Water Research 129 (2018) 1–10

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Bioaugmentation of rapid sand filters by microbiome priming with a nitrifying consortium will optimize production of drinking water from groundwater

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ARTICLE INFO

Article history:
Received 5 July 2017
Received in revised form
3 November 2017
Accepted 3 November 2017
Available online 6 November 2017

Keywords:
Rapid sand filters
Waterworks
Nitrification
Bioaugmentation
Microbiome priming

ABSTRACT

Ammonium oxidation to nitrite and then to nitrate (nitrification) is a key process in many waterworks treating groundwater to make it potable. In rapid sand filters, nitrifying microbial communities may evolve naturally from groundwater bacteria entering the filters. However, in new filters this may take several months, and in some cases the nitrification process is never sufficiently rapid to be efficient or is only performed partially, with nitrite as an undesired end product. The present study reports the first successful priming of nitrification in a rapid sand filter treating groundwater. It is shown that nitrifying communities could be enriched by microbiomes from well-functioning rapid sand filters in waterworks and that the enriched nitrifying consortium could be used to inoculate fresh filters, significantly shortening the time taken for the nitrification process to start. The key nitrifiers in the enrichment were different from those in the well-functioning filter, but similar to those that initiated the nitrification process in fresh filters without inoculation. Whether or not the nitrification was primed with the enriched nitrifying consortium, the bacteria performing the nitrification process during start-up appeared to be slowly outcompeted by *Nitrospira*, the dominant nitrifying bacterium in well-functioning rapid sand filters.

1. Introduction

Ammonium oxidation to nitrite and then to nitrate (nitrification) is a key process in many waterworks treating groundwater to make it potable. In Northern Europe in particular, but in many other places as well, microbial oxidation is preferred over chemical oxidation with chlorine. In rapid sand filters, which are widespread in the purification of groundwater for use as drinking water, nitrifying microbial communities may evolve naturally from the groundwater bacteria that enter the filters (Lytle et al., 2007; Rittmann et al., 2012; Tekerlekopoulou et al., 2013). In most cases nitrification starts within one to four months of operation, during which time the water cannot be distributed to consumers (Lytle et al., 2007; Tekerlekopoulou et al., 2013). In some cases the nitrification process is never rapid enough to be efficient or is only partial, with nitrite as an undesired end product (e.g. Van der Aa et al., 2002; De Vet et al., 2011; De Vet et al., 2012; Lytle et al., 2013). In Denmark, for example, 20% of waterworks experience challenges with the nitrification process (Lee, 2014). In any event, a faster and more reliable start of the nitrification process in waterworks sand filters would be of great benefit to drinking water production companies.

Microbial nitrification has traditionally been viewed as two separate processes. Ammonium oxidation was thought to be carried out by *Nitrosomonas*, a betaproteobacterium (White et al., 2012; Tekerlekopoulou et al., 2013), and nitrite-oxidising bacteria such as *Nitrospira* and *Nitrobacter* were expected to complete the nitrification process (De Vet et al., 2009; Tekerlekopoulou et al., 2013; Albers et al., 2015a). Recently, however, it has been shown that the complete nitrification process may be carried out by comammox *Nitrospira* bacteria (Van Kessel et al., 2015; Daims et al.,

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https://doi.org/10.1016/j.watres.2017.11.009
0043-1354/

2015) and that these may be present in sand filters used for water purification (Pinto et al., 2016; Palomo et al., 2016). The scientific literature on nitrification in rapid sand filters treating groundwater is vast, but few studies have been published on attempts to prime the nitrification process during the start-up of new filters. In the few cases where priming of the nitrification process has been attempted, the approach was to stimulate the nitrifying microbes by adding trace elements such as phosphorous and copper (Van der Aa et al., 2002; De Vet et al., 2012; Lytle et al., 2013; Wagner et al., 2016) or by subsurface aeration (De Vet et al., 2011). Attempts have also been made to transfer nitrifying microorganisms from well-functioning sand filters, either as sand material or backwash water (Stembal et al., 2004; Lytle et al., 2007), although a positive effect on nitrification compared to non-inoculated filters has not been observed.

In the present study we took a different and novel approach. This involved (i) enriching a nitrifying bacterial consortium from a well-functioning sand filter, and (ii) adding (inoculating) the enriched consortium to new filters. The primary purpose of the inoculation was to ensure rapid nitrification, either by functioning until the local groundwater bacteria took over the process or as a substitute for local groundwater bacteria if these were unable to establish a nitrifying population. Furthermore, the study aimed to describe the evolution of nitrifying communities in new sand filters with and without inoculation. This was done by extracting DNA from the original filter material, from the enrichment and during the start-up of new filters and then by characterising the bacterial communities by 16S rRNA gene amplicon sequencing.

2. Materials & methods

2.1. Chemical analyses

Commercial spectrophotometric kits and a UV-1800 Spectrophotometer (Shimadzu, Japan) were used to analyse ammonium (Merck Spectroquant 1.14752, Merck KGaA, Germany) and nitrite (Merck Spectroquant 1.14776). The limits of quantification derived from standard curves in tap water and milliQ water were 0.02 mg/L ammonium-N and 0.01 mg/L nitrite-N. Nitrate was analysed by anion chromatography (Metrohm 819 IC detector with a Metrosep A 150/4.0 column, Metrohm, Switzerland) with a limit of quantification of 0.01 mg/L nitrate-N. Samples for the analysis of ammonium and nitrate were stored cold and analysed on the day of sampling. Samples for analysis of nitrate were kept frozen until analysis.

2.2. Enrichment and maintenance of nitrifying bacteria

The nitrifying consortium was enriched from filter material that originated in a rapid sand filter located at Islevbro Waterworks (Copenhagen, Denmark). The sand filter community in this particular filter has previously been described and compared with other rapid sand filters in Danish waterworks (Albers et al., 2015a). The filter is 27 years old and its primary function is to remove manganese and ammonium. It receives water from a chalk aquifer (11–93 m depth below surface), and before the groundwater enters the filter it is aerated and most of the dissolved iron removed. The concentration of ammonium in the filter's inlet water is ~0.3 mg/L. In March 2015, filter sand was sampled from the top of the second filter, i.e. after pre-filtration, into sterilised glass jars and stored at 5 °C until enrichment of the nitrifying consortium.

The enrichment procedure started in sterile 500 mL blue cap bottles with 200 g sterilised quartz sand (1.2–2.0 mm) inoculated with 10 g original filter material supplemented with 250 mL mineral growth medium according to Pitcher et al. (2010). The ammonium concentration used was 1 mM (18 mg L^{-1}). After two weeks all ammonium in the bottle was degraded and 2 g of sand was transferred to a bottle with 200 g sterilised quartz sand and 250 mL fresh medium. This transfer was repeated four times at intervals of 8–15 days following complete ammonium degradation in the bottle. The enrichment was undertaken in darkness at 10 °C on an orbital shaker.

The enriched nitrifying consortium was maintained on quartz sand (1.2–2.0 mm) in an incubator at 10 °C. The growth medium in the bottles was refreshed at 14-days intervals by removing the supernatant and adding fresh medium. Attempts to make cryocultures or to freeze-dry the consortium failed to yield regrowth of a functional nitrifying consortium. It was therefore essential to maintain the active nitrifying consortium. Production of larger volumes of the enriched nitrifying consortium for laboratory columns and mesocosm sand filter experiment starter culture was done under similar conditions as the enrichment described above but in 1 L bottles containing 750 g sterilised quartz sand and 500 mL growth medium.

2.3. Laboratory column setup

The nitrifying consortium was further tested and enriched in laboratory columns (h20 × Ø1.6 cm) run at 10 °C with a continuous upward flow of 4.8 mL/h, which gave an approximate water residence time of 3 h. The medium used was the same as that used during isolation, but was diluted five times to give an ammonium concentration of 3.6 mg/L. Quartz sand (0.8–1.4 mm, 99.0% SiO_2, DKI, Denmark) was steam sterilised, mixed with sand containing the enriched nitrifying consortium and wet packed into the columns sterilised with 0.01 M NaOH. The columns were not operated under strictly sterile conditions.

At the end of the experiment, sand was sampled in triplicate from the inlet, middle and outlet parts of the columns for extraction of DNA and further processing by qPCR and sequencing. DNA was extracted using the PowerLyzer PowerSoil DNA Isolation 159 Kit (MoBio, Carlsbad, California) as previously described by Ellegaard-Jensen et al. (2016).

2.4. Mesocosm sand filter experiment in a waterworks

A pilot-scale sand filter system was set up at the same waterworks from where the enriched nitrifying consortium originated (Islevbro, Copenhagen) and was run for eight weeks. The system consisted of four parallel filter columns, each made of a Plexiglas cylinder (h200 × Ø18 cm) operated with adjustable downward flow (Fig. 1). The columns were filled with 78 cm quartz sand (1.2–2.0 mm, 99.3% SiO_2, DKI, Denmark) on top of 20 cm coarser material (10 cm 50–80 mm quartz and 10 cm 30–50 mm quartz). After one day of operation and one backwashing procedure, 4 cm (1 L) of sand containing the enriched nitrifying consortium was added on top of two filters and 4 cm of non-inoculated sand was added to two control columns.

The sand filters received water from the waterworks just after aeration of the abstracted raw water with no pre-filtration. The key water parameters of the inlet water were: 0.3–0.4 mg/L ammonium, 2 mg/L iron, 0.04 mg/L manganese, 10 mg/L oxygen, 2 mg/L non-volatile organic carbon, <0.02 mg/L nitrite, <0.05 mg/L nitrate, <0.02 mg/L methane, pH of 7.3 and a temperature of 9–10 °C.

The water flow was 70 L/h, which corresponded to 2.8 m/h or an empty bed contact time in the 80 cm filters of 17 min. This corresponded to a water residence time of 7 min assuming a porosity of 0.40. Backwashing was performed every four days with filtrated water only (8 min, 20 L/min).