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Phosphorus limitation in nitrifying groundwater filters

W.W.J.M. de Vet [a,b,c,*], M.C.M. van Loosdrecht [b], L.C. Rietveld [c]

[a] Oasen Drinking Water Company, PO box 122, 2800 AC Gouda, The Netherlands
[b] Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands
[c] Department of Water Management, Delft University of Technology, Stevinweg 1, 2628 CN Delft, The Netherlands

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ABSTRACT

Phosphorus limitation has been demonstrated for heterotrophic growth in groundwater, in drinking water production and distribution systems, and for nitrification of surface water treatment at low temperatures. In this study, phosphorus limitation was tested, in the Netherlands, for nitrification of anaerobic groundwater rich in iron, ammonium and orthophosphate. The bioassay method developed by Lehtola et al. (1999) was adapted to determine the microbially available phosphorus (MAP) for nitrification. In standardized batch experiments with an enriched mixed culture inoculum, the formation of nitrite and nitrate and ATP and the growth of ammonia-oxidizing bacteria (AOB; as indicated by qPCR targeting the *amoA*-coding gene) were determined for MAP concentrations between 0 and 100 μg PO_4–P L^{-1}. The nitrification and microbial growth rates were limited at under 100 μg PO_4–P L^{-1} and virtually stopped at under 10 μg PO_4–P L^{-1}. In the range between 10 and 50 μg PO_4–P L^{-1}, a linear relationship was found between MAP and the maximum nitrification rate. AOB cell growth and ATP formation were proportional to the total ammonia oxidized. Contrary to Lehtola et al. (1999), biological growth was very slow for MAP concentrations less than 25 μg PO_4–P L^{-1}. No full conversion nor maximum cell numbers were reached within 19 days. In full-scale groundwater filters, most of the orthophosphate was removed alongside with iron. The remaining orthophosphate appeared to have only limited availability for microbial growth and activity. In some groundwater filters, nitrification was almost totally prevented by limitation of MAP. In batch experiments with filtrate water from these filters, the nitrification process could be effectively stimulated by adding phosphoric acid.

1. Introduction

In drinking water production from anaerobic groundwater in the Netherlands and Flanders, iron, manganese and ammonium are often removed in one trickling filtration step without adding strong oxidants. Molecular research into full- and pilot-scale filters showed that not only nitrification, but also iron and manganese oxidation may be biological processes (de Vet et al., 2010). In some of these groundwater filters at the drinking water company Oasen in the Netherlands, nitrification poses regular problems. In a groundwater filter with incomplete nitrification, the cell-specific nitrification rate was much lower than in a filter with full nitrification (de Vet et al., 2011).

* *Corresponding author*. Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands. Tel.: +31 610927947; fax: +31 152782355.
E-mail addresses: weren.de.vet@oasen.nl, W.W.J.M.deVet@tudelft.nl (W.W.J.M. de Vet), M.C.M.vanLoosdrecht@tudelft.nl (M.C.M. van Loosdrecht), L.C.Rietveld@tudelft.nl (L.C. Rietveld).
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In addition to the catabolic substrates, all microorganisms require anabolic substrates, such as (in)organic carbon, oxygen, ammonium (or nitrate)-nitrogen, phosphate and trace elements, for growth. Phosphorus (P) is an essential element in life. All cells contain phosphorus and require this element for growth, activity and regulation. Microorganisms may replace phosphorus with other elements in a P-limited environment, but only to a certain extent. The phosphorus content of a *Bacillus subtilis* may vary between 1.7 and 3.2% of the dry weight depending on the P availability (Harder and Dijkhuizen, 1983; and references therein).

Phosphorus in the environment is mainly present in the form of organic phosphate esters or inorganic phosphate. Phosphate availability in water has been the subject of many studies. There is a wide range in the phosphate content in natural water depending on the input from deposits, fertilizers, waste streams, and the dissolution of phosphate-containing ground minerals. In poor natural waters in boreal areas, the phosphate content may be so low (Miettinen et al., 1997) that it is the primary limiting compound for both prokaryotic and eukaryotic growth. Not all phosphate may be available for use by microorganisms. Next to the ionic orthophosphate form, phosphate may be present in dissolved organic complexes or in particulate form as metal or organic complexes (Scherrenberg et al., 2008). Drinking water references concerning phosphate deal with the (unwanted) heterotrophic growth in distribution and membrane systems (Vrouwenvelder et al., 2010; and references therein), and focus on the limitation of phosphate to control and minimize heterotrophic growth. Phosphate-limited nitrification has been reported in a wastewater polishing filter (Scherrenberg et al., 2009). During drinking water production, phosphate-limited nitrification is usually related to low temperatures (Andersson et al., 2001; Kors et al., 1998; van der Aa et al., 2002). In several nitrifying filter systems, phosphate was dosed effectively to remove the limitation. Yoshizaki and Ozaki (1993) reported dosing 50 μg PO_4^{3-}–P to a demonstration plant, van der Aa et al. (2002) 5–50 μg PO_4^{3-}–P to several pilot- and full-scale filter systems.

Here, nitrification at the Oasen water treatment plant (WTP) Lekkerkerk was studied. In the Oasen groundwater filters, the water temperature is moderately low and constant (13 °C), but the availability of phosphate is not straightforward. Orthophosphate content is relatively high in the groundwater, but reduced in the de-ironing trickling filters (see Table 1). Phosphate may be removed by (surface) precipitation (Ler and Stanforth, 2003) and adsorption (Lijklema, 1980) on iron (oxy)hydroxides in these filters. Makris et al. (2004) found minimal desorption of phosphate from iron-containing drinking water residuals and suggested that this stability was related to the immobilization of phosphate in abundantly present micropores. Next to the chemical removal of phosphate by iron (oxy) hydroxides, competing biological processes and specifically biological iron oxidation might further hamper nitrification by competing for phosphate.

To assess the microbial availability of phosphate for heterotrophic growth, Lehtola et al. (1999) developed a sensitive bioassay method. They found a constant cell yield per unit of phosphate, which was confirmed by other researchers (Polanska et al., 2005), 3.7×10^8 and 3.2×10^8 cfu per μg PO_4–P. Both teams worked with a pure culture of the heterotrophic *Pseudomonas fluorescens* P17 growing on acetate and enumerated with plate counts. In this paper, we hypothesize that phosphate may be limiting nitrification in de-ironing groundwater filters. To assess the microbially available phosphorus (MAP) for nitrifiers, we adapted the method developed by Lehtola et al. (1999) and applied it to full-scale nitrifying filters that performed well and poorly. The effect of phosphorous dosing on nitrification was evaluated in batch experiments.

2. Material and methods

2.1. Standardization of microbially available phosphorus for nitrifiers

The experimental method for the determination of MAP was based on the approach of Lehtola et al. (1999). These researchers enumerated the maximum cell numbers by spread plating of a pure culture of heterotrophic *P. fluorescence P17* bacteria grown in batch tests with environmental samples and standardization samples containing different P concentrations. The adaptations, we made for the application on nitrifying bacteria were as follows:

The setup consisted of Labinco multi-position magnetic stirring plateaus with 1 L glass bottles that each contained a steel sampling needle through a foam stopper and a magnetic stirring rod.

Table 1 – Orthophosphate, final ATP concentrations and cell numbers, maximum nitrification rates and calculated MAP in MAP batch measurements with filtrate samples from full-scale groundwater filters at WTP Lekkerkerk.

Origin filtrate sample	Nitrification in full-scale filters	PO_4–P μg L^{-1}	ATP yield pg mL^{-1}	AOB yield Cells mL^{-1}	Max. nitrification rate mg N L^{-1} h^{-1}	MAP μg P L^{-1}
Effluent of						
1st filter 1[a]	Full	80	257 ± 81	16×10^3	0.09	16
2nd filter 1	Full	80	25 ± 2	1.9×10^3	0.03	11
1st filter 5[b]	Incomplete	30	117 ± 27	0.4×10^3	0.00	≤10
2nd filter 5	Incomplete	20	6	0.05×10^3	0.00	≤10

a Groundwater for first filter 1 contained 545 ± 144 μg PO_4–P L^{-1} (average and st. deviation for 22 values in 2010).
b Groundwater for first filter 5 contained 742 ± 156 μg PO_4–P L^{-1} (average and st. deviation for 22 values in 2010).