Rich microbial communities inhabit water treatment biofilters and are differentially affected by filter type and sampling depth

S. A. Wakelin, D. W. Page, P. Pavelic, A. L. Gregg and P. J. Dillon

ABSTRACT

Factors affecting microbial diversity (richness) and community structure in biofilter columns were investigated. At a pilot filtration plant, granular activated carbon (GAC), anthracite and sand-based filters were used to treat stormwater from an urban catchment. After 12 weeks operation, sand media filters clogged (hydraulic conductivity declining by 90%) and all filters were destructively sampled. All biofilters had similar levels of polysaccharide in the surface layer, however only the sand columns clogged. This clogging may have been due to a combination of polysaccharide and small particle size, the development of a sand-specific microbial community, or other biogeochemical interactions. DNA fingerprinting was used to show that bacterial, archaeal and eukaryotic communities were present in all filter types and at all sampling depths (to 45 cm). The bacterial community was far richer (Margalefs index, \(d\), 1.5–2) than the other groups. This was consistent across filter types and sampling depths. The structure of the bacteria and archaea communities in sand filters differed to those in GAC and anthracite filters (\(P < 0.05\)). In contrast, eukaryotic communities were similar in surface biofilm layers, irrespective of filter type. As such, physicochemical properties of filters differentially influence the microbial community. Furthermore, we have established that archaea are distributed throughout biofilters; the role of these microorganisms in water treatment and filter function, particularly clogging, requires attention.

Key words | anthracite, archaea, bacteria, biofilter, clogging, community structure, diversity, eukarya, filter type, GAC, PCR-DGGE

INTRODUCTION

Biofiltration of water through beds of porous material is a widely used technology for water treatment, including supply of public drinking water (Wotton 2002). The relatively simple and robust nature of the technology has enabled its widespread application from large modern cities to isolated rural villages.

In biofiltration, much of the water treatment process takes place though the activities of microorganisms present on the surfaces of the filter media. Biological processes involved in water treatment include assimilative removal of major nutrients (Campos et al. 2002), entrapment of metals (Muhammad et al. 1998), degradation of toxins (Bourne et al. 2006), and removal of organisms including algae, bacteria, protozoa and viruses (Poynter & Slade 1977; Bellamy et al. 1985; Weber-Shirk & Dick 1999). Conversely, biological activity in the filter—and especially slow sand filters—can negatively impact on filter performance. This is most evident as filter clogging, a loss of filter hydraulic conductivity (\(K_s\)) and a concomitant reduction in volume of water treated (Vandevivere & Baveye 1992a,b).

The biological elements and the processes they support are, therefore, central to effective filter operation.
Given the increasing range of application for biofilters in water treatment (e.g. degradation of organic chemicals and micropollutants), understanding microbiology of biofilters, effects of biotic and environmental interactions, and how these factors combine to affect filter function is important. In addition, there remains potential to explore biogeochemical processes occurring on and within biofilters as experimental analogues towards understanding bio-clogging mechanisms in managed aquifer recharge applications such as aquifer storage and recovery (ASR) (Dillon 2005).

Surprisingly, there is a paucity of knowledge as to the range (diversity) of prokaryotic and eukaryotic organisms present in biofilters. Most descriptions relating to microbial species composition and diversity in such systems have been limited to culture-based or microscopic investigations. As the majority of environmental microorganisms have yet to be cultured (Hugenholtz et al. 1998) previous work is likely to have vastly underestimated the range of species present. The use of culture-based techniques raises the potential to erroneously ascribe functioning within biofilters. This knowledge gap is heightened by the recent discovery of mesophilic archaea being widespread in many environments (e.g. Buckley et al. 1998). The diversity and role of archaea in biofilters is yet to be explored.

In this paper we present insights into the degree of microbial diversity in biofilters and test how biotic communities are affected by filter bed type and sampling depth. Molecular (DNA) methods were used to investigate the community structures of microorganisms from each of the three domains (bacteria, archaea and the eukarya) within biofilters containing three different media type commonly used in water treatment applications (sand, anthracite and granular activated carbon) in a replicated experimental framework.

**MATERIALS AND METHODS**

**Biofiltration plant: setup and running**

A biofiltration experiment was conducted at the Urrbrae Wetland (Adelaide, South Australia) during June–August (winter) 2006. The biofilters were used to treat urban stormwater with the aim to produce water of a quality suitable for sustainable ASR. Water sourced from the storage basin had previously been used for ASR applications with only rapid sand filtration, however was unsuccessful due to rapid clogging of the injection well (Pavelic et al. 2008).

Stormwater initially enters a settling basin before entering the main wetland lagoon and finally the storage basin (Figure 1). The water was then pumped into a raised $8m^3$ concrete tank (Figure 1) for use in the biofiltration experimental facility. Water was passed through a series of four up-flow roughing filters to physically remove...
algae and reduce turbidity. The filters, consisting of acrylic columns of 67 cm in length and 6.3 cm in internal diameter, contained gravel sand and quartz media of sequentially decreasing size (7.55 mm, 7.55 mm 5.18 mm 2.18 mm average diameter) on a base of coarse (1 cm) gravel. Details of the design, testing and operation of the roughing filter are fully described by Lin et al. (2006, 2008).

Biofilters were set up in duplicate to evaluate the effects of filter media material on microbial community structure. Three filter media types were evaluated: sand, granular activated carbon (GAC) and anthracite. Washed river sand (0.2 mm particle size) was sourced from River Sands Pty Ltd, coal-based GAC (1.1 mm) was sourced from PICA under the trade name ‘Picacarb’, and anthracite (0.9 mm) was supplied by United Water International Pty. Ltd. (Adelaide) from the Little Para Water Treatment Plant. Filter bed media was packed into 180 cm long \( \times \) 6 cm i.d. columns constructed from acrylic plastic (Figure 2). Each column included a flange located 90 cm from the top to facilitate sampling of the biofilm material. Sampling ports were located at filter depth of 0, 5, 20 and 45 cm.

Water treated by roughing filters was held in header tanks and delivered to the biofilters at a constant head-pressure (90 cm above the surface of the filter media; Figures 1 and 2). For consistency across the different filter media types, all biofilters were initially operated to achieve target hydraulic loading rates of 0.1–0.3 m/hr, as is typical for the operation of slow sand filters. This flow was maintained by adjusting a valve at the base of the columns (Figure 2) until the valve was in a fully open position. The biofilters were run for 12 weeks over which time water quality measures were taken to ensure filters were functioning, and measurements of flow were taken to determine the rate of filter clogging. Hydraulic conductivity \( (K_s) \) in the sand biofilters were taken to determine the rate of filter clogging. \( K_s \) was determined from Darcys law (Equation (1)) by measuring differences in hydraulic head (\( \Delta H \)) between the top and bottom manometers (Figure 2), water flow rate (\( Q \)), filter area-cross section (\( A \)) and the distance between manometers (\( L \)).

\[
K_s = \frac{Q}{A} \frac{L}{\Delta H} \tag{1}
\]

\( K_s \) decline was only observed from the sand biofilters. When the sand media biofilters had become clogged, all biofilters were destructively sampled for polysaccharide analysis and microbial community characterization.

Water quality analysis

Water quality samples were collected from the inflow and outflow of the biofilters and characterised for general (conductivity, turbidity, pH and total suspended solids, true colour) microbial (total coliforms, thermo-tolerant coliforms, \( E. \ coli \)) and nutrient (amount and forms of N, P, and C) properties according to standard methods (APHA 2005).

Polysaccharide analysis

When the sand-media biofilms had become clogged (defined here as > 90% loss in \( K_s \)), the polysaccharide
content of each biofilter was determined at the surface (also known as the schmutzdecke) layer and at 5 cm, 20 cm and 45 cm depths using the phenol-sulfuric acid method (Dubois et al. 1956). To each 0.5 g sub-sample of moist filter material or glucose standard, distilled water (1 ml) and 5% phenol w/v (1 mL) were added. The samples were vortexed, 5 mL of sulfuric acid (specific gravity 1.84) added, and mixed again. Suspensions were cooled to room temperature, left to stand for 30 min, centrifuged at 120 g for 5 min and the absorbance measured at 485 nm using a UV/visible spectrophotometer (Ultraspec II, LKB Biochrom, Cambridge, England).

### Microbial community profiling

The effects of filter media type (sand, GAC, and anthracite) and filter depth (0, 5, 20 and 45 cm) on species of bacteria, archaea, and eukaryotic organisms were determined. DNA was extracted from 0.7 g samples of filter material collected from the 0 (schmutzdecke) and at 5, 20 and 45 cm depths of each filter. Extraction of DNA was based on the MoBio UltraClean Soil DNA extraction kit (MoBio Laboratories Inc.), with additional use of a FastPrep (QBiogene Inc.) bead beater to aid extraction efficiency. DNA was quantified using the QuantIT PicoGreen dsDNA system (Molecular Probes) against a λ-phage DNA mass standard curve that had been calibrated on a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies).

Polymerase chain reaction (PCR) was used to specifically amplify variable regions of bacterial, archaeal and eukaryotic small subunit (SSU) rRNA genes from biofilter DNA extracts. For archaea, the nested PCR method of Øvreås et al. (1997), using primers PRA46f and PREA1100r and then PARCh340f (with GC clamp) and PARCh519r was employed. Bacterial 16S rRNA gene fragments were amplified using PCR primers F968-GC and R1401 (Duineveld et al. 1998). Laboratory specific conditions and chemistry for bacterial and archaeal PCR-DGGE gene fragments have been described elsewhere (Wakelin et al. 2008).

For eukaryae, 18S rRNA amplification was based on the method of van Heenen et al. (1998). Primers were used at 0.6 μM each. PCR chemistry was based on Qiagen hot star Taq (1 U/25 uL reaction). After hot-start activation, PCR conditions consisted of 30 cycles of ‘94°C for 30 s, 52°C for 1 min, and 72°C for 90 s’. A final extension step of 72°C for 30 min was used for all PCRs to reduce double banding phenomenon in subsequent DGGE (Janse et al. 2004). DGGE of eukaryotic PCR fragments was conducted in 7% w/v acrylamide:bis-acrylamide gels using a 30–50% urea:formamide denaturing gradient. Further DGGE conditions, staining, and band capture have been described elsewhere (Wakelin et al. 2008).

Band intensity and position data were exported into the Primer6 software package for statistical analysis. Each band was inferred to be an operational taxonomic unit which, within the context of this work, we hereon refer to as a species. Community structure, integrating both species occurrence and abundance data, were compared across filter media types and with filter depths. Raw intensity values were 4th-root transformed to down-weight the contributions of highly abundant taxa to overall community composition. Similarity between communities was measured using the Bray-Curtis method, and visualized by non-metric multidimensional scaling (MDS) ordination. The effects of filter and depth on community structure were determined using analysis of similarities (ANOSIM) (Clarke 1993). The output of ANOSIM provides a global R value describing the magnitude of effect ($R \leq 0 = \text{no effect}, -1 = \text{strong effect}$) and a likelihood of the R value occurring by chance (analogous to the ‘traditional’ $P$ value). Diversity, *sensu* species richness, of the microbial communities in the filter samples were determined using Margalef’s richness index ($d$; Equation (2)):

$$d = \frac{(S - 1)}{\log(N)}$$

Where $S =$ number of species (DGGE bands) and $N =$ number of individuals (non-transformed band intensity values). As such, this index considers sampling effort when determining richness of species present. The application of richness indices to DGGE and related data sets has been discussed previously (e.g. Bent et al. 2007 and associated material).

The effects of filter matrix type and sampling depths on community richness ($d$), polysaccharide production and total DNA were tested using 2-way ANOVA. Fishers protected LSD test was used as a post hoc test for treatment
effects. When interaction effects were significant, these were interpreted exclusively. Statistical testing was conducted in GenStat (v8.1; Lawes Agricultural Trust, Rothamsted Experimental Station), while charts were generated using GraphPad Prism (v5; GraphPad Software, San Diego, California).

RESULTS

Filter functioning, water quality and clogging

Water quality measures were taken during the operation of the biofilters and were used to establish effective functioning of the filters (Table 1). Overall, the biofilters were effective in the removing bacterial pathogen indicators but only moderate in terms of nutrients (Table 1), with performance similar to those reported in the literature (Barrett et al. 1991).

Over time, $K_s$ decreased exponentially in the sand filters (Table 1) and, by the end of the experiment (12 weeks operation), the filters were considered clogged; defined as a > 90% reduction in $K_s$. Filters with GAC or anthracite media showed no clogging for the duration of the experiment (Table 1).

Polysaccharide and DNA quantification

Levels of polysaccharide were significantly affected by both filter media and sampling depth (interaction $P < 0.004$; Figure 3(A)). Irrespective of filter media type, the quantity of polysaccharide material present at the

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**Table 1** | Treatment performance of biofilters

<table>
<thead>
<tr>
<th>Filter properties</th>
<th>Sand</th>
<th>Anthracite</th>
<th>GAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size (mm)</td>
<td>0.2</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Bulk density (g/cm³)</td>
<td>1.7</td>
<td>0.9</td>
<td>0.5</td>
</tr>
<tr>
<td>$K_s$ (initial)</td>
<td>19–56</td>
<td>~110⁻⁷</td>
<td>~110⁻⁷</td>
</tr>
<tr>
<td>$K_s$ (final)</td>
<td>0.05–3</td>
<td>~110⁻⁷</td>
<td>~110⁻⁷</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Water treatment</th>
<th>Influent</th>
<th>Effluent</th>
<th>Influent</th>
<th>Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliforms (cfu/100 mL)</td>
<td>5,800 (3,500)</td>
<td>5 (6)</td>
<td>145 (113)</td>
<td>204 (350)</td>
</tr>
<tr>
<td>Thermotolerant coliforms (cfu/100 mL)</td>
<td>150 (86)</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>E. coli (cfu/100 mL)</td>
<td>130 (86)</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>3–28</td>
<td>7.4 (0.4)</td>
<td>7.7</td>
<td>7.6 (0.04)</td>
</tr>
<tr>
<td>pH</td>
<td>7.4 (0.4)</td>
<td>7.7</td>
<td>7.6 (0.04)</td>
<td>7.7</td>
</tr>
<tr>
<td>Colour (HU)</td>
<td>10 (0)</td>
<td>8 (0)</td>
<td>10</td>
<td>5.3 (1.7)</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>5.7 (3.7)</td>
<td>1.9 (3.5)</td>
<td>0.6 (0.2)</td>
<td>0.5</td>
</tr>
<tr>
<td>Total dissolved solids (mg/L)</td>
<td>83 (37)</td>
<td>132 (23)</td>
<td>112 (4)</td>
<td>112 (5)</td>
</tr>
<tr>
<td>Suspended solids (mg/L)</td>
<td>4.1 (4.3)</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Total organic carbon (mg/L)</td>
<td>4.7 (0.5)</td>
<td>4.4 (2.5)</td>
<td>4.0 (0.4)</td>
<td>2.2 (0.5)</td>
</tr>
<tr>
<td>Total phosphorous (mg/L)</td>
<td>0.13 (0.06)</td>
<td>0.26 (0.53)</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Dissolved phosphorous (mg/L)</td>
<td>0.07 (0.02)</td>
<td>0.06 (0.05)</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>Total nitrogen (mg/L)</td>
<td>0.56 (0.29)</td>
<td>0.88 (0.42)</td>
<td>0.58 (0.03)</td>
<td>0.63 (0.09)</td>
</tr>
<tr>
<td>Ammonia (mg/L)</td>
<td>0.07 (0.05)</td>
<td>0.03 (0.02)</td>
<td>0.001 (0.002)</td>
<td>0.006 (0.001)</td>
</tr>
<tr>
<td>Nitrate + nitrite (mg/L)</td>
<td>0.12 (0.09)</td>
<td>0.56 (0.35)</td>
<td>0.32 (0.03)</td>
<td>0.32 (0.21)</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen (mg/L)</td>
<td>0.55 (0.28)</td>
<td>0.46 (0.44)</td>
<td>0.26 (0.01)</td>
<td>0.16 (0.03)</td>
</tr>
</tbody>
</table>

$K_s$ was maintained at ~110 m/day by means of a valve at the bottom of the filter to achieve a flow of 14 L/day per filter. All water quality data are presented as means, brackets represent 1 standard deviation.
surface layer (∼0 cm) layer was greater than lower in the filter ($P < 0.05$). The sharpest decline in polysaccharide with depth was evident in the sand filter (Figure 3(A)). The small particle size may have acted as a barrier to downward migration of surficial polysaccharide in the filter. Alternatively, physicochemical parameters deeper in the sand filter may not have been conducive to \textit{in situ} polysaccharide formation.

Total extractable DNA in the filters also significantly varied by filter media type and depth (interaction $P < 0.008$; Figure 3(B)). Highest concentrations were generally present near the surface (Figure 3(B)). However, in the GAC and anthracite filters the amount of DNA in the surface layer was not significantly different to samples taken from 5 cm depth. Across all data, a positive linear correlation existed between DNA concentration and polysaccharide ($P < 0.001$; $R^2 = 0.615$).

**Microbial community structure**

After loss of filter functioning ($K_s$ decline), the microbial communities in the surface and lower portions of the filters were sampled for analysis. Total bacterial, archaeal and eukaryotic species (DGGE gel band positions) were high (Figure 4), indicating a substantial range of species present across all domains.

Filter media type was the primary factor influencing microbial community structure, significantly affecting the types of bacteria, archaea and eukarya present in the samples ($P < 0.001$; Table 2). For bacteria and archaea, the community compositions were similar between the anthracite and GAC media, but were distinct to those in sand media filters ($P = 0.022$). The effect of filter media is apparent in the MDS ordination plots (Figure 5) where the sand communities are delineated from those present in GAC and anthracite. Sampling depth did not significantly affect the composition of bacterial or archaeal species in the biofilters (Table 2; Figure 5).

For the eukarya, the effect of filter media, whilst statistically significant, was not as strong at determining community composition as observed for the bacterial and archaeal communities ($R = 0.588$; Table 2). However, each of the filter bed media supported a distinct community ($P = 0.029$ across all pair-wise comparisons). Again, this separation of communities according to filter media is evident in the MDS plot (Figure 5). Sampling depth did not significantly affect the composition of bacterial or archaeal species in the biofilters (Table 2; Figure 5).
Species richness

The richness ($d$) of bacteria was affected by an interaction of filter media type and sampling depth (interaction $P = 0.037$; Figure 6(A)). The highest levels of richness were at the lowest level (40 cm) in the sand filter ($d = 2.245$). In the GAC filters, bacterial richness did not change with sampling depth. In the anthracite filters, bacterial richness was greatest in the middle portions of the filter.

The richness of archaeal species was not affected by filter media or depth (Figure 6(B)) and measures were similar to those for the eukarya (Figure 6(C)). Richness measures of archaea and eukarya were both lower than those for bacteria (Figure 6(A)).

The richness of eukaryotic organisms in the biofilters was affected by filter media and depth (interaction $P = 0.006$; Figure 6(C)). Richness was highest in the GAC columns, except for the GAC surface layer which was significantly lower than other GAC samples. Eukaryotic richness in the sand columns was not significantly affected by sampling depth (Figure 6(C)).

DISCUSSION

Our work has shown that biofilters support richly and structurally complex communities of bacteria, archaea and eukarya. The level of biological complexity observed across the three domains has not been previously realized for these environments. As such, this has significant implications for understanding processes involved in water treatment, filter function (particularly clogging) and using biofilters as experimental analogues for ASR well clogging.

Basic water quality measures were used to verify that the filters were functioning as previously reported in the

Table 2  Summary results from two-way ANOSIM testing effects of filter bed material and sampling depth on the structure of the bacterial, eukaryotic, and archaeal communities

<table>
<thead>
<tr>
<th>Factor</th>
<th>Bacteria</th>
<th>Eukarya</th>
<th>Archaea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filter type</td>
<td>$R = 0.611$, $P = 0.001$</td>
<td>$R = 0.588$, $P = 0.001$</td>
<td>$R = 0.704$, $P = 0.001$</td>
</tr>
<tr>
<td>Filter depth</td>
<td>$R = 0.139$, $P = 0.233$</td>
<td>$R = 0.306$, $P = 0.036$</td>
<td>$R = -0.233$, $P = 0.963$</td>
</tr>
</tbody>
</table>

$R$ value = strength of the treatment effect, where 1 = strongest effect; $P$ value = statistical significance, i.e. likelihood of $R$ value occurring by chance.
Filter clogging is widely attributed to physical and/or biological clogging mechanisms such as bacterial polysaccharide production \cite{Logsdon2002}. We found that total polysaccharide production in the surface layer, where clogging typically occurs, was similar irrespective of filter media type. As only sand filters exhibited $K_s$ loss, the particle size of the media was obviously the critical factor. In sand filters, microbial community size, determined by DNA extracted from the filter bed material, correlated to polysaccharide production. Depending on particle sizes within the filter bed, microbial community size, polysaccharide production and clogging are likely to be tightly linked. The majority of bioclogging related research has focused on polysaccharide production, yet other microbial extracellular polymeric substances including proteins may also be involved. As such, production of polysaccharide, whilst related to declining $K_s$, may potentially be an indicator for other process and not causal per se. Wider characterization of microbial extracellular polymeric substances involved in $K_s$ loss and a detailed understanding of the interactions between biomass formation, microbial processes, and clogging would enable the development of mechanistic models for functioning within SSF systems. Ultimately, this may be used to improve the operational management of SSF through the prediction of head loss rate, and the frequency of sand cleaning and renewal. In functioning SSF’s, flow is restored by scraping off the surface layer and, followed by a ripening period during which microbial communities re-establish, filter operation is restored \cite{Logsdon2002}.

Across the three microbial domains, bacterial communities richest was greatest. The maintenance of diversity spatially in sand biofilters is supported by findings of Calvo-Bado et al. \cite{Calvo2005}. Although continuous biomass distribution through more porous media, such as GAC filters, has also been reported \cite{Servais1991, Velten2007}, biodiversity within GAC and anthracite columns has not received significant attention. Surprisingly, the overall community structures were similar through filter depth for the bacteria and archaea microorganisms. This was unexpected, as the physicochemical conditions are likely to vary greatly, particularly with respect to oxygen and nutrient availability. The primary drivers affecting

![Figure 5](image-url)
species composition of prokaryotic communities, therefore, can be identified as filter media type and also inflow water parameters (Calvo-Bado et al. 2003; Petry-Hansen et al. 2006).

This work has found archaea to be common inhabitants within biofilters. The community of archaea differed between filter matrix, but not with depth, showing that archaeal species exhibit habitat preference within these environments. To date, most work investigating the role of archaea in water treatment has focused on degradation of specific toxins or chemicals in bio-reactor type systems (e.g. Connaughton et al. 2006; de Bok et al. 2006). Archaea also have a more general role in the treatment of water (i.e. at WWTP’s). In particular, these including the removal of carbon (Gray et al. 2002) and also nitrogen (You et al. 2009) through the activities of ammonia oxidizing archaea (Park et al. 2006). Investigation of the wider role of these microorganisms in the functioning of water treatment systems warrants attention.

Eukaryotic organisms, comprising algae, protozoa, fungi, and metazoa such as midges and worms are common in biofilters (Wotton 2002). The beneficial and deleterious role of eukaryotic organisms in SSF has been widely explored for biofilms, but there is less knowledge relating to the community at depth, or in differing media types. In comparison to prokaryotic communities, filter bed depth did significantly influence the eukaryotic community structure. This may be attributable to the larger relative size of eukaryotic organisms compared to bacteria and archaea, and the subsequent impact of depth in the filter for physical colonization/habitation. Despite this, diverse rRNA genotypes from eukaryotic organisms were present throughout all samples, suggesting a role for these organisms throughout biofilters.

There is potential to use filter columns as experimental units to explore clogging mechanisms in a range of applications of managed aquifer recharge, including ASR (injection/recovery) wells. In ASR wells, a number of clogging causes have been documented. These have been summarised previously (Rinck-Pfeiffer et al. 2000) to include bacterial production of exo-polymers, physical entrapment of suspended solids, gaseous reactions and clay dispersion. In addition, biogeochemical processes such as formation of iron or aluminium hydroxides.
(James-Smith et al. 2005) or calcite precipitation/dissolution (Rinck-Pfeiffer et al. 2000; Pavelic et al. 2007) are also important. Given the complexity of interactions and the difficulty in accessing the media around ASR injection wells, it is difficult to explore in situ processes involved in ASR well clogging. As such, there is interest in using laboratory columns as experimental analogs (Rinck-Pfeiffer et al. 2000). The success of this approach is dependent on a number of factors, particularly generation of conditions reflective of ASR-type clogging. With respect to biological clogging, the experimental unit must support both a similar microbial community (species present) and also conditions for bio-clogging processes (polymer production, geochemical transformation, production of gas). The methods used in this work can aid in addressing these questions. In particular, we have shown that a key factor affecting microbial species composition is the nature of the support material. As such, when using column experiments to explore ASR research questions, it is important that the filter bed material should closely represent the physical and perhaps geochemical properties of the aquifer matrix.

CONCLUSIONS

(1) Filter material was a key factor determining the occurrence of microbial species.
(2) Rich communities of microorganisms were distributed throughout the bio-filters, however only the eukaryotic components showed significant species change with filter depth. This is likely to be related to the larger size of these organisms and the increased effect of filter material packing on growth.
(3) Clogging only occurred in filters with sand media, yet all filters had equivalent amounts of polysaccharide in the surface layer. It is likely that the sand media filters clogged due to the low particle size of the sand grains, however clogging may also have been due to other attributes associated with the evolution of SSF-distinct microbial communities. A biogeochemical experimental approach which assesses microbial community structure, exopolymer production, mineral precipitation and dissolution, and Ks over time can be used to help resolve this.
(4) Archaea are a previously unrealized component of the microbial community in water treatment filters. Given the known role of archaea in terrestrial and aquatic biogeochemical cycles, it is important to consider their contribution to filter function, both in terms of water treatment and filter clogging.

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