

## Extended functionality of microbial communities in rapid sand filters – inference from community and metagenomic analysis.

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Rapid sand filtration is a commonly used method for the production of potable water from groundwater, and is the primary method for drinking water production in Denmark. It is traditionally believed that the main microbiological process in rapid sand filters is nitrification: the oxidation of ammonium to nitrate. Our recent findings suggest that the microbial communities in rapid sand filters have a broader range of functionalities than previously appreciated. We surveyed the microbial communities of 12 filters distributed across Denmark using 16S rRNA gene amplicon sequencing, qPCR analysis and metagenomic sequencing. The core community, comprising taxa present in all filters, consists of 9 genera which made up between 57-96% of the filter communities, but only 10.6% of the total sequence variants. These genera, in order of decreasing abundance are *Nitrospira*, *Blastocatella*, *Methylogobulus*, *Woodsholea*, *Hyphomicrobium*, *Pedomicrobium*, *Nitrosomonas*, *Zymomonas*, and *Variibacter*. Putative functions associated with these genera include nitrification (*Nitrospira* and *Nitrosomonas*), methane oxidation (*Methyloglobulus*), metal (Fe and/or Mn) oxidation (*Hyphomicrobium* and *Pedomicrobium*). The communities are dominated by *Nitrospira* spp. (mean=35%), especially the recently described completely nitrifying comammox *Nitrospira* (28-100% of the total *Nitrospira*). Using stable isotope probing, comammox *Nitrospira* were found to be the most active nitrifying bacteria in one filter, however in all filters they coexist with both Betaproteobacterial AOB (average= 1.8%) and AOA (<1%). In addition to nitrifying bacteria, we assembled draft genomes that encode methane and sulfide oxidation pathways, as well as organisms closely related to known Fe<sup>2+</sup> and Mn<sup>2+</sup> oxidizers, suggesting that these organisms contribute to the removal of these contaminants in the filters. Furthermore, iron oxidizing bacteria have been enriched from filter material, including *Gallionella* spp., *Ferriphaeselus* spp. and putative iron oxidizers *Undibacterium* sp. and *Curvibacter* sp.. In addition to the removal of inorganic contaminants, the filter communities are also capable of the removal of organic compounds. An important source of organics within the filter are microbial cells derived from either the groundwater, or that grow during the treatment process itself, which are removed over the course of filtration. The presence of pathways for the oxidation of aromatic compounds including catechol, phthalates and benzoate suggest that the filter communities also have the potential to remove a range of organic compounds that may include substances native to the subsurface such as lignin and lignin degradation products, as well as organic pollutants including pesticides, xenobiotics and organic micropollutants.