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Gülay, Arda; Musovic, Sanin; Albrechtsen, Hans-Jørgen; Smets, Barth F.

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Corresponding Author:	Arda Gülay, PhD DTU Environment Lyngby, Copenhage DENMARK
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	DTU Environment
Corresponding Author's Secondary Institution:	
First Author:	Arda Gülay, PhD
First Author Secondary Information:	
Order of Authors:	Arda Gülay, PhD
	Sanin Musovic, PhD
	Hans Jørgen Albrechtsen, Professor
	Barth Franciscus Smets, Professor
Order of Authors Secondary Information:	
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Abstract:	Rapid sand filtration is a traditional and common technology for drinking water purification from groundwater. Despite its wide scale and long-term use, the diversity and characterization of microbial communities in these engineered systems have remained unexplored and their roles in removal performances yet to be discovered. In order to explore the microbial ecology of these systems, we conducted 16S rRNA gene (rDNA) based 454 pyrosequencing as a deep sequencing approach to 94 sample cores retrieved from 5 different waterworks including proper biological replication. This comprehensive sampling of replicate rapid sand filters across many waterworks together with high-throughput sequencing provides a first glimpse into the microbial communities in rapid sand filters and their potential roles in the treatment process.
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Microbial community structure and a core microbiome in biological rapid sand filters at Danish waterworks

A. Gülay[1], S. Musovic[1], H.J. Albrechtsen[1], B.F. Smets[1]

[1] Department of Environmental Engineering, Technical University of Denmark, Building 113, Miljøvej, 2800 Kongens Lyngby, Denmark

Abstract

Rapid sand filtration is a traditional and common technology for drinking water purification from groundwater. Despite its wide scale and long-term use, the diversity and characterization of microbial communities in these engineered systems have remained unexplored and their roles in removal performances yet to be discovered. In order to explore the microbial ecology of these systems, we conducted 16S rRNA gene (rDNA) based 454 pyrosequencing as a deep sequencing approach to 94 sample cores retrieved from 5 different waterworks including proper biological replication. This comprehensive sampling of replicate rapid sand filters across many waterworks together with high-throughput sequencing provides a first glimpse into the microbial communities in rapid sand filters and their potential roles in the treatment process.

Keywords: Pyrosequencing; diversity; microbial community

Introduction

Producing drinking water to adequate purity for human consumption is a vital engineering task. In Europe and several other countries, biological rapid sand filtration is a common treatment technique to produce drinking water from relatively high-quality groundwaters. The chemical composition of the extracted groundwaters varies on geographical location but NH_4^+, Fe^{2+}, Mn^{2+}, CH_4, H_2S are typically the main electron donors which require removal down to a certain effluent criterion. Rapid sand filters are appropriate environments to support attached microbial growth, as the sand and associated mineral deposits providing substratum and protection. Although it is a long-standing technology in many countries, filter performances may be temporally instable and intrafilter spatial variability in performance has been measured, both of which may cause undesirable exceedence of effluent water criteria. In order to control filter performance and minimize the variability in treated water quality, knowledge of the microbial composition, diversity and its functional potential are essential. This knowledge is, to a large extent, absent in the scientific community. This deficiency stems from (a) a lack of comprehensive surveys on full-scale drinking water plants, (b) poor sampling strategies, (c) inadequate biological and technical replication and (d) a lack of robust microbiological and molecular techniques.

Here we applied 16S rRNA gene (rDNA) based 454 pyrosequencing as a deep sequencing approach to 94 core samples (each divided in a near surface and below surface sample) from 5 different waterworks together with proper biological replication. We chose the waterworks to represent different groundwater influent conditions and with records of good or bad performance. We aimed to answer following questions: Which taxa are dominant in rapid sand filters? How does phylogenetic structure differ between waterworks and waterworks units? Can we identify a core (shared) microbiome among selected waterworks? Are there any differences in terms of diversity between specific functional guilds (Ammonium, Nitrite, Iron and Methane Oxidizing Bacteria) among different units and waterworks?

Material and Methods

Sample collection and Genomic DNA isolation

Sediment samples were collected on prefilter and afterfilters at 5 different waterworks using a 60cm long core sampler. Each core was sectioned on site at 5 to 10 cm using a metal spatula and immediately stored on ice. Sections were directly transferred to laboratory and homogenized in sterile plastic bags using a rotating drum. A 0.5 g aliquot of homogenized sediment (from a certain depth) was subjected to subsequent genomic DNA extraction using MP FastDNA™ SPIN Kit (MP Biomedicals LLC., Solon, USA) according to manufacturer's instructions. The concentration and purity of extracted DNA was checked by NanoDrop (ThermoFisher Scientific). Extracted DNA was amplified Phusion (Pfu) DNA polymerase (Finnzymes, Finland) and the 16S rRNA gene targeted (rDNA) universal primers PRK341F (5'-CCTAYGGGRBGCAACAG-3') and PRK806R (5'-GGACTACNNGGGTATCTAAT-3') (Yu et al., 2005) with 25 annealing and elongation cycles. 16S

rDNA fragments comprising the V3 and V4 hypervariable regions were pyrosequenced using a 454 FLX Titanium sequencer (Roche, USA).

Bioinformatic analyses

All raw 16S rRNA amplicons were processed and classified using the QIIME software package (Caporaso et al., 2010). Chimera checking and denoising were performed with the software Ampliconnoise (Quince et al., 2011) and UCHIME (Edgar et al., 2011). Sequences were clustered into OTUs at 97% phylogenetic similarity and aligned against the Greengenes database (DeSantis et al., 2006) using the Pynast (Caporaso et al., 2010) algorithm. Particular phylogenetic trees were created in ARB using library sequences of interest together with selected representatives from a SSU reference library (SSU Ref. Nr. 111 Silva). Statistical calculations and phylogenetic comparisons were carried out using R software (Core Team, 2012) and QIIME software.

Results and Conclusions

A total of 857835 sequences passed all quality checks and 33.019 $OTUs_{0.03}$ were identified at 97% phylogenetic similarity. Among these $OTUs_{0.03}$, 32627 (99%) could be assigned to known taxonomies. Subsampling the $OTUs_{0.03}$ revealed that in nearly all waterworks (excluding Sjælsø South after-filters), the sampling effort is sufficient to cover the diversity. Shannon diversity results (Table 1) showed differences between prefilter and afterfilter communities (except at Langerød waterworks). A surprising high diversity of microbes was detected in rapid sand filters compared to activated sludge and freshwater systems. Evenness results followed the same pattern as diversity and revealed differences in pre- and afterfilters. We found that the microbial structure at different waterworks were similar both in taxa identity and relative abundances. The community at Sjaelsø North was very different, probably due to the high groundwater methane concentrations, only found at this site. Intriguingly, the most abundant taxa across the waterworks were *Nitrospirae* (*Nitrospirales*) (fig.1: green) in most of the after filter samples and *gamma-Proteobacteria* (*Oceanospirillales*) (fig.1: blue) in most of the pre-filters. Core microbiome analysis revealed 34 (4% of total $OTUs_{0.03}$) and 26 (3% of total $OTUs_{0.03}$) shared $OTUs_{0.03}$ in pre and after filters of all waterworks respectively. The core microbiome accounted for 80% of the total sequence abundances in all units. This comprehensive sampling of the rapid sand filters across many waterworks together with high-throughput sequencing provides us for the first time an insight into the microbial communities in rapid sand filters.

Table 1: Evenness (Gini) and diversity (Shannon) of each waterworks and their units

Gini Corr.	Shannon	Zone	Unit	Water works
0.841	8.5±0.02		Gw	
0.890±0.007	4.1±0.18	top	Pre	Langerød West
0.881±0.01	4.2±0.24	bot.		
0.862±0.01	4.8±0.17	top	After	
0.855±0.01	4.8±0.4	bot.		
0.683	6.1±0.03		Gw	
0.895±0.007	4.3±0.2	top	Pre	Langerød East
0.888±0.01	4.5±0.05	bot.		
0.862±0.01	4.6±0.09	top	After	
0.868±0.007	4.5±0.02	bot.		
0.735	7.5±0.03		Gw	Islevbro
0.909±0.01	3.4±0.36	top	After	
0.859±0.04	4.6±1.02	bot.		
0.775±0.03	6.8±0.04		Gw	
0.929±0.007	3.1±0.60	top	Pre	Sjaelsø North
0.879±0.04	3.3±0.43	bot.		
0.721±0.03	7.6±0.01	top	After	
0.716±0.03	7.5±0.41	bot.		
0.667	8.6±0.03		Gw	
0.879±0.01	4.0±0.37	top	Pre	Sjaelsø South
0.852±0.01	4.6±0.26	bot.		
0.784±0.02	6.4±0.08	top	After	
0.792±0.01	6.3±0.47	bot.		

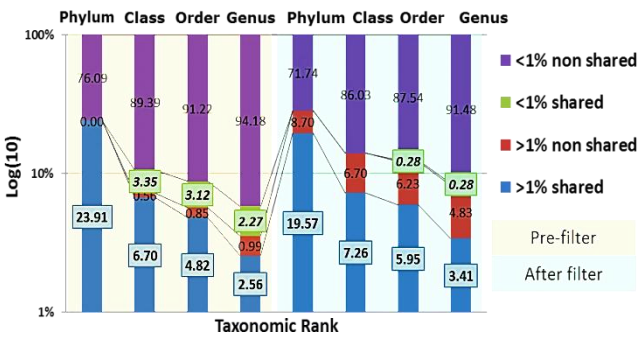

Figure 2: Fractions of shared and unshared $OTUs_{0.03}$ in dominant (>1%) and rare (<1%) microbiome at the levels of phylum, class, order and genus

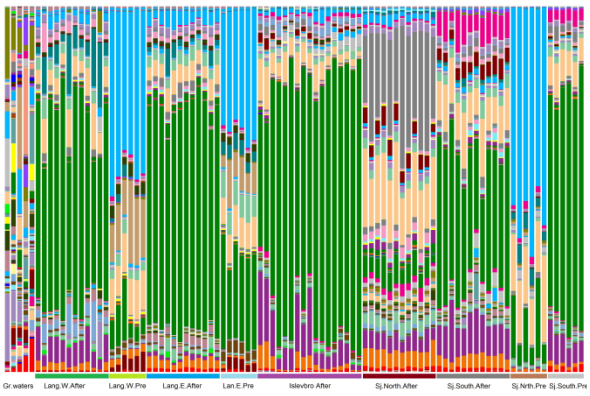

Figure 1: Relative abundances of 5 different waterworks at the level of order

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