOC; 52 days after inoculation the VOC varied from 2.5 to 7.5 g m\(^{-3}\) for hexane and 11 to 24 g m\(^{-3}\) for ethanol. The humidified VOC–air mixture was passed through the biofilter in a down-flow mode and the VOC was removed by naturally immobilised microorganisms on the packing material. A defined mineral medium was added to the top of the biofilter periodically to prevent drying. Analysis of gas samples was performed using a Hewlett-Packard 5890 Series II gas chromatograph (GC).

2.2. Sampling

Samples of compost were removed from side access ports at the top and bottom of the biofilter. 0.9 g (wet) of sample was added to 5 ml of buffer (1.236 g Na\(_2\)HPO\(_4\) (BDH); 0.18 g NaH\(_2\)PO\(_4\) (Sigma); 8.5 g NaCl (Caledon) per liter of water, filter sterilised) and shaken for 3 h at room temperature. The resulting suspension was filtered using tissue pre-soaked with 2 ml of buffer. The o.d. of the resulting filtrate was roughly controlled to be 0.19 at 420 nm, and each well in the BIOLOG ECO-plates inoculated with 0.15 ml. The BIOLOG plates were incubated with shaking at 23 °C and the o.d. of each well at 590 nm read periodically.

Samples were taken 22, 36, 51 and 69 days after inoculation of the biofilter using hexane as the VOC; 52 days after inoculation the VOC varied from 2.5 to 7.5 g m\(^{-3}\) for hexane and 11 to 24 g m\(^{-3}\) for ethanol. The humidified VOC–air mixture was passed through the biofilter in a down-flow mode and the VOC was removed by naturally immobilised microorganisms on the packing material. A defined mineral medium was added to the top of the biofilter periodically to prevent drying. Analysis of gas samples was performed using a Hewlett-Packard 5890 Series II gas chromatograph (GC).

2.3. Statistical methods

Statistical analysis of the BIOLOG ECO microplate data was performed using principle component (PC) analysis, following the procedure described by Glimm et al. [6]. Each 96-well plate consists of three replicates, each comprising 31 sole carbon sources and a water blank. The optical density readings were first truncated to lie in the range \([0, 2]\), since values below 0 are clearly erroneous and values above 2 have been shown to be dominated by measurement error. The reading for each well was then corrected by subtracting the value of the water blank for that replicate, and standardised by dividing by the average well colour development (AWCD) for the replicate, in line with the recommendations of Garland [9]. If OD\(_{i(t)}\) represents the corrected o.d. for well \(i\) of replicate \(j\) at time \(t\), then the AWCD for replicate \(j\) at time \(t\) is given as,

\[
\text{AWCD}_j = \frac{1}{J \times T} \sum_{t=1}^{T} \text{OD}_{i(t)}
\]

The standardised o.d. values are then given by,

\[
\text{OD}_{i(t)} = \frac{\text{OD}_{i(t)} - \text{AWCD}_j}{\text{AWCD}_j}
\]

The time point for analysis is selected by counting the number of wells for which corrected o.d. exceeds 0.1 (over all plates) and selecting the time with the greatest change, i.e. \(t\) is selected such that \(|n[t] - n(t - 1)|\) is maximised where \(n[t]\) represents the number of wells, counting over all replicates, with corrected o.d. > 0.1 at time \(t\).

Principal component analysis was then performed on the data, using SAS v8.01 on a personal computer, at the selected time point using the covariance matrix (so that scale is maintained). The number of components to analyse was determined using a scree plot. Fisher’s least significant difference test was used to determine differences in the communities. Wilks’ \(F\) test was first used to test the null hypothesis of no difference between any of the communities, and then Hotelling’s \(T^2\) was used for all pairwise comparisons if the null hypothesis is rejected by the first test.

The carbon sources most important in distinguishing between the different samples were identified from the diagonal elements of the estimated factor loading matrix and univariate \(F\)-tests on the individual carbon sources, using the procedure described by Glimm et al. [6].

3. Results and discussion

3.1. Multivariate tests for differences between the communities

The time selected for analysis using the method described above was 19 h. Five PCs were selected to be retained from a scree plot and these are able to account for over 90% of the variance in the data. The first two PCs are plotted against each other in Fig. 1 for illustration. The third data point for the sample from the outlet on day 36 (O36 points in Fig. 1) was judged to be an outlier and removed from the
Fig. 1. The first and second principal components for each of the samples. Outline markers represent inlet and solid markers outlet samples. The legend indicates inlet (I) or outlet (O), followed by the day of operation on which the sample was taken.

Analysis. Analysis of the data with the data point included gives very similar results. Table 1 and Fig. 1 indicate some interesting trends in the biofilter microbial community development over time. At day 22, samples from the inlet and outlet appear to have markedly different metabolic activities, as indicated by the wide spread of the two data clusters. As time progresses (days 36 and 51) the clusters move closer together, suggesting that the hexane feed stream leads to a selective pressure towards a single microbial community metabolic profile. After switching to ethanol in the feed stream (day 69), there is again a dramatic change and difference between the inlet and outlet samples. Therefore the technique appears to show promise as a monitoring tool for biofilters at the metabolic level.

Fisher’s LSD test resulted in an approximate $F$-statistic of 41.93 at 35, 49 d.f. for the comparison of the eight groups. This result is highly significant ($P < 0.0001$), and rejects the null hypothesis of no variation between the groups. Results of all pairwise group comparisons are given in Table 1. All groups are significantly different to one another with the exception of the inlet and outlet samples taken on day 51 of operation. This result should not be over interpreted since the data is from replicate treatments of samples, not from replicate samples.

### 3.2. Determination of important carbon sources

The factor weights and results of univariate $F$-tests for the individual carbon sources are given in Table 2. Only those carbon sources in the upper quartile of the factor weight distribution are listed. The Spearman correlation between the factor weights and the $F$-values is 0.62, which falls within the range 0.43–0.67 previously reported [6]. This level rejects the null hypothesis of no correlation between the results.

<table>
<thead>
<tr>
<th>$O_{22}$</th>
<th>I6</th>
<th>O36</th>
<th>I51</th>
<th>O51</th>
<th>I69E</th>
<th>O69E</th>
</tr>
</thead>
<tbody>
<tr>
<td>47.107 ($&lt;0.001$)</td>
<td>9.619 ($&lt;0.001$)</td>
<td>12.68 ($&lt;0.001$)</td>
<td>116.031 ($&lt;0.001$)</td>
<td>0.839 ($0.001$)</td>
<td>244.735 ($&lt;0.001$)</td>
<td>101.953 ($&lt;0.001$)</td>
</tr>
</tbody>
</table>

Compared at 5.12 d.f. Groups are indicated as either inlet (I) or outlet (O) and the day after inoculation that the sample was taken.
Table 2
Factor weights and results of univariate F-tests for the individual carbon sources

<table>
<thead>
<tr>
<th>Carbon Source</th>
<th>Factor Weight</th>
<th>F (Rank)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Methyl-D-ribose</td>
<td>0.111 (15.1%)</td>
<td>41.3 (3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>D-Malic acid</td>
<td>0.073 (9.9%)</td>
<td>22.6 (9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>4-Hydroxy benzoic acid</td>
<td>0.061 (8.3%)</td>
<td>24.2 (8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>N-Acetyl-D-glucosamine</td>
<td>0.049 (7.6%)</td>
<td>15.8 (15)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L-Asparagine</td>
<td>0.046 (6.2%)</td>
<td>24.9 (7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>D-Galacturonic acid</td>
<td>0.042 (5.7%)</td>
<td>22.2 (10)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Putrescine</td>
<td>0.038 (5.2%)</td>
<td>16.6 (14)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>D-Cellobiose</td>
<td>0.036 (4.8%)</td>
<td>34.8 (4)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The overall contribution of the carbon source to explaining the variance in the first five PCs is given as a percentage in brackets.

at the 0.2% level. Note that the results of the F-test here should not be interpreted in the usual sense of statistical significance, but act only as indicators of the carbon sources most responsible for the differences in response observed.

4. Conclusions

Assessment of variation in the community structure of a biofilter can be rapidly and easily made using CLPP with BIOLOG ECO-plates. These results demonstrate the applicability of the method to a biofiltration system. The results are consistent with variation in the microbial population both spatially and temporally within a biofilter; however, the number of samples taken in this study is insufficient to draw that conclusion in a robust statistical sense. Triplication of carbon-sources on the plates provides a measure of the within-sample variation, which should be small; many samples are required to gain a measure of the functional diversity of the biofilter at one time and position. These samples may show significant variation due to the presence or absence of minority members of the bacterial community in the particular sample being assessed; although this may seem to cloud the analysis, significant information is contained in such between-sample variations.

The technique might find application in monitoring the health of the microbial population in the biofilter and it might be expected that significant changes in the community structure precede a "crash" of an operating unit. In the laboratory, it provides a simple method for assessment of the stability of the microbial community in a biofilter; for assessing the effect of different inoculum strategies on the microbial community that develops in the biofilter; and, for assessing the changes in community brought about in response to various stressors (such as drought, periodic exposure to increased VOC concentration, changes in the VOC etc.). The technique might also find application in searching for similarities and differences in the functional diversity of microbial communities in different biofilters operating under similar conditions. Once the interpretation of the statistical tests has been understood, their implementation is easy using a standard statistical program, such as SAS or SPSS, on a personal computer.

References