Biological active groundwater filters: exploiting natural diversity

Weren W. J. M. de Vet, Willem Jan Knibbe, Luuk C. Rietveld and Mark C. M. van Loosdrecht

ABSTRACT

In the Netherlands, biological trickling filters without chemical pre-oxidation are generally applied to treat anaerobic groundwater, containing methane, iron, ammonium and manganese. Previous research showed that all compounds can be removed in one filter step and that not only the ammonia oxidation (by nitrification), but also the iron oxidation is often a biological process, despite oxygen saturated conditions and neutral pH. However, the optimal conditions for each process differs. In this paper, we report the preliminary results of a demonstration plant (40 $m^3 h^{-1}$) with two consecutive trickling filtration steps. The first highly loaded filter removed 1–1.5 ppm of methane and 5–6 ppm of iron with filtration rates up to 30 m h^{-1}. The second filter step removed 5–6 ppm of ammonium and 0.5–0.6 ppm of manganese virtually completely at 2 m h^{-1}. Quantitative (real time) polymerase chain reaction (qPCR) indicated that the growth of methane-oxidizing bacteria was marginal, but biological iron oxidation by *Gallionella* bacteria accounted for a quarter to over half of the total iron conversion.

Key words | biological filtration, *Gallionella*, groundwater, methane stripping, nitrification, qPCR

Weren W. J. M. de Vet (corresponding author)
Willem Jan Knibbe
Oasen Drinking Water Company,
Post Box 122, 2800 AC Gouda,
The Netherlands
E-mail: *weren.de.vet@oasen.nl*

Weren W. J. M. de Vet
Luuk C. Rietveld
Department of Water Management,
Delft University of Technology,
Stevinweg 1, 2628 CN Delft,
The Netherlands

Weren W. J. M. de Vet
Mark C. M. van Loosdrecht
Department of Biotechnology,
Delft University of Technology,
Julianalaan 67, 2628 BC Delft,
The Netherlands

INTRODUCTION

Drinking water treatment is generally characterized by physical or chemical processes. These processes, with a design based on either scientifically or empirically obtained knowledge, sufficient for many treatment setups. Biological processes are generally less well understood in drinking water treatment. They are even often considered undesirable since biological activity is associated with health risks. Only some bacteriological applications, such as nitrification, biological activated carbon filtration and some denitrification processes, are generally accepted in drinking water treatment. This differs greatly from the situation in wastewater treatment, where biological processes are considered an important tool for the improvement of water quality. Still, microorganisms are ubiquitous in natural and engineered systems and may be effectively employed in drinking water treatment for the removal of a wide range of inorganic and organic compounds.

This paper focuses on the removal of methane, iron, manganese and ammonium from anaerobic groundwater in trickling filters. The Dutch Drinking Water Company Oasen produces drinking water from river bank filtrated groundwater. This groundwater is characterized by relatively high concentrations of ammonium (up to over 10 mg L^{-1}) in combination with iron, methane and manganese. Because of the elevated levels of methane and ammonium in the water, trickling instead of submerged filters are used as a first filtration step.

The biological nitrification process, by which the ammonium is removed, sometimes fails. Cost and labor-intensive maintenance, such as frequent replacement or external washing of the filter material, forms the usual approach to maintain the nitrification at an acceptable level (de Vet *et al.* 2009a). Investigations into probable causes and solutions have taken place during the past decade in order to find enhancements of nitrification in trickling filters. Using molecular techniques, kinetics and activity measurements, the biological populations and processes in groundwater trickling filters were studied, with a

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focus on iron and ammonia oxidation (de Vet 2011a). The application of denaturing gradient gel electrophoresis (DGGE; Muyzer *et al.* 1993) and quantitative (real time) polymerase chain reaction (qPCR; Hierro *et al.* 2006) proved very successful in elucidating the nature of iron, ammonium and manganese removal in these trickling filters. The research confirmed the work of other researchers that different bacteria and archaea than the generally assumed genera perform the nitrification process under the oligotrophic conditions present in groundwater filters (de Vet *et al.* 2009b). The identification of different microorganisms performing different transformation processes made it possible to shed light on their interaction. Against expectation, the process of iron and manganese oxidation in groundwater trickling filters was found to be at least partly biological. The strictly lithotrophic *Gallionella* spp. played an important role in the iron removal in the groundwater trickling filters, despite neutral to slightly alkaline and the oxygen-saturated process conditions (de Vet *et al.* 2011b). The observed nitrification problems were caused by the inactivity and retarded growth of the ammonia-oxidizing bacteria (AOB; de Vet *et al.* 2011c) resulting from phosphate limitation in de-ironing groundwater filters. Under circumstances found in the filters of Oasen, the bacteria performing the nitrification process suffered from phosphate limitation (de Vet *et al.* 2012). Phosphate is removed very efficiently by iron-oxidizing bacteria and their highly adsorptive biogenic iron precipitates (Rentz *et al.* 2009). Iron- and ammonia-oxidizing bacteria compete for a limited food supply, and in these cases iron removers outcompete the ammonium removers.

The enhancement of the nitrification process by the dosage of phosphate is uncommon but not unprecedented in surface water treatment (Yoshizaki & Ozaki 1993; van der Aa *et al.* 2002). It has also been applied successfully on a laboratory scale with filtered groundwater (de Vet *et al.* 2012), but direct addition to groundwater prior to iron removal is hampered by the coprecipitation of phosphate with the iron(oxy)hydroxides.

The kinetics of iron, manganese and ammonia oxidation and the growth dynamics of the microorganisms involved differ. Rapid growth of iron- (and manganese-) oxidizing bacteria and accumulation of voluminous iron precipitates require frequent backwashing, which may disturb the slower growing ammonia-oxidizing microbial populations. Separation of these processes in consecutive filtration steps may solve these problems. Separation of the removal of iron, manganese and ammonium is possible in consecutive filter steps by the variation of the filtration rate. A separate highly loaded filtration step should remove the methane and iron first. The technique of separate iron removal is applied in submerged filters by the Flemish Drinking Water Company Pidpa. With methane-containing groundwater, the high filtration step needs to be trickling instead of submerged to minimize the biological methane oxidation. Biological removal of methane leading to excessive biomass growth and filter clogging should be avoided in favor of physical stripping (de Vet *et al.* 2010).

This paper presents and discusses the first results of a pilot study by Oasen, in cooperation with Pidpa, of a two stage biological trickling filtration for consecutive methane/iron and manganese/ammonium removal. The major innovation in this research is the application of highly loaded trickling filters to combine methane stripping and iron oxidation in one filter step. After the removal of methane and iron, nutrient limitation for nitrification can be effectively removed by dosage between the two filter steps, if necessary.

METHODS

Installation

The separation of the iron and ammonium removal processes was tested by the installation of two highly loaded trickling filters in front of an existing trickling filter at the Oasen water treatment plant (WTP), Lekkerkerk. A scheme of the experimental setup is shown in Figure 1.

The main characteristics of the filters involved are given in Table 1.

For the highly loaded first filters, water flows, turbidity of the effluent of both filters and the pH and oxidation-reduction potential (ORP) were monitored online with an interval of 1–10 min. Sampling points were available for water samples in the filter influent, effluent and backwash water, in the ventilation blow off and at three depths over the filer bed height. For both the first and second filters,

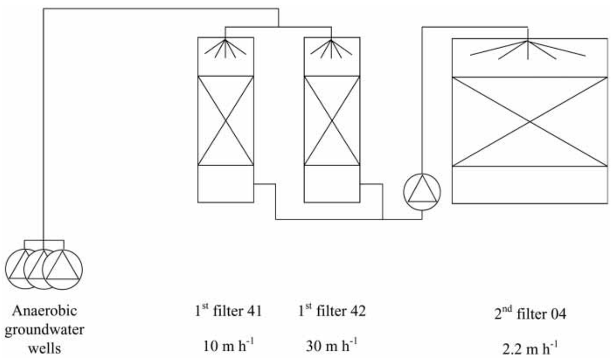


Figure 1 | Scheme of the experimental pilot installation with consecutive high and trickling filters.

samples were taken from the effluent once or twice a week and during special measurement campaigns (see Table 2).

The timeline of the experiments is summarized in Table 2.

Measurements

Enumeration and microbial balances of methane- and iron-oxidizing bacteria in filters

Methane-oxidizing bacteria (MOB) and iron-oxidizing bacteria (FeOB) were enumerated by qPCR. For the methods description for the *Gallionella* spp. (belonging to FeOB), we refer to de Vet *et al.* (2011b). Further protocols and primer sequences are available from the corresponding author, on request. From the cell numbers in and flow rates of all in- and outgoing flows (influent, effluent and backwash water), balances were made for MOB and *Gallionella* spp. over one filter runtime. The net outflow was assumed to match the growth of MOB and *Gallionella* spp. during that filter runtime. The fraction of biologically oxidized methane and iron was estimated from the total methane and iron removed, the measured cell yield, the cell dry weight (DW) from the literature (for *Gallionella* spp.: Hallbeck & Pedersen 1991) and the maximal theoretical DW yield for both types of bacteria from the literature (MOB: Leak & Dalton 1986; *Gallionella* spp.: Lütters & Hanert 1989; Neubauer *et al.* 2002). The cell DW of MOB was assumed to be similar to that of *Gallionella* spp.

Chemical analyses

Methane was determined by gas chromatography from the headspace of collected samples. The concentration in the water phase was calculated to be in equilibrium with the gas phase. Iron was determined by inductively coupled plasma mass spectrometry (ICP-MS). Samples for iron analysis were taken directly into nitric acid containing bottles to set the pH below 2. Ammonium was determined by colorimetric measurement.

Table 1 | Characteristics of first and second trickling filters at WTP Lekkerkerk

		Highly loaded filters for iron and methane removal: First filter 41	First filter 42	Filter for ammonium and manganese removal: Second filter 04
Filter material		Anthracite 1.4–2.5 mm		Dual layer: anthracite 1.4–2.5 mm/sand 0.8–1.25 mm
Filter area	m^2	1	1	18
Bed height	m	1.5	1.5	1 + 1
Production flow (from 27 May 2011)	m^3/h	10	30	40
Filtration rate	m/h	10	30	2.2
Filter runtime	h	72	24	504
Backwash rate	m/h	55		800
Aeration and ventilation	Spraying followed by trickling filtration with forced ventilation			
RQ (air to water ratio)		1		10

Table 2 | Overview of events and special measurements during the experiments with two stage biological filtration for iron and ammonium removal

Date	Event or special measurement
4 April 2011	Startup with limited flow
18 April–27 May 27 2011	Buildup of production flow
7–15 April 2011 *and* 16–19 May 2011 *and* 20–24 June 2011	Special measurements on highly loaded iron and methane removing filters: • Microbial balances • Carousel sampling
30 May–20 June 2011	Special measurements on ammonium and manganese removing filter: • Carousel sampling

Carousel sampling

To evaluate the filter performance over the filter runtime, samples of the filter effluent were taken by an automatic sampling device at regular intervals during one runtime.

RESULTS AND DISCUSSION

Methane and iron removal by highly loaded trickling filtration

Methane removal

The influent and effluent measurements for methane of both filters are shown in Figure 2 for the applied RQ of 1. The average methane removal efficiency was 99 and 93%, and the average effluent methane concentration was 34 ± 25 and $85 \pm 33\ \mu g\ L^{-1}$ for the first filter 41 ($5–10\ m\ h^{-1}$) and first filter 42 ($5–30\ m\ h^{-1}$), respectively.

One of the prerequisites for a stable functioning of the highly loaded first filter step is minimal growth of MOB. The MOB cell numbers, cumulated over one filter runtime, for the influent and both outgoing water flows (effluent and backwash water) are shown in Figure 3.

The enumeration of MOB cells in filter samples showed a marginal growth of these bacteria in both highly loaded filters. After 2½ months of operation (June) the growth in first filter 42 ($30\ m\ h^{-1}$) was even slightly lower than in 41

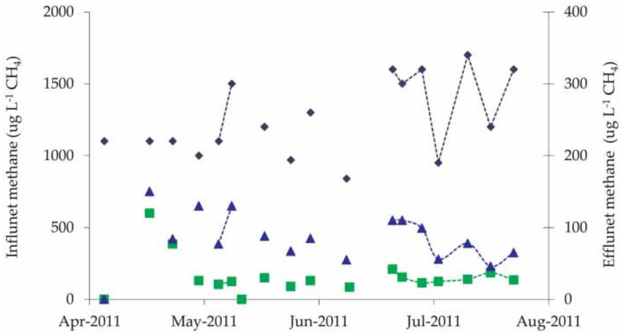


Figure 2 | Methane concentrations in influent (◆, left axis) and effluent (right axis) of highly loaded first filter 41 (■) and 42 (▲).

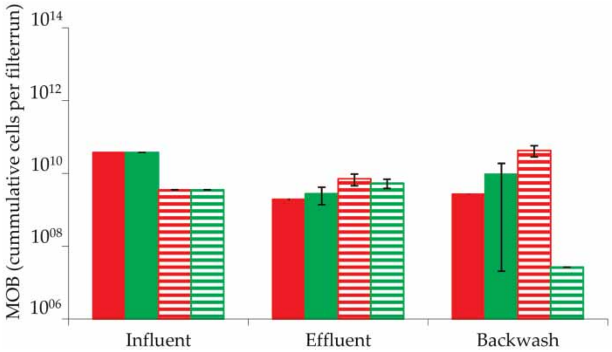


Figure 3 | Methane-oxidizing bacteria in filter influent, effluent and backwash water, cumulated over one filter runtime; dark grey (red online) bars first filter 41; light grey (green online) bars first filter 42; solid bars May 2011 (first filter 41, $10\ m\ h^{-1}$; first filter 42, $25\ m\ h^{-1}$), striped bars June 2011 (first filter 41, $10\ m\ h^{-1}$; first filter 42, $30\ m\ h^{-1}$). A colour version of this figure is available in the online version of the paper, at http://www.iwaponline.com/ws/toc.htm.

($10\ m\ h^{-1}$). The maximum cell growth was observed for first filter 41 in June 2011: 5×10^{10} cells per 504 g of CH_4 removed during one filter runtime. This corresponds to a yield of 1×10^8 cells or 1×10^{-5} g DW per g of CH_4 removed (with an assumed similar specific cell DW of 1.2×10^{-13} g $cell^{-1}$ from Hallbeck & Pedersen 1991). Leak & Dalton (1986) found a biomass yield of 0.5–0.8 g DW g^{-1} CH_4. This suggests that even at the applied low RQ of 1, the methane removal in the highly loaded filters is mainly physical.

Iron removal

The iron removal of the highly loaded trickling filters is shown in Figure 4. The effluent samples are taken at the end of the filter runtime.

A clear pattern in iron and turbidity was seen for both filters. Figure 5 shows this pattern for first filter 42 ($25\ m\ h^{-1}$)

Figure 4 | Iron concentrations in influent (◆), effluent of highly loaded first filter 41 (■) and of 42 (▲); dotted lines indicate – from left to right – the increase in filtration rate to 10 (for both filters) and 15, 20, 25, 30 for first filter 42 m h^{-1} respectively.

in May. A linear relation between iron analyses and online turbidity measurements was found, showing the relevance of the turbidity measurement for filter monitoring.

The *Gallionella* spp. cell numbers in the in- and outgoing water flows and in the filter samples are given in Figure 6. The left graph shows the cumulative cell numbers over the filter runtime, which are used to estimate the share of biological iron oxidation in these filters (see Table 3). The right graph gives cell numbers over the depth of the filter bed. *Gallionella* spp. grew strongly in both filters and reached an almost steady state after 1 month of operation.

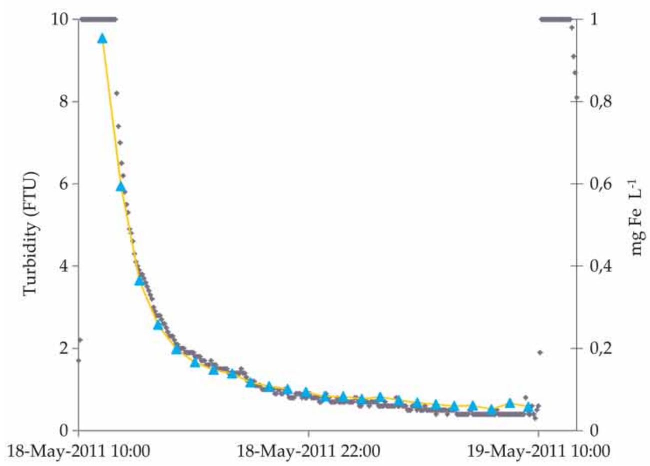
Based on the balanced increase of cells during one filter runtime, the indicative share of the biological process to the total iron oxidation is presented in Table 3. According to this approach, biological iron oxidation accounts for a quarter to over half of the total iron conversion.

Ammonium (and manganese) removal in second trickling filtration step

Ammonium and manganese were almost completely removed in the second filtration step after a startup period (see Figure 7). Hardly any removal of these compounds occurred in the highly loaded first trickling filters, unlike the removal of methane and iron. This indicates different (slower) kinetics for nitrification and manganese removal compared to stripping of methane and (bio)adsorptive oxidation and precipitation of iron, which makes the separation of iron and methane removal from nitrification and manganese removal feasible during the treatment of anaerobic groundwater.

The ammonium concentration in the filter effluent (see Figure 8) also shows a variation during the filter runtime, with an increase after backwashing. The carousel measurement was performed at the end of the startup period, so the ammonium profile over the filter runtime may have improved afterwards.

The long filter runtime of 3 weeks provided a stable nitrification with lower backwash water losses compared to the previous situation with combined removal of iron and ammonium (data not shown). The relapse in nitrification



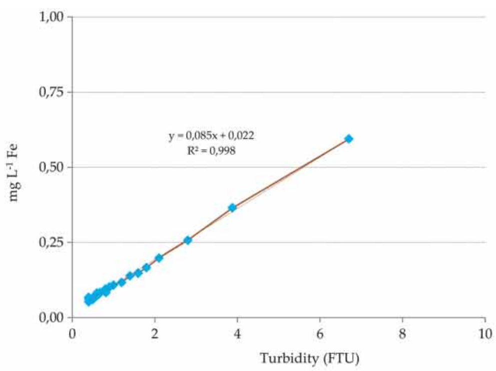


Figure 5 | Iron (▲) and turbidity (◆) in the effluent of highly loaded de-ironing first filter 42 during one filter runtime (left graph); calibration line for the relation of turbidity and iron for the same filter runtime (right graph).

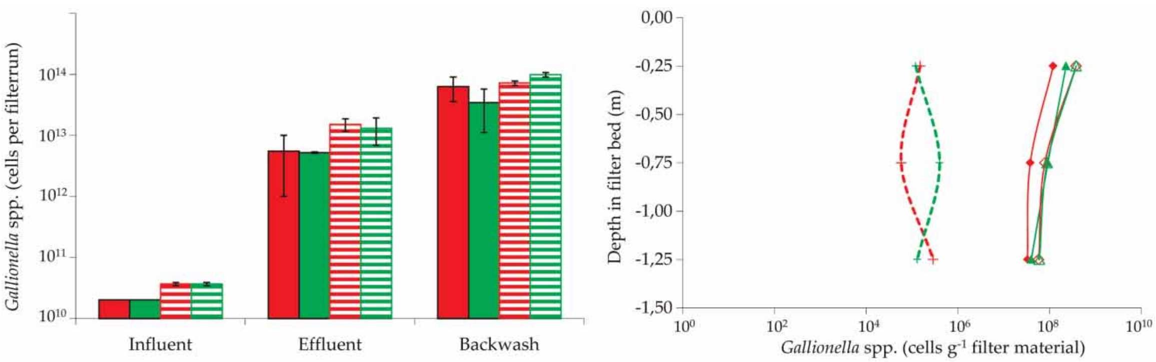


Figure 6 | *Gallionella* cells numbers in highly loaded trickling filters; left graph: cumulative cell numbers over one filter runtime, dark grey (red online) bars first filter 41, light grey (green online) bars first filter 42 ; solid bars May 2011 (first filter 41, 10 m h^{-1}; first filter 42, 25 m h^{-1}), striped bars June 2011 (first filter 41, 10 m h^{-1}; first filter 42, 30 m h^{-1}); right graph: cell numbers per g of filter material over the depth of the filter bed, dark grey (red online) lines: first filter 41, light grey (green online) lines: first filter 42; dotted lines April 2011 (initial state), solid symbols May 2011, open symbols June 2011. A colour version of this figure is available in the online version of the paper, at http://www.iwaponline.com/ws/toc.htm.

Table 3 | Removed iron, cell yield and estimation of the share of biological iron oxidation

		Iron removed		Yield		Fraction of Fe oxidized biological based on the maximal theoretical yield (gDW g^{-1} Fe)	
			per filter run			(Lütters & Hanert 1989)	(Neubauer *et al.* 2002)
	First filter	**gFe m^{-3}**	**gFe**	**Cells g^{-1} Fe**	**gDW[d] g^{-1} Fe**	**0.006**	**0.013**
May	41[a]	5.3	2,427	2.8×10^{10}	0.003	57%	26%
	42[b]	5.3	2,930	1.4×10^{10}	0.002	27%	12%
June	41[a]	5.3	3,429	2.5×10^{10}	0.003	51%	24%
	42[c]	5.3	3,657	3.1×10^{10}	0.004	61%	28%

[a]10 m h^{-1}, [b]25 m h^{-1},[c]30 m h^{-1}, [d]Specific cell DW 1.2×10^{-13} g cell $^{-1}$ (Hallbeck & Pedersen 1991).

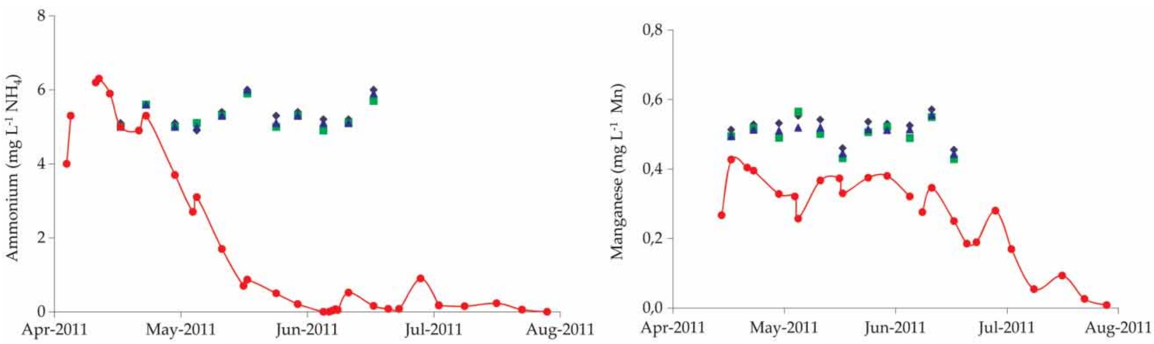


Figure 7 | Ammonium and manganese in influent and effluent of second filter; concentrations in groundwater (◆), effluent of highly loaded first filter 41 (■) and of 42 (▲) and effluent of second filter 04 (•).

after backwash suggests the washout of active biomass and asks for further optimization of the process operation. Dosing of phosphate to speed up the restoration of full nitrification after backwash and optimization of the backwash program to minimize biomass loss during backwash are being considered to this end.

CONCLUSIONS

The main conclusions from this research are:

- effective separation of removal processes in two consecutive trickling filters steps;

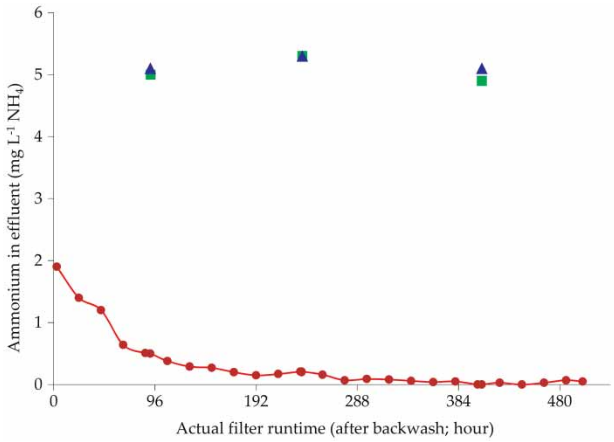


Figure 8 | Ammonium concentration over one filter runtime of 3 weeks (May 30–June 20) in the effluent of the second trickling filter following the highly loaded de-ironing filters; effluent of highly loaded first filter 41 (■) and of 42 (▲) and effluent of second filter 04 (•).

- over 90% methane and iron removal from groundwater in highly loaded (10–30 m h^{-1}) first trickling filters;
- no removal of ammonium and manganese in highly loaded first filters but almost complete removal in 2nd trickling filter with long filter runtime;
- methane physically stripped in first filters even at a low RQ of 1; limited growth of MOB;
- iron removal in first filters partially biological by *Gallionella* spp.

In summary, trickling filters are full of biological activity which gives opportunities for research breakthroughs, as well as interesting options for process improvements.

REFERENCES

van der Aa, L. T. J., Kors, L. J., Wind, A. P. M., Hofman, J. A. M. H. & Rietveld, L. C. 2002 Nitrification in rapid sand filter: Phosphate limitation at low temperatures. *Water Science and Technology: Water Supply* **2** (1), 37–46.

Hallbeck, L. & Pedersen, K. 1991 Autotrophic and mixotrophic growth of *Gallionella ferruginea*. *Journal of General Microbiology* **137** (11), 2657–2661.

Hierro, N., Esteve-Zarzoso, B., González, A., Mas, A. & Guillamón, J. M. 2006 Real-time quantitative PCR (QPCR) and reverse transcription-QPCR for detection and enumeration of total yeasts in wine. *Applied and Environmental Microbiology* **72** (11), 7148–7155.

Leak, D. J. & Dalton, H. 1986 Growth yields of methanotrophs. I. Effect of copper on the energetics of methane oxidation. *Applied Microbiology and Biotechnology* **23** (6), 470–476.

Lütters, S. & Hanert, H. H. 1989 The ultrastructure of chemolithoautotrophic *Gallionella ferruginea* and *Thiobacillus ferrooxidans* as revealed by chemical fixation and freeze-etching. *Archives of Microbiology* **151** (3), 245–251.

Muyzer, G., De Waal, E. C. & Uitterlinden, A. G. 1993 Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology* **59** (3), 695–700.

Neubauer, S. C., Emerson, D. & Megonigal, J. P. 2002 Life at the energetic edge: kinetics of circumneutral iron oxidation by lithotrophic iron-oxidizing bacteria isolated from the wetland-plant rhizosphere. *Applied and Environmental Microbiology* **68** (8), 3988–3995.

Rentz, J. A., Turner, I. P. & Ullman, J. L. 2009 Removal of phosphorus from solution using biogenic iron oxides. *Water Research* **43** (7), 2029–2035.

de Vet, W. W. J. M., Rietveld, L. C. & van Loosdrecht, M. C. M. 2009a Influence of iron on nitrification in full-scale drinking water filters. *Journal of Water Supply: Research and Technology – AQUA* **58** (4), 247–256.

de Vet, W. W. J. M., Dinkla, I. J. T., Muyzer, G., Rietveld, L. C. & van Loosdrecht, M. C. M. 2009b Molecular characterization of microbial populations in groundwater sources and sand filters for drinking water production. *Water Research* **43** (1), 182–194.

de Vet, W. W. J. M., van Genuchten, C. C. A., van Loosdrecht, M. C. M. & van Dijk, J. C. 2010 Water quality and treatment of river bank filtrate. *Drinking Water Engineering Science* **3** (1), 79–90.

de Vet, W. W. J. M. 2011a Biological Drinking Water Treatment of Anaerobic Groundwater in Trickling Filters. Dissertation, Technical University Delft, The Netherlands. Available from: http://repository.tudelft.nl.

de Vet, W. W. J. M., Dinkla, I. J. T., Rietveld, L. C. & van Loosdrecht, M. C. M. 2011b Biological iron oxidation by *Gallionella* spp. in drinking water production under fully aerated conditions. *Water Research* **45** (17), 5389–5398.

de Vet, W. W. J. M., Kleerebezem, R., van der Wielen, P. W. J. J., Rietveld, L. C. & van Loosdrecht, M. C. M. 2011c Assessment of nitrification in groundwater filters for drinking water production by qPCR and activity measurement. *Water Research* **45** (13), 4008–4018.

de Vet, W. W. J. M., van Loosdrecht, M. C. M. & Rietveld, L. C. 2012 Phosphorus limitation in nitrifying groundwater filters. *Water Research* **46** (4), 1061–1069.

Yoshizaki, T. & Ozaki, M. 1993 On removal of ammonia nitrogen by addition of phosphoric acid in ozone-granulated active carbon treatment. *Water Supply* **11** (3–4), 321–330.

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