

## Discovery and description of complete ammonium oxidizers in groundwater-fed rapid sand filters - DTU Orbit (09/11/2017)

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Microbial communities are directly linked with process performance in several engineered systems. In the last century, intense study of microorganisms has contributed to optimize important environmental biotechnologies such as the activated sludge process or anaerobic digestion. However, less attention has been paid to the role of microorganisms in drinking water treatment technologies. In contrast, much effort has been devoted to eliminate potential pathogens in the drinking water treatment and supply systems. Nevertheless, the role of microbes in some drinking water treatments systems as biological filtration has long been acknowledged and recently been investigated. Biological filtration technology is widely used around the world and is especially important in Denmark as groundwater is the main source water for drinking water production. Because the groundwater has a relative high-quality, aeration followed by biological filtration is the only required treatment before distribution. In the last years, the microbial communities in rapid gravity sand filters, the typical biological filter used in Denmark, have been characterized, but little knowledge had been required about their physiological activity and roles in compound removal from the source water.

This PhD project focused on a comprehensive investigation of the microbial communities in rapid sand filters beyond their purely taxonomical identification. For this purpose, samples collected from a rapid sand filter were subjected to metagenomics analysis and genome recovery to identify the genetic capacities of the dominant types in the microbial community. Fourteen near-complete population genomes representing the dominant community were recovered comprising the capacity to grow on the typical compounds found in groundwater. The identified population genomes contained capabilities to oxidize ammonium, nitrite, methane, hydrogen sulfide, iron and manganese as well as to assimilate organic compounds. A composite population genome was assigned to *Nitrospira*. This genus had previously been found in multiple rapid sand filters at an unexplained high abundance. *Nitrospira* spp. are known to perform the second step of nitrification: oxidation of nitrite to nitrate. The two-step nitrification process disclosed at the end of the 19th century was assumed to be carried out by two different functional groups, ammonia oxidizing prokaryotes and nitrite oxidizing bacteria. Strikingly, the *Nitrospira* composite population genome not only contained the genes to oxidize nitrite to nitrate, but also the genetic potential to execute the first step of nitrification. Exhaustive bioinformatics investigation ruled out the possibility of genomic contamination and confirmed that the *Nitrospira* composite population genome harboured the complete ammonium oxidation (comammox) pathway. At the same time, evidence of a single microbe's capacity to carry out complete nitrification was obtained by three other groups; in all cases the comammox type belonged to the *Nitrospira* genus.

To further investigate the genomic capacities of comammox *Nitrospira*, the *Nitrospira* composite genome was separated into individual population genomes using a differential coverage binning approach. As a result, five individual genomes were recovered, four of them containing the complete ammonium oxidation pathway. These genomes together with 11 high-quality publically available *Nitrospira* genomes (seven comammox and four strict nitrite oxidizers) were subject to a comparative genomics analysis. This examination showed specific genomic features for comammox, strict nitrite oxidizers and the two comammox clades. Thus, comammox *Nitrospira* harbour a higher variety of genes related to adaptation to nutrient-limited environments. The two comammox clades differ in their ammonium uptake affinity systems. Additionally, comammox *Nitrospira* genomes lack the genetic capacity to use nitrite as the only nitrogen source.

The evolutionary history of comammox *Nitrospira* was also examined based on protein dissimilarity, gene arrangement and reconciliation analysis. We detected a high probability of horizontal gene transfer events from betaproteobacterial ammonia oxidizers to comammox *Nitrospira* for genes belonging to the ammonium oxidation pathway as well as from comammox clade B to clade A for a subset of genes.

I investigated the abundance of comammox *Nitrospira* in rapid sand filters at 12 different waterworks in Denmark. As these new microorganisms are taxonomically similar to strict *Nitrospira* nitrite oxidizers, we developed specific primers to exclusively target comammox based on their gene encoding the ammonia monooxygenase subunit A. With these primers, we detected comammox *Nitrospira* as the dominant nitrifier in the biofilters with an abundance typically one order of magnitude higher than canonical ammonium oxidizing prokaryotes.

Lastly, I carried out lab-scale experiments with filter material from the top and bottom layers of a rapid sand filter containing different proportions of comammox *Nitrospira*, and strict nitrite and ammonia oxidizing prokaryotes under different loading conditions. Specifically, I exposed the filter material to distinct ammonium loading, under presence or absence of external carbon source as well as under oxygen limitation. In relation to the nitrifying community three main findings were made: (i) simultaneous growth of comammox *Nitrospira* and ammonium oxidizing prokaryotes; (ii) lower fitness of ammonium oxidizing archaea at higher temperatures; (iii) selection of comammox clade A over clade B at increasing ammonium loadings at reference temperature.

Overall, this PhD has provided insights into the genomic capabilities of the main types in the microbial community of a groundwater-fed biological filter. Moreover, the previously observed high abundances of *Nitrospira* spp. in rapid sand filters, has now been explained, by the discovery of complete ammonium oxidizing (comammox) *Nitrospira* from metagenomics analysis. In addition, this thesis presents the first extensive analysis of the genomic capabilities of comammox *Nitrospira* compared to canonical ammonium and nitrite oxidizers.

### General information

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