Prokaryotic communities in drinking water biofilters using alternative filter medium
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**INTRODUCTION**

Biofilters are often the heart of drinking water treatment systems in Europe [1]. Research in biofilters has contributed to optimize water treatment. Often filter media consist of washed, dried and sieved quartz sand. The function of alternative filter media for drinking filters have been investigated [2-4]. These media include carbon products, ceramics, industrial waste products, and synthetic organics such as plastics, expanded clay, coated sands, and metal oxides. The duration of the start-up process has also been investigated [5-6]. However, the effect that alternative filter media may have on the microbial attachment and development of biofilm and therefore in the duration of the start-up period has received little attention.

The overall objective is to investigate the microbial communities on the filter media coating during the start-up of biofilters.

**METHODS**

The investigations described in this poster were carried out at Frederborg waterworks near Skanderborg, Denmark. The waterworks treats anaerobic groundwater using a simple process of aeration and filtration (2 filters in series).

**Setup**

After evaluation of several filter media properties, a filter column of calcium carbonate was selected to remove manganese using as inlet water between the waterworks filters. The water was spiked with manganese to achieve a constant concentration of 0.27 mg L\(^{-1}\).

**Water and Filter media samples**

Filter medium properties of quartz sand, calcium carbonate, anthracite and manganese oxide were determined using gravimetric methods and a photometric particle analyzer (Camsizer664, Retech Technology GmbH).

Water samples of the setup inlet (water between filters from the waterworks) were taken during the experiment and analyzed for standard parameters.

Water samples (water between the filters and clean water from the waterworks) and filter media samples (second filter of the waterworks and filter column) were collected and analyzed DNA extraction, qPCR with broad range bacterial primers, and amplicon sequencing using 16S rRNA primers. The analysis identified the most abundant amphilic Phyla and Genera in the samples.

**RESULTS**

**Filter media properties**

**Water quality before filtration**

Water extraction, qPCR and pyrosequencing results

<table>
<thead>
<tr>
<th>Filter media properties</th>
<th>Water quality before filtration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filter media</td>
<td>Characteristic</td>
</tr>
<tr>
<td>Quartz</td>
<td>0.96</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>1.12</td>
</tr>
<tr>
<td>Anthracite</td>
<td>0.82</td>
</tr>
<tr>
<td>Manganese oxide</td>
<td>0.62</td>
</tr>
</tbody>
</table>

**DISCUSSION**

DNA extraction, qPCR and pyrosequencing results

- **Table 3** - Sample overview and qPCR results.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ext. DNA</th>
<th>Lib. DNA</th>
<th>qPCR</th>
<th>Genomes</th>
<th>GC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM from filter 1</td>
<td>1.5</td>
<td>7.7</td>
<td>1.2</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>TM from setup</td>
<td>17.3</td>
<td>14.9</td>
<td>2.3</td>
<td>1.0</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Figure 3 shows that microbial analyses detected the presence of commonly reported prokaryotic groups: Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Acidobacteria and Gammaproteobacteria [7].

Pyrosequencing showed attachment of bacteria present in the inoculant, water between filters of the waterworks, on the filter medium surface (Figure 4).

Alphaproteobacteria (AOB's) were much more abundant (2-3 times) on the calcium carbonate column than in the water between filters (used as inlet).

**CONCLUSIONS**

- After 30 days of start-up, 20% of the manganese in the inlet was removed (contact time of 20 min). Even with low manganese removal, there was a selection for some taxonomic groups on the filter material relative to the inlet.

- 16S rRNA amplicon sequencing showed attachment of bacteria commonly reported as MnOB's and AOB's on the medium coating. These included bacteria present in the inoculant (water between filters). Further, bacteria underdetected in the inoculant suggest that other MnOB's were formed in the coating of the medium, such as: Hyphomicrobium and Hyphomonas.

- Further investigations on microbial communities evolving on different filter media can have great importance for start up and management of biofilters for drinking water treatment.


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