Microbial Communities on Different Packing Media in Biofilter

Xiaomeng Wang, Xudong Li, Jiangping Qiu, Yinsheng Li

School of Agriculture and biology, Shanghai Jiao Tong University, Shanghai 200240, China

Corresponding Author Email: yinshengli@sjtu.edu.cn

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ABSTRACT

Many scholars have explored the treatment effect of biofilters with different packing media. However, there is little report on the microbial communities of the packing media. Therefore, this paper attempts to disclose the differences between two popular packing media of biofilter, namely, volcanic rock and ceramiste, in terms of the attached bacteria. Firstly, two multi-layer biofilters (MBFs) were designed, and respectively packed with volcanic rock and ceramiste. The packing media were collected after the MBFs entered stable operation. The DNA was extracted from the media and pyro-sequenced. After that, the community structure and diversity of the microorganisms were analyzed in details. The main results are as follows: The values of ACE estimator, Chao1 estimator, and Simpson’s diversity show that volcanic rock had the higher bacterial diversity. The libraries with volcanic rock and ceramiste respectively consist of 1,810 and 1,352 operational taxonomic units (OTUs). Surprisingly, the Venn diagram of OTUs indicates that the two-packing media only share 13.2% OTUs. The volcanic rock contained sequences in 16 phyla while ceramiste only covered sequences in 11 phyla. Among all phyla, Tenericutes was unique to ceramiste, while Chloroflexi, GN02, NKB19, Thermi, Chlorobi and TM7 were only observed in volcanic rock. Ceramiste contained more Proteobacteria (67.06% vs. 64.12%), Bacteroidetes (25.11% vs. 22.72%) and Firmicutes (6.20% vs. 4.55%) than volcanic rock. The research findings clarify the research direction and provide the theoretical basis for improving ceramiste technology and developing alternatives for volcanic rock.

1. INTRODUCTION

Volcanic rock and ceramiste are two common materials in biofilters [1, 2]. The former is a natural product which is abundant on Earth, and the latter is a novel artificial material [3]. Both volcanic rock and ceramiste have good bearing capacity and corrosion resistance. In addition, the two materials enjoy high gas permeability, a huge specific surface area, and thus the ability to absorb lots of microorganisms. As a result, volcanic rock and ceramiste are excellent materials for wastewater treatment. Comparatively, volcanic rock has better treatment effect, while ceramiste is easier to obtain. Many scholars have explored the treatment effect of the two materials, but failing to compare the microorganism communities between them [4-7].

In 2018, Jiang et al. [8] studied the features and composition of nitrobacteria (NOB) and ammonia oxidizing bacteria (AOB) in a partial nitrification-anammox biofilter (PN/AF) system, revealing that the NOB is more stable when dissolved oxygen (DO) is high. Yang et al. [9] investigated the denitrification performance of volcanic rock in aerated biofilters, and found that the AOB can coexist with anammox bacteria. Gao et al. [10] developed and implemented a novel volcanic rock-based filter, discovered the positive correlation between DO and the operating efficiency of the filter, and disclosed the importance of Nitromonas, Microsporomand Candida krusei in the removal of organic matter and ammonia nitrogen. These studies mainly focus on the NOB in volcanic rock medium, paying less attention to the total microbial communities. In addition, there is little report on the ceramiste medium or multi-layer biofilter (MBF).

With good conservation and variability, the 16S rRNA is an important tool to analyze microbial diversity. For instance, Yasuda et al. [11] relied on this tool to explore the community structure and total bacterial count of denitrifying bacteria (DNB) in ammonia-nitrogen biofilters. Similarly, the high-throughput sequencing (HTS), a rapidly evolving method, has been widely applied to analyze the diversity and composition of environmental microorganisms [12]. Brinkman et al. [13] adopted the HTS to monitor the seasonal changes of the virus in wastewater. Martínez et al. [14] examined the abundance variation of archaea in sludge by the HTS. All these studies have shown that the HTS is extremely efficient and convenient in determining the properties of environmental microorganisms [15, 16].

In this paper, two pilot scale MBFs are designed, respectively packed with volcanic rock and ceramiste. Under steady operation, the two MBFs were compared in terms of operating state, microbial features, dominant population and microbial diversity. The research findings clarify the research direction and provide the theoretical basis for improving ceramiste technology and developing alternatives for volcanic rock.
2. MATERIALS AND METHODS

2.1 Pilot scale MBFs

Two pilot scale MBFs were fabricated with stainless steel (L×W×H: 42cm×31cm×2,200cm). Each MBF consists of six equal-height chambers. The inside dimension of each chamber is 250mm×200mm×220mm. The total volume of the packing medium is 66L. For natural ventilation, both the shell and the inner layers are highly porous. One of the MBF was packed with volcanic rock and the other with ceramiste. The particle size of both materials is about 1cm. The structure of each pilot scale MBF is shown in Figure 1.

![Figure 1. Sketch map of a pilot scale MBF](image)

Note: 1. Wastewater tank; 2. Peristaltic pump; 3. Main body of trickling filter; 4. Chamber; 5. Perforated rubber hose

2.2 Water quality analysis

To keep the water quality consistent, the wastewater was prepared every two days. The effluent was collected by a plate beneath the MBF. The COD, NH₄-N and TN were analyzed by the methods specified in Chinese national standards [17-19].

2.3 Sample collection and processing

At 60d, the biofilm samples were collected from every layer of the two MBF. The samples from the same MBF were mixed together. The mixture was subjected to 5min ultrasonic treatment, and then flushed with deionized water. After that, the sample was relocated to a 50mL tube, and centrifuged at 8,000×g for 10min. The sediments were stored at -80°C until DNA extraction.

2.4 DNA extraction and polymerase chain reactions (PCRs)

The total genomic DNA was extracted by E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, US). To set up the clone library, the primer sets 341F (5’- CCTACGGGAGGCAGCAG-3’) and 907R (5’- CCCTCAATTCTMTTARGT-3’) were selected to amplify the hypervariable V3~V5 region (about 560bp) of bacterial 16S rRNA gene. To identify each sample in the mixed reaction, the fused forward primer contains a10-base long barcode, inserted between the 454 adapter and the 341F primer.

The PCRs were carried out in a 25 μL volume containing 2.5 μL 10×buffer, 2 μL dNTP, 1 μL of each primer (10 μM),2 μL of DNA(20 ng/μL) and 0.125 μL 5 pyrobrestpolymerase(5 U/μL, Takara, China). The thermocycling was carried out at 94 °C for 4 min, followed by 27 cycles at 95 °C for 30s, 55 °C for 45s, 72 °C for 1 min and a final extension at 72 °C for 7 min. The number of PCR cycles was reduced for the accuracy and reliability of the subsequent analysis. The PCR products were obtained with a 0.8 % agarose gel, with the amplicon size of 500bp.

2.5 Pyrosequencing

The PCR products were purified with AMPureXP beads, quantified by Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, US), and finally pyro-sequenced on 454 Gs Fx Titanium Platform (Roche, US).

2.6 Sequence analysis

The genetic sequences were analyzed on QIIME, an open-source bioinformatics pipeline for performing microbiome analysis from raw DNA sequencing data. Any sequences failing to meet the following requirements were considered ineffective and removed: the length falls within 200~1,000bp, fewer than 6 consecutive bases are identical, no ambiguous base is included, and the quality score is above 2. Next, the sequences with greater-than-97 % similarities were clustered into one operational taxonomic unit (OTU), using the QIIME program. Community richness and diversity index, such as Chao1 estimator, ACE estimator and Simpson’s diversity, and rarefaction curves were obtained with mothur. The species in each OTU were identified through blasting with the genes in Greengene database. The Venn diagram of each OTU was drawn by Venny 2.1.0.

3. RESULTS AND DISCUSSION

3.1 MBF performance

Table 1 lists the water quality indices of the two MBFs under stable operation. As shown in the Table, the pH value only change slightly through the monitoring. The influent is basically pH neutral (6.827), while the effluents of both packing media is faintly alkaline (7.897 for volcanic rock and 7.692 for ceramiste). The DO of both MBFs is on the rise, signifying the increase in the content of dissolved oxygen. The effluent of volcanic rock contains more dissolved oxygen than that of ceramiste.

The removal rates of COD, NH₄-N and TN of the two MBFs are shown in Figure 2. It can be seen that, the COD of volcanic rock (76.8 %) was higher than that of ceramist (60.8 %).
Table 1. Water quality indices (temperature: 23.45±0.49 °C)

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>DO (mg/L)</th>
<th>COD (mg/L)</th>
<th>NH$_3$-N (mg/L)</th>
<th>TN (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>6.827</td>
<td>1.31</td>
<td>360</td>
<td>13.21</td>
<td>12.53</td>
</tr>
<tr>
<td>Effluent (volcanic rock)</td>
<td>7.897</td>
<td>4.14</td>
<td>77</td>
<td>2.79</td>
<td>2.72</td>
</tr>
<tr>
<td>Effluent (ceramsite)</td>
<td>7.692</td>
<td>3.78</td>
<td>141</td>
<td>2.18</td>
<td>7.91</td>
</tr>
</tbody>
</table>

In terms of NH$_3$-N removal rate, ceramiste slightly outperformed volcanic rock (83.5 % vs. 78.8 %). The high NH$_3$-N content in volcanic rock is the result of weak nitrification, which is an aerobic reaction. The more intense the reaction, the lower the DO of the effluent. Thus, it is speculated that volcanic rock has more but smaller pores than ceramiste, which favors anoxic reaction. The inverse is also true. This explains the relatively weak nitrification in volcanic rock.

By contrast, the effluent of volcanic rock had a TN removal rate (78.3 %) much higher than that of ceramiste (36.8 %). This means volcanic rock has a stronger denitrification effect than ceramiste. Denitrification is an anaerobic reaction. The result further validates the previous speculation.

3.2 Bacteria diversity

The data in Table 2 show that 11,844 and 10,904 sequences with high-quality 16S rRNA genes were recovered from volcanic rock and ceramsite, respectively, and subjected to community analysis. The libraries with volcanic rock and ceramsite respectively consist of 1,810 and 1,352 OTUs.

Table 2. Community richness and diversity indices

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of effective sequences</th>
<th>Number of high-quality sequences</th>
<th>OTUs</th>
<th>ACE estimator</th>
<th>Chao1 estimator</th>
<th>Simpson’s diversity</th>
<th>Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volcanic rock</td>
<td>12,576</td>
<td>11,844</td>
<td>1,810</td>
<td>3,082.413</td>
<td>3,008.355</td>
<td>0.0206</td>
<td>0.909</td>
</tr>
<tr>
<td>Ceramsite</td>
<td>11,431</td>
<td>10,904</td>
<td>1,352</td>
<td>3,125.282</td>
<td>2,287.846</td>
<td>0.0715</td>
<td>0.929</td>
</tr>
</tbody>
</table>

It can also be seen from Table 2 that volcanic rock contained richer species than ceramiste. In addition, the values of ACE estimator, Chao1 estimator, and Simpson’s diversity show that volcanic rock had the higher bacterial diversity.

The comparison of bacterial diversity reflects the features of the packing media. The volcanic rock has more but smaller pores than ceramiste, which reduce the impact of water flow. Thus, microorganisms are more likely to stay on volcanic rock for a long time than ceramiste, pushing up the total bacterial count and diversity on this medium. This agrees well with the number of OTUs in the two packing media.

The two samples are ranked by OTU abundance. Then, the distribution curves of OTU abundance are drawn and recorded as Figure 4. For each curve, the horizontal length reflects community richness, and the shape describes the species evenness. The curve of ceramiste is flatter than that of volcanic rock, revealing that the ceramiste medium is more evenly distributed than volcanic rock medium.

As shown in Figure 5, the two packing media only share 13.2 % OTUs, reflecting a huge difference in species between volcanic rock and ceramiste. The difference may be attributable to the origin of the two materials; volcanic rock is...
a natural packing material, while ceramiste is artificially prepared. As a natural material, volcanic rock may originally contain some microorganisms [21-24]. Thus, the microorganisms in ceramiste mainly come from the local environment, while only a part of those in volcanic rock has the same origin.

3.4 Bacteria composition

As shown in Figure 6, the volcanic rock contains sequences in 16 phyla while ceramiste only covers sequences in 11 phyla. This is an indirect evidence to the relatively high concentration of microbial species in ceramiste. Among all phyla, Tenericutes was unique to ceramiste, while Chloroflexi, GN02, NKB19, Thermi, Chlorobi and TM7 were only observed in volcanic rock. The top five phyla in the packing media were Proteobacteria, Bacteroidetes, Firmicutes, Gemmatimonadetes and Actinobacteria in turn. Ceramiste contained more Proteobacteria (67.06 % vs. 64.12 %), Bacteroidetes (25.11 % vs. 22.72 %) and Firmicutes (6.20 % vs. 4.55 %) than volcanic rock, but fewer Gemmatimonadetes (1.18 % vs. 3.87 %) and way fewer Actinobacteria.

4. CONCLUSIONS

This paper pioneers the comparison of microbial communities between pilot scale MBFs packed with volcanic rock and ceramiste, respectively. The analysis results show that, compared with ceramiste, volcanic rock can reduce COD and TN effectively, and ensure a high DO in the effluent. However, ceramiste has a slight edge in the removal of NH3-N. A possible reason for these results lies in the uniform shape of and wide gap between ceramiste particles. In the biofilter, ceramiste medium contains more oxygen than volcanic rock medium, and thus acquires strong nitrification effect and weak denitrification effect.

In terms of microbial features, volcanic rock contains more diverse microorganisms than ceramiste. This is because the multiple pores on and inside volcanic rock reduce the shear force of water flow, allowing more kinds of microorganisms to remain on its surface. Phyla analysis shows that the two packing media have similar dominant populations, but vary greatly in the proportion of each phylum. The above results show that ceramiste technique can be improved by optimizing the particle shape and increasing the particle porosity.

REFERENCES


