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ABSTRACT

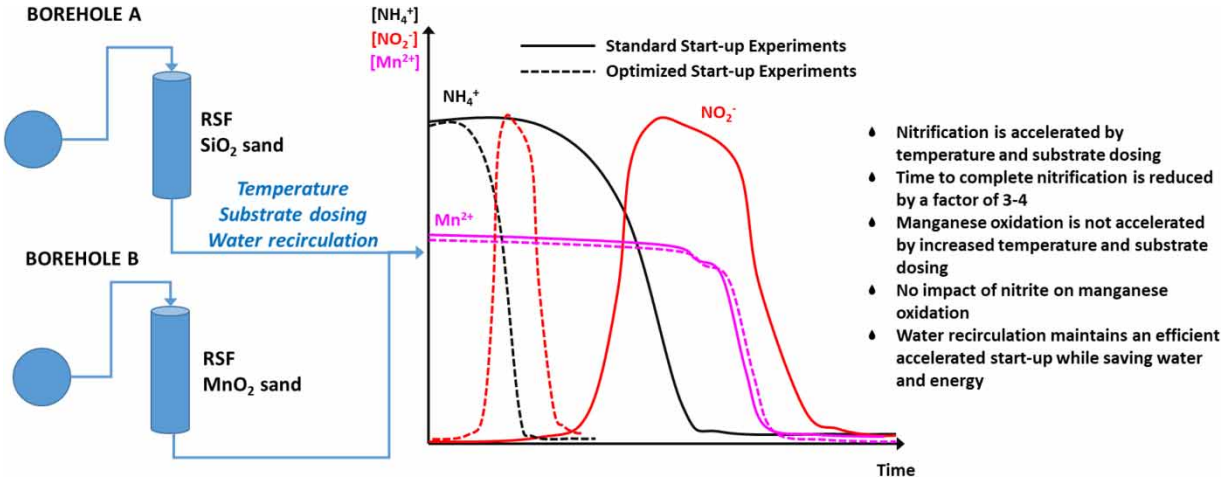
In drinking water production from groundwaters, biological rapid sand filters can be used for ammonium and manganese removal in aerobic conditions. However, in some boreholes, a start-up duration of several months is required to reach the required removal capacity, leading to significant water losses. Moreover, in specific industrial cases no exogenous biomass under the form of backwash water or activated sludge can be added to accelerate the process, and different approaches are seldom considered in literature. With the aim of saving water, start-up strategies coupling water temperature increase and substrate dosing were studied to accelerate the installation of biological activities, in a pilot plant fed with borehole water. These set-ups enabled a substantial acceleration of nitrification but no improvement of manganese oxidation in the experimental conditions, although the experiments showed no clear negative influence of nitrification, through nitrite accumulation, on biological manganese oxidation. To further save energy and reduce water loss, outlet water recirculation at a rate of 75% during the start-up phase was validated. The proposed start-up strategy enabled the complete installation of active biofilms with a mean start-up time reduction of 36% and water use reduction of 84% compared to the reference natural conditions.

Key words: ammonium, biofiltration, drinking water, aanganese, start-up, water saving

HIGHLIGHTS

- Accelerated start-up strategy for nitrification and manganese oxidation for the production of drinking water (84% of water saved in the best configuration tested).
- Temperature increase and substrate dosing have a better impact on nitrification than on manganese oxidation.
- No clear negative interaction between nitrification and manganese oxidation.

GRAPHICAL ABSTRACT



INTRODUCTION

Rapid sand filters are a common treatment process in drinking water production, for selective separation of unstable elements in groundwaters. They can combine particle removal by filtration with biological and physical-chemical processes to eliminate mostly nitrogen compounds, iron and manganese (Tekerlekopoulou *et al.* 2013). Presence of dissolved oxygen allows biological nitrification, which has to be complete with the conversion of all ammonium and nitrite into nitrate, due to the toxicity of nitrite at low levels. Manganese removal can be proceeded through two pathways: (1) biological manganese oxidation in presence of oxygen, (2) physical-chemical oxidation of soluble manganese (Mn^{2+}) into particulate manganese oxides by adsorption on reactive sand composed or coated by manganese oxides. An important operational issue with these biofilters is the long period required to achieve a stable and efficient elimination of the targeted compounds, leading to a considerable loss of water. In addition, physical-chemical oxidation of manganese requires frequent chemical regeneration of the reactive sand oxidation potential, which represents industrial costs and constraints.

Nitrification is a well-known process in water treatment, commonly described in two-step biological process involving bacteria and archaea (Niu *et al.* 2013). In presence of oxygen, ammonia-oxidizing bacteria AOB (the majority being part of the *Nitrosomonadaceae* family under the *Betaproteobacteria* class) (Klotz & Stein 2011) and archaea AOA (among them, the phyla *Crenarchaeota* and *Thaumarchaeota*) (Pester *et al.* 2011; Urakawa *et al.* 2011; Kitzinger *et al.* 2019), oxidize ammonium to nitrite. Then, nitrite oxidizing bacteria NOB (including *Nitrobacter* and *Nitrospira* genera) (Ehrlich *et al.* 1995; Tränckner *et al.* 2008) oxidize nitrite to nitrate. Since several years, microorganims named *commamox* (of *Nitrospira* genera) were also identified to operate complete nitrification (Daims *et al.* 2015; Kessel *et al.* 2015).

Manganese adsorption and oxidation on reactive sands allows rapid and efficient manganese removal. However, regular interruptions of the process and addition of chemical agents (strong oxidant agents such as Cl_2 or $KMnO_4$) are needed to regenerate the catalytic oxidative potential of the reactive sand. Biological manganese oxidation is a slow process which leads to particulate manganese oxides which in turn promote the chemical oxidation pathway similarly to reactive sands (Bruins *et al.* 2015a; Breda *et al.* 2019). Evidence of such processes was reported for *Pseudomonas manganoxidans* (Gage *et al.* 2001) and *Leptothrix dischopora* SS1 (Zhang *et al.* 2002) or for *Bacillus specie* in the case of sewage activated sludge (Hasan 2010).

Biological manganese oxidation is interesting because it limits the addition of chemical agents. Moreover, co-elimination of ammonium and manganese is feasible (Bruins *et al.* 2014; Cai *et al.* 2015; Zeng *et al.* 2020). However, a negative influence of nitrification on biological manganese oxidation has been reported in literature. The rate of biological manganese oxidation could be reduced in presence of nitrification, due to oxygen competition, nitrite accumulation (Vandenabeele *et al.* 1995; Cheng *et al.* 2016) and/or pH and ORP conditions (Abu Hasan *et al.* 2012; Han *et al.* 2013). In all cases, biological activities and biofilm development for nitrification and manganese oxidation are dependent on environmental and/or operating conditions (such as temperature, inlet concentrations, pH and oxygen) (Bruins *et al.* 2014). In real conditions, low temperature

and low concentrations of ammonium and manganese lead to slow start-up of industrial biofilters (Ciancio Casalini *et al.* 2020). Durations between 25 and 40 days for complete nitrification were reported by (Vandenabeele *et al.* 1995; Cheng 2015; Albers *et al.* 2018) and between 15 and 75 days for complete manganese oxidation (Bruins *et al.* 2015a; Cheng *et al.* 2018; Breda *et al.* 2019). Hence, high volumes of water are wasted during this settling phase, as it is unsuitable for production. To reduce start-up time, acceleration of the biological activities in terms of microbial filter colonization and growth must be achieved. Strategies to reach this goal must therefore be investigated.

Biological activity in nature or in industrial processes is the combination of the local biomass quantity and inherent biological properties, such as specific growth rate, which strongly depends on environmental conditions (temperature, pH, and substrate concentrations mainly). Mathematically, biological growth can be commonly represented by Equation (1) using Monod definition, where [X] is the concentration of active microorganisms (in mg/L), [S] is the concentration of substrate (mg/L), μ_{max}(T, pH) is the maximal specific growth rate dependent of temperature and pH, Ks is the half saturation constant (in mg/L).

$$\frac{d[X]}{dt} = \mu_{max}(T, pH).\frac{[S]}{Ks + [S]}.[X] \quad (1)$$

Thus, for initial biofilm settlement enhancement, two different strategies can be considered: (1) start with inoculated sand from another location to enhance the initial biomass quantity (X) (Cai *et al.* 2015; Albers *et al.* 2018; Ciancio Casalini *et al.* 2020); (2) modify the initial environmental conditions (T, [S]) to improve biological rates (Queinnec *et al.* 2006). For natural mineral waters, biomass addition is impossible due to regulations that forbid any modification of the natural microbial ecosystem (Directive 2009/54/EC of the European Parliament). Hence, only some modifications of the environmental conditions are acceptable. Therefore, to improve initial biological rates, enhancement of temperature and/or substrates concentration can be preferentially considered. These modifications could only be done off production during the start-up period. Temperature is a well-known parameter, efficiently influencing biological rates of nitrification (Antoniou *et al.* 1990; Kaelin *et al.* 2009) and of manganese oxidation (Tipping 1984; Zhang *et al.* 2002). However, the temperature dependency of these biological rates is not known and depends on the microbial populations presents in the system (Tipping 1984). Similarly, specific growth rate dependency to the substrate concentration in connection with the value of substrate affinity has not been studied in the case of groundwaters. However, it is dependent on the type of microbial population.

Our study focused on the evaluation of start-up strategies to accelerate nitrification and manganese removal without added exogenous biomass. Start-up strategies based on an increase in water temperature or in substrates concentration were examined in an experimental approach. A biofilter fed with groundwater from industrial boreholes was operated with the possibility to modulate inlet temperature and substrate concentrations (ammonium, nitrite, or manganese) leading to various start-up experiments. Pulses of nitrite were operated in some experiments to investigate nitrite influence on manganese oxidation. The start-up experiments were compared to reference ones where the same biofilter was operated in conventional natural conditions (no modification of the inlet water) and using the same borehole. Results were confirmed for boreholes situated in different locations.

MATERIALS AND METHODS

Rapid sand filter pilot

Figure 1 presents the experimental pilot composed of two identical stainless-steel columns (1.5 m height, inlet diameter 0.15 m) filled with sand. The height of the sand bed was 1.25 m with a porosity of 60.2%. The inlet flowrate was fixed to obtain a water velocity identical to the industrial sand filters used at the borehole to adsorb manganese. Sampling ports were placed along the height of the filters at 0.15 m, 0.53 m, 0.73 m, 0.93 m and 1.130 m from the top). To ensure aerobic biological activities, oxygen was provided continuously by a venturi system, sucking air through a sterile filter to avoid any microbial contamination of water. A sanitation of the column was performed before every experiment, consisting in the use of chlorine, or a mix of peracetic acid and hydrogen peroxide, followed by a thorough rinsing.

Raw waters properties (boreholes A and B)

First, reference experiments and start-up experiments were operated on an industrial borehole, labelled borehole A. Then, the start-up strategy was repeated with an identical pilot, on an industrial borehole located in another location, labelled borehole B.

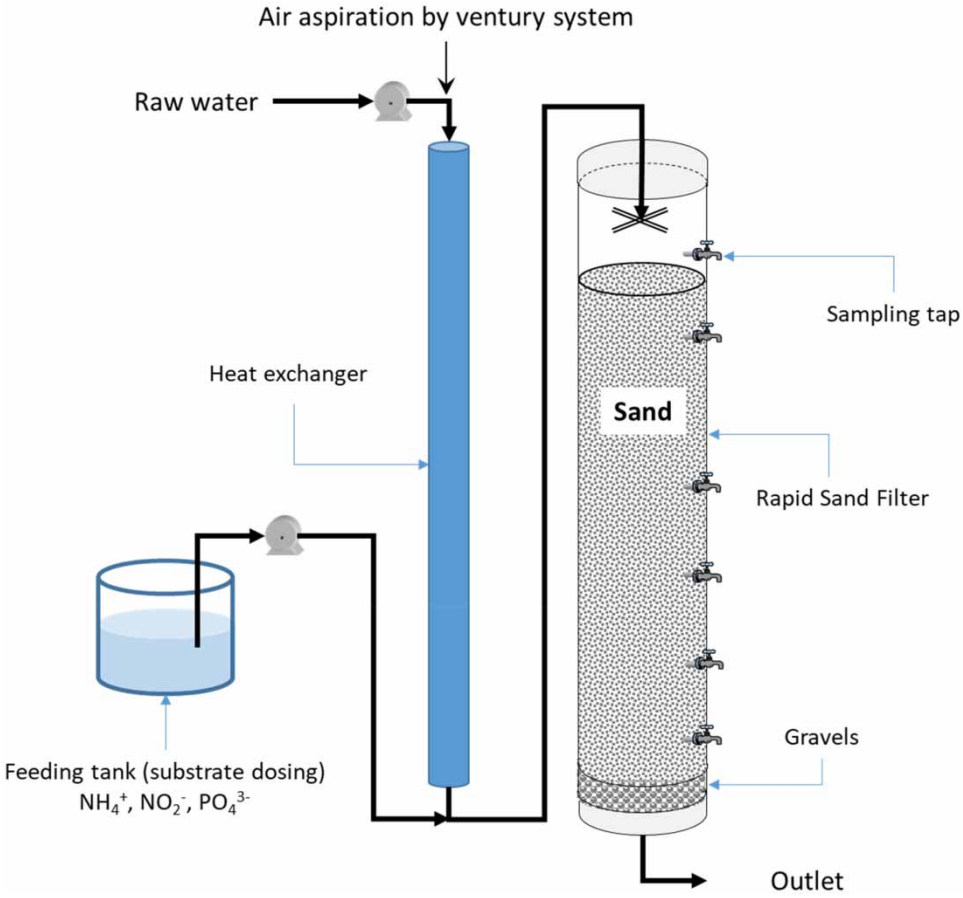


Figure 1 | Scheme of the rapid sand filter pilot used in the experiments. Water path is indicated with the black arrows.

The Table 1 presents the main properties of the groundwater filtered on these two sites. As a side note, nitrate concentration was around 500 μgN-NO_3^-/L; this parameter was measured to validate the complete oxidation of the nitrogen and validate nitrogen mass balances (data not shown).

Experiments

Start-up experiments

The following Table 2 presents the experiments with the key conditions during the implantation of the biological activities. The experiments are labelled with a code name: 'Ref' indicates experiment in natural conditions, 'SU' start-up experiment, and A or B indicating the borehole location). Due to the low concentration of phosphate in the groundwaters, the start-up experiment were conducted with a continuous addition of phosphate (5 μgP/L) (Kors *et al.* 1998). The experimental plan (Table 2) shows experiments with temperature increase, with or without substrate dosing, with or without recirculation of the filtered water, for two industrial sites. In all cases the start-up was considered completed when the following parameters were achieved: ammonium below 7 μgN-NH_4^+/L and nitrite below 2 μgN-NO_2^-/L for nitrification experiences, and

Table 1 | Composition of the groundwater for the borehole A and the borehole B for the parameters considered in this study

	Temperature (°C) Mean (min, max)	pH Mean ±	[N-NH_4^+] (μgN/L) Mean ±	[N-NO_2^-] (μgN/L) Mean ±	[Mn^{2+}] (μg/L) Mean ±
Borehole A	11.9 (11.2, 13,5)	7.4 ± 0.2	100	<2	100 ± 50
Borehole B	12 (11.3, 13.9)	7.4 ± 0.1	<7	<2	0

Table 2 | Experiments and experimental conditions during the implantation of the biological activities (chemical oxidation of manganese was performed with reactive manganese oxide sand)

	Sand	T°C	[N-NH_4^+] (μgN/L)	[N-NO_2^-] (μgN/L)	[Mn^{2+}] (μg/L)	Recirculation
RefA1	SiO_2	12	85	5	120	0%
RefA2	SiO_2	12.2	65	0	55	0%
SUA1	SiO_2	**30**	**500**	**50**	100	0%
SUA2	SiO_2	**29.7**	**450**	**55**	60	0%
SUA3	SiO_2	**29.3**	**500**	**40**	55	0%
SUA4	SiO_2	**29.5**	85	5	**450**	0%
SUB1	MnO_2	**21.2**	**550**	**70**	Chem. Ox.	0%
SUB2	MnO_2	**22.2**	**550**	**70**	Chem. Ox.	0%
SUB3	MnO_2	17	**500**	0	Chem. Ox.	75%
SUB4	MnO_2	11	**500**	0	Chem. Ox.	75%

'Ref' experiments were performed in natural conditions. Bold values indicate modifications such as substrate supplementation or artificially increased temperature.

manganese below 10 μgMn^{2+}/L for Mn oxidation. Substrates used were under the chemical forms of $(NH_4)_2SO_4$, $NaNO_2$, and $MnSO_4(H_2O)$.

Influence of nitrite injection on manganese oxidation

During the experiments SUA2 and SUA3, nitrite pulses were operated to obtain nitrite concentration in all the depth of the filter, to evaluate the impact of different nitrite concentrations on manganese oxidation. Nitrite and manganese profiles were realized before, during and after the nitrite pulses. The nitrite concentration applied for the pulses ranged from 110 μgN-NO_2^-/L to 1,280 μgN-NO_2^-/L, to be compared with Cheng *et al.* (2016) who observed nitrite influence on manganese oxidation for nitrite concentration up to 930 μgN-NO_2^-/L.

Sampling methods

During the experiments, water volumes were sampled at different times since the beginning of the experiment at the inlet and at the outlet of the filter columns and at different heights of the columns. Before and during sampling, stainless steel tubes of the sampling valves were regularly sterilized with a blowtorch to avoid any contamination from the exterior. Samples were stored at 5 °C and analyzed in a maximum of 3 h from the sampling time.

Analytical methods

Chemical analysis

Ammonium, nitrite and manganese concentration was analyzed by spectrophotometry using Spectroquant Ammonium Test kit 114752 (0.007–3.00 mgN-NH_4^+/L), Nitrite Test kit 114776 (0.002–1.00 mgN-NO_2^-/L), and Manganese Test kit 114770 (0.010–10.00 mgMn^{2+}/L), respectively, on the spectrophotometer Spectroquant Pharo 300 from Merck.

As nitrate is the final product of the nitrification, measurements were realized to validate the completeness of nitrification by mass balance. For all experiments, nitrites were fully converted into nitrate, thus data for nitrate concentration are not presented.

Oxygen was controlled by measurement at the inlet and the outlet of the filter by electrochemical sensor (Memosens COS81D). The system was aerobic during all the experiments, except for some short periods due to technical incidents.

pH, temperature, and conductivity were measured at the inlet and the outlet of the columns for control by sensors (Tophit CPS471D, Easytemp TMR35 and Indumax CLS54D, respectively) respectively, with no significant variations, hence data are not provided.

Sand characterization

Virgin sand (composed by a minimum of 96% silica) from CAS filtration was used in the pilot filters (granulometry: 1.18–0.60 mm; mineral hardness; 6–7 Moh; bulk density: 1,560–1,600 kg·m^{-3}; specific gravity: 2.65).

Before filtration experiments, virgin quartz sand was exposed in batch reactor to ammonium and then manganese concentrations to investigate the potential interactions between the sand and these compounds. The ammonium and the manganese concentrations did not change during these tests. No physical-chemical interaction was observed between the sand and the targeted compounds.

RESULTS AND DISCUSSION

Reference experiments

Nitrification in reference experiments

Two reference experiments, RefA1 in 2019 and RefA2 in 2020, were operated in reference conditions at the borehole A, for 530 days and 80 days, respectively. Those experiments were purposely run at different periods to assess the reproducibility of reference start-up data; in addition, these experiments were ran at the same time as optimized start-up experiments to yield a comparison with the same water parameters. Figure 2 presents ammonium and nitrites concentrations measured at the outlet of the filter for RefA1 (Figure 2(a)) and RefA2 (Figure 2(b)). During RefA1, complete ammonia removal took 40 days. The outlet nitrite concentration first rose until a maximum at day 42, then decreased to values under the detection limit at day 92. During this experiment disrupting events occurred (especially stop of the water feeding pump at day 60), resulting in a temporal loss of ammonium oxidation activity (around 80% of the nitritation activity), and to a decrease in the accumulation of nitrite. However, complete nitritation was restored in 5 days. For RefA2 experiment (Figure 2(b)), nitritation was complete

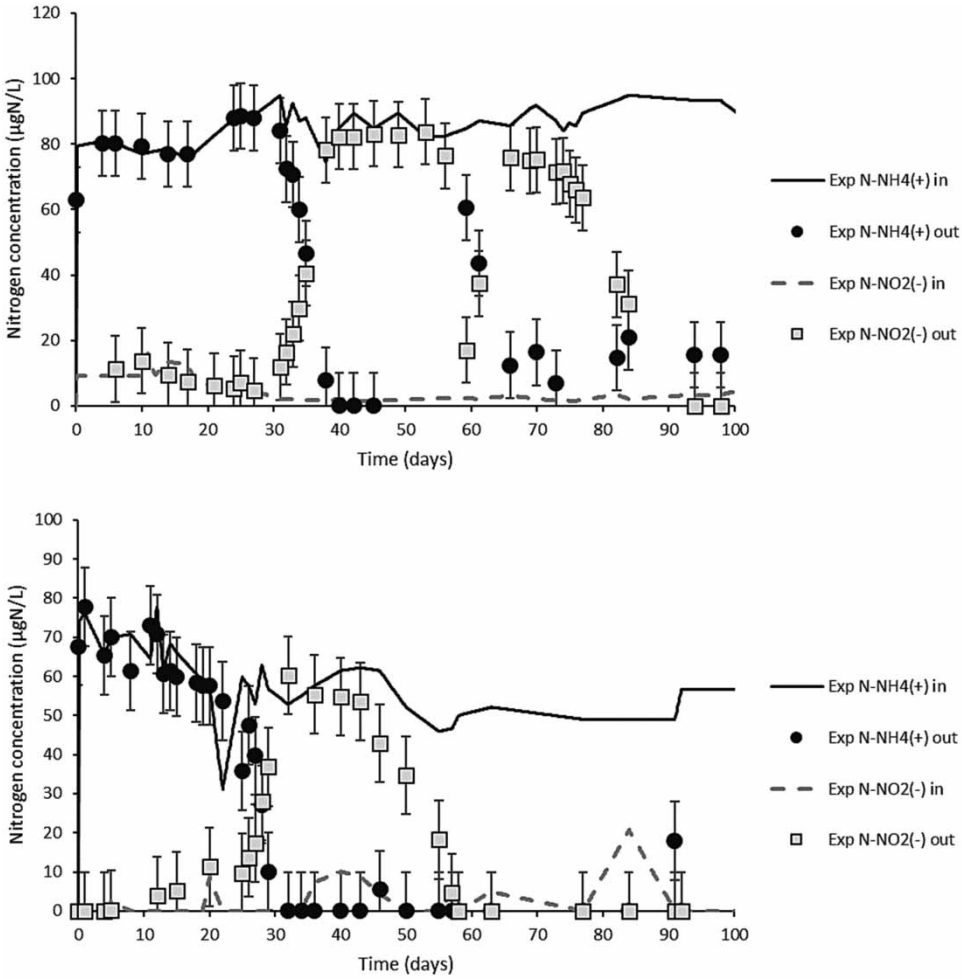


Figure 2 | Ammonium and nitrite concentration at the inlet and the outlet of the pilot filter for the reference experiments (a – RefA1, b – RefA2).

in 32 days, and nitratation in 63 days. Thus, during natural start-up period, nitrification process proceeded in two-step as commonly described (Niu *et al.* 2013) and a long duration for start-up is confirmed. The start-up duration significantly varied (complete nitritation needed 30–40 days and complete nitrification, 63–92 days). The range in variability could be partly explained, especially for nitritation, by an incident causing a technical stop for a few days at day 60 for experiment RefA1. These durations are high compared to the ones reported in the literature, probably due to the low temperature (~12 °C) and the very low concentration of ammonium (~80 μgN-NH_4^+/L) in borehole A. The duration variation could be also explained by the sensitivity of microbial activities to changes in environmental conditions, including the concentration of microelements, and the initial concentration of the active microbial population (which is the only responsible for the seeding of the filter).

Manganese oxidation in reference experiments

The following Figure 3 presents the outlet manganese concentrations for RefA1 (Figure 3(a)) and RefA2 (Figure 3(b)). For RefA1, manganese concentration showed no significant evolution for 32 days, but then started to decrease below detection limit at day 41. For RefA2 experiment (Figure 3(b)), high variations in inlet manganese concentrations during the first 15 days were observed. 80% of manganese concentration was removed at day 25, which was 16 days faster than in RefA1. Difference in the inlet soluble manganese concentration is the main difference between the two experiments (~120 μMn/L for RefA and ~ 55 μgMn/L for RefA2). As for nitrification, the duration required to complete manganese oxidation is variable, with a higher activity for RefA2. This could be explained by the high variations in inlet concentration. Finally, it can be concluded

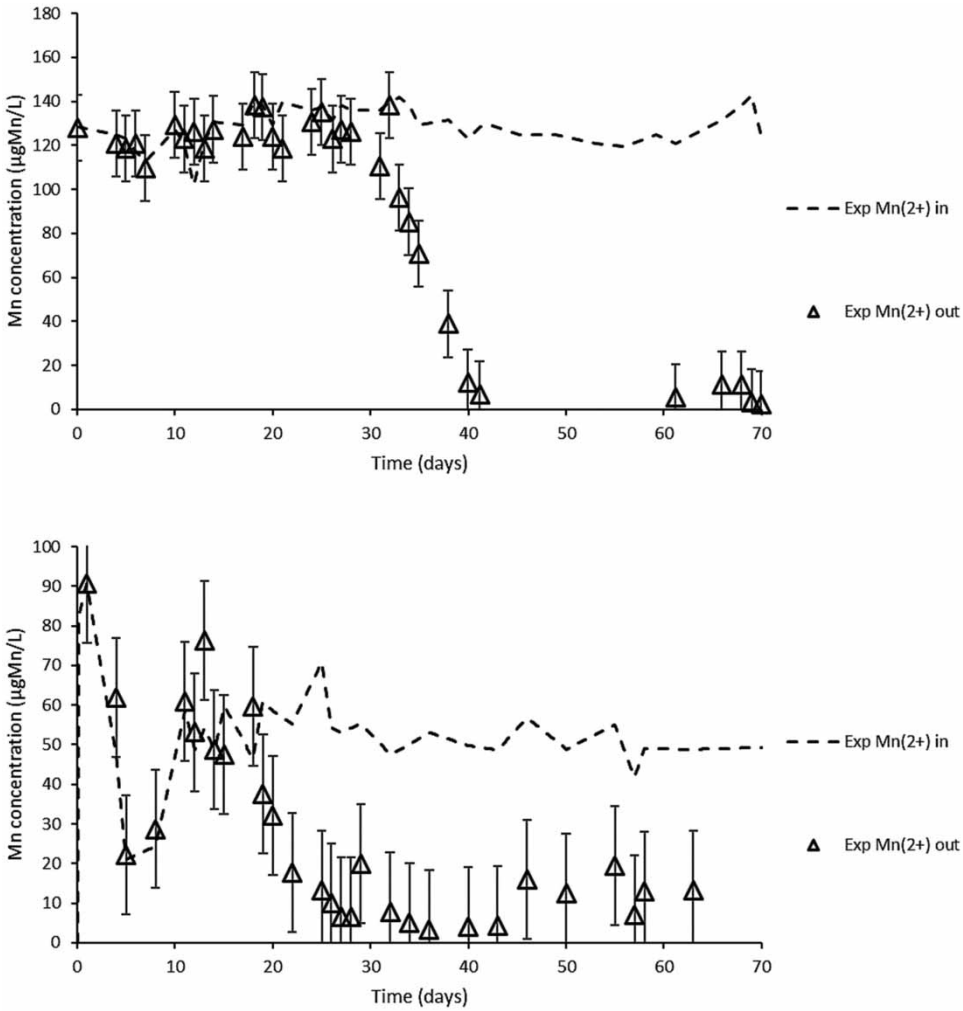


Figure 3 | Manganese concentration at the inlet and the outlet of the pilot filter for the reference experiments (a – RefA1, b – RefA2).

that the durations required to complete manganese oxidation (between 25 and 40 days) are in the range of values found in literature (15–75 days (Bruins *et al.* 2015b; Cheng *et al.* 2018; Breda *et al.* 2019)).

Nitrification and manganese oxidation in reference experiments

Experiments RefA1 and RefA2 have shown the feasibility of ammonium, nitrite and manganese co-elimination with unreactive sand, confirming previous observations by various authors (Bruins *et al.* 2014; Cai *et al.* 2015; Zeng *et al.* 2020). To go deeper into our understanding of the removal processes, concentration profiles were realized along the depth of the filter for RefA2 at different times (day 4 and day 13, no measurable biological activity; day 27, start of the nitritation and the manganese oxidation; day 40 complete nitritation and complete manganese oxidation; and day 65, complete nitritation, end of nitratation and complete manganese oxidation (Figure 4). From these profiles, it can be observed that biological activities are initially distributed along all the filter depth (see day 27 for ammonium and manganese oxidation, with a regular consumption of the substrate in the depth of the filter). However, over time, the biological activities concentrate at the top of the filter, confirming the observations of 9. Additionally, the concentration profiles for nitrite and nitrate confirmed a nitrification in two steps during start up.

Considering a maximal growth yield for nitrification of 0.18 $gVSS.gN^{-1}$ ((Blackburne *et al.* 2007), and a mean manganese content in bacteria of $5e^{-3}$ gMn/gVSS (Novoselov *et al.* 2013), the manganese consumed by the complete nitrification correspond to 0.4 $\mu gMn^{2+}/L$, thus between 0.3 and 0.7% of the manganese concentration in the raw water. Moreover, the virgin quartz sand showed no interaction with manganese. Therefore, specific biological activity should be responsible of the initiation of manganese oxidation. In the present study, manganese oxidation occurred simultaneously with nitritation, which is not in agreement with results of various authors (Vandenabeele *et al.* 1995; Han *et al.* 2013; Cheng *et al.* 2016). It can be seen in Figure 4, at day 40, that nitrite accumulation occurred in the filter depth without any effect on manganese oxidation. Thus, no inhibiting effect of nitrite on manganese oxidation could be observed in these experimental conditions. However, it can be underlined that ammonia concentrations in raw water (max 90 $\mu gN\text{-}NH_4^+/L$) were low compared to the concentrations reported in the literature (for example Cheng *et al.* 2016 described concentrations up to 930 $\mu gN\text{-}NH_4^+/L$). These observations tend to be in line with a recent study showing no influence of ammonium pulses on manganese oxidation under 0.9 mg/L of ammonia (Tang *et al.* 2021).

Accelerated start-up experiments

Borehole A

Start-Up strategies were applied to borehole A with temperature increase and substrate dosing as presented in Table 2. Four experiments were carried out (SUA1, SUA2, SUA3 and SUA4). Figure 5 presents the results for ammonium, nitrite, and manganese concentrations at the outlet of the filter during and after the start-up, when the filter was operated back into reference conditions (raw water temperature and concentrations, no substrate added). For experiments SUA1, SUA2 and SUA3, temperature increases were applied with only nitrogen dosing (ammonium and nitrite). Nitritation and nitratation periods

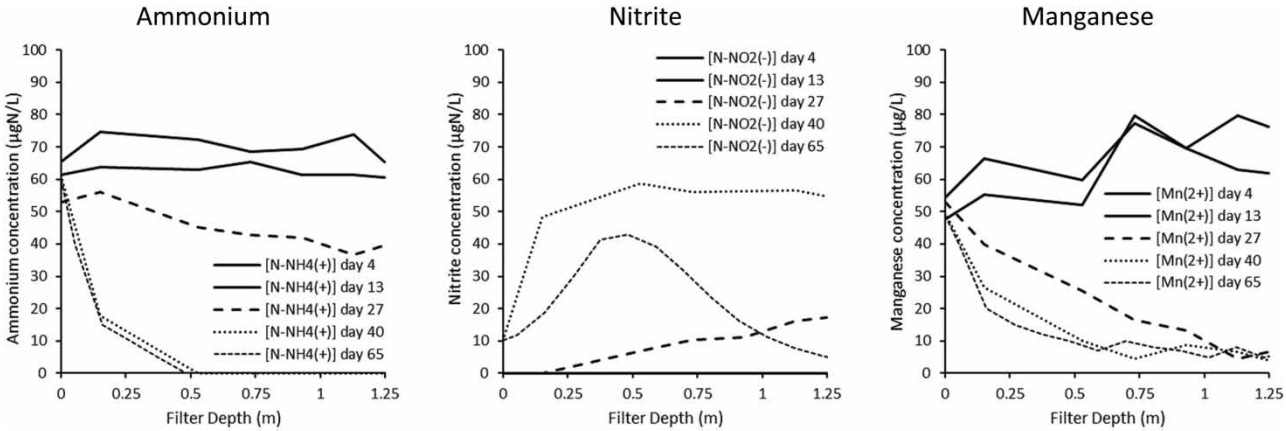


Figure 4 | Ammonium, nitrite and manganese concentrations profiles in the depth of the filter for the RefA2 experiment at day 4, 13, 27, 40 and 65.

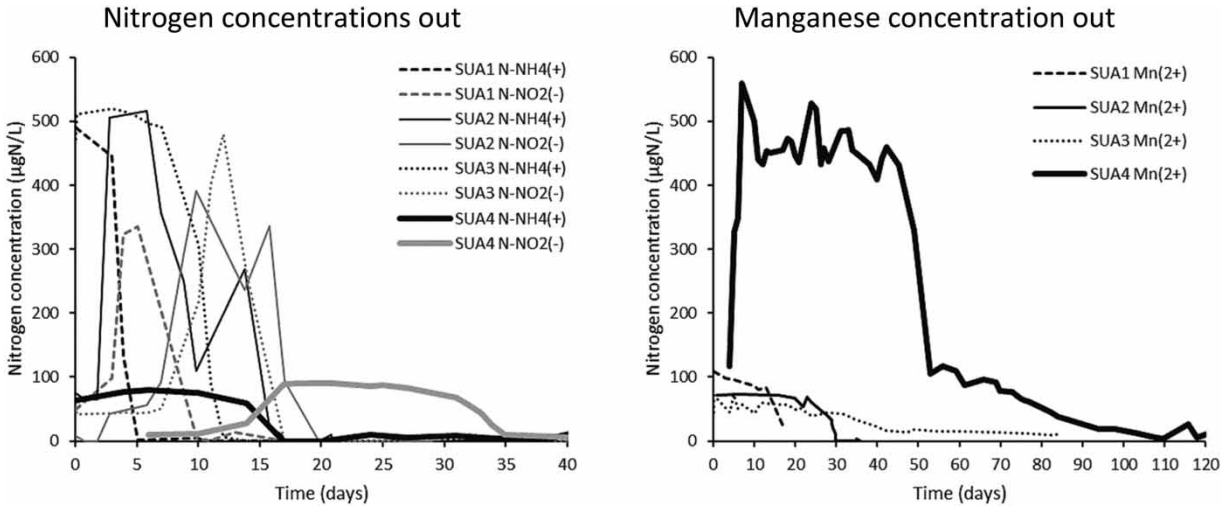


Figure 5 | Ammonium, nitrite and manganese concentrations at the outlet of the filter during the start-up experiments (SUA1, SUA2, SUA3 and SUA4).

were reduced to 5–17 days (compared to 30–40 days in reference conditions), and to 10–20 days (compared to 63–92 days in reference conditions), respectively, thus yielding a gain of 43–72 days to obtain the same removal efficiency for nitrification. For manganese oxidation SUA1, SUA2 and SUA3 experiments (Figure 5), with only temperature increase, showed a complete removal in 20–40 days (compared to 25–40 days in reference conditions). Hence, no clear impact of a temperature increase on the manganese oxidation was highlighted. Experiment SUA4 was operated with temperature increase and with manganese dosing only. During manganese dosing (500 $\mu gMn^{2+}/L$ during the 50 first days), no manganese conversion was measured. After 50 days, only temperature increase was applied and manganese dosing was stopped, then manganese oxidation started, highlighting a potential inhibiting effect of the inlet manganese concentration on the biological oxidation (self-substrate inhibition existing for manganese oxidizers but at higher manganese levels was reported by (Therdkiattikul *et al.* 2020)). For nitrification, with no nitrogen dosing, experiment SUA4 showed (Figure 5) an acceleration of nitritation (17 days) and nitratation (35 days), due to the temperature increase. Nonetheless, acceleration factors were lower than with both temperature increase and substrate dosing.

After the start-up period, the filter was brought back into reference operating conditions (for all the experiments), to evaluate the potential of the accelerated start-ups to develop efficient biological activities at lower temperatures. For all the start-up experiments on borehole A, nitrogen and manganese removals were at the same level in natural conditions than at the end of the start-up period. Start-up strategy with temperature increase and substrate dosing showed a clear positive effect on nitrification. No gain was obtained for manganese oxidation in the tested experimental conditions. However, manganese oxidation in reference conditions was achieved before complete nitrification, thus acceleration of the nitrification may allow to the reduction of time required to install the biological activities for the co-removal of nitrogen and manganese. Moreover, in these conditions, temperature increase, without substrate dosing, enables accelerating start-up period for nitrification (SUA4). Compared to experiment RefA2 (63 days to complete nitrification), 40 days to install all the biological activities (highest duration recorded for manganese oxidation without dosing), enables 36% of water saving, with only temperature increase (30 °C).

For all experiments (except SUA4), the nitrite peak was around 500 $\mu gN\text{-}NO_2^-/L$ and manganese oxidation always occurred after complete nitrification, thus indicating a possible interaction of manganese oxidation with nitrification as described in the literature (Vandenabeele *et al.* 1995; Cheng *et al.* 2016). However, Han *et al.* have showed that ammonium shots logically increase the nitrification activity, with no negative influence on the simultaneous removal of ammonium and manganese (Han *et al.* 2013). In our experiments, manganese oxidation does not appear to be influenced by the chosen parameters for the start-up strategy, unlike nitrification. Therefore, the results could be only linked to a different behavior of the involved specific microorganisms towards environmental conditions. Further investigation is needed to improve knowledge on the interactions between these biological activities.

Nitrite dosing experiments

During the start-up period. During the initial start-up phase, we have confirmed that manganese oxidation is biologically supported, and we have observed that manganese oxidation always occurred after nitritation. To further investigate the influence of nitrification on manganese oxidation, nitrite dosing experiments were conducted. Figure 6 presents manganese concentrations in influent, effluent, and along the depth of the filter during experiment SUA2. In this experiment, successive pulses of nitrite at the pilot inlet have been operated during the installation of biological manganese oxidation, with the aim to detect potential negative interactions between the biological processes, at day 29, day 30 and day 35. Data clearly refute any influence of nitrite on biological manganese removal during the start-up phase of manganese oxidation, in our experimental conditions.

After the start-up period. Manganese oxidation in rapid sand filters is a complex phenomenon including biological oxidation and chemical heterogenous catalysis due to the production during biological reaction of solid manganese oxides with catalytic properties for Mn^{2+} oxidation (Tekerlekopoulou *et al.* 2013; Bruins *et al.* 2014; Breda *et al.* 2019). To investigate the influence of nitrite on the global manganese oxidation process, two pulses of nitrite were realized in influent water of the biofilter during experiment SUA3 at day 119 and day 122 (no modification of the raw water conditions). Results from Figure 7 show that concentration profiles of manganese before and after the injections did not significantly differ at both concentrations. In our experimental conditions, nitrite seems to have no significant impact on global manganese oxidation, in contrast with some results that are often cited from litterature (Vandenabeele *et al.* 1995; Cheng *et al.* 2016). Interestingly, more recent data have similarly shown no influence of ammonium pulses on manganese

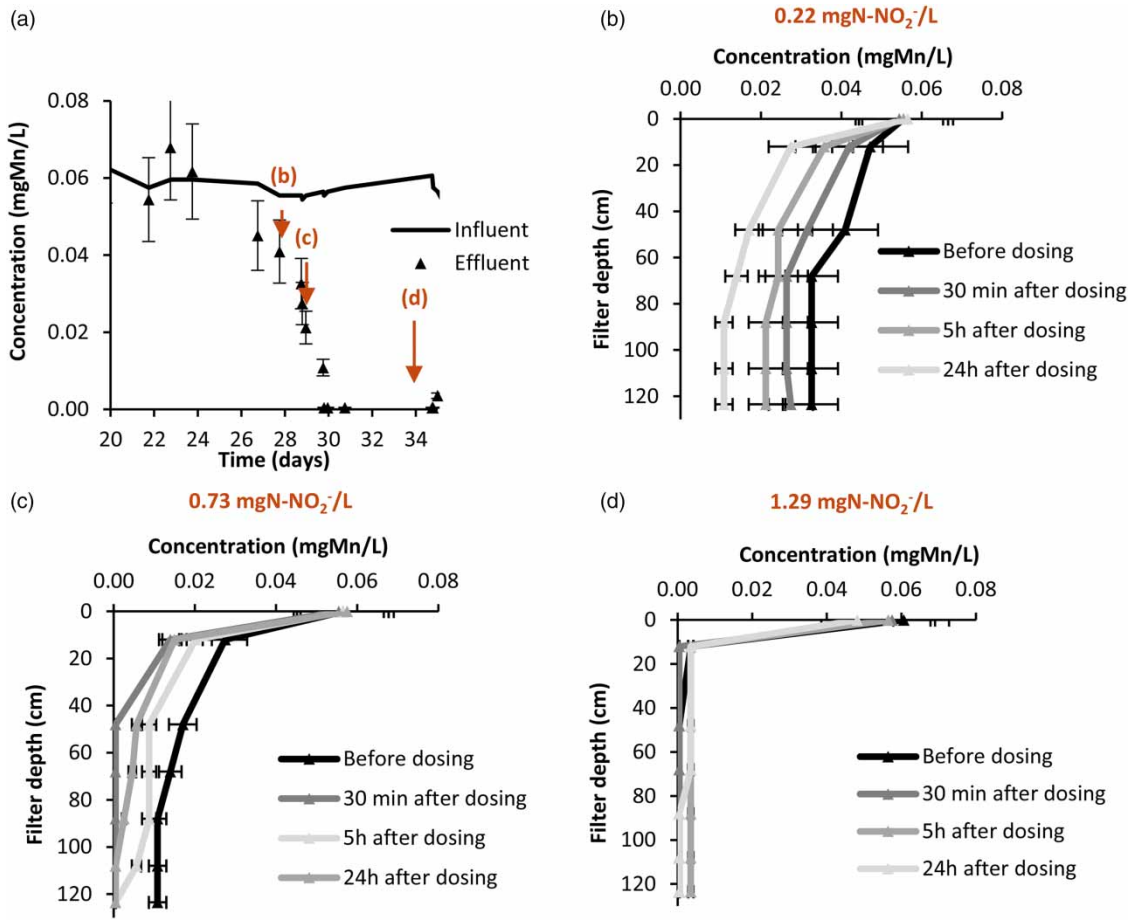


Figure 6 | Concentrations of manganese (a) in influent and effluent water with several injections of nitrite (arrows) and (b), (c), (d) in the depth of the biofilter with nitrite injections from days 29 to 35, during experiment SUA2.

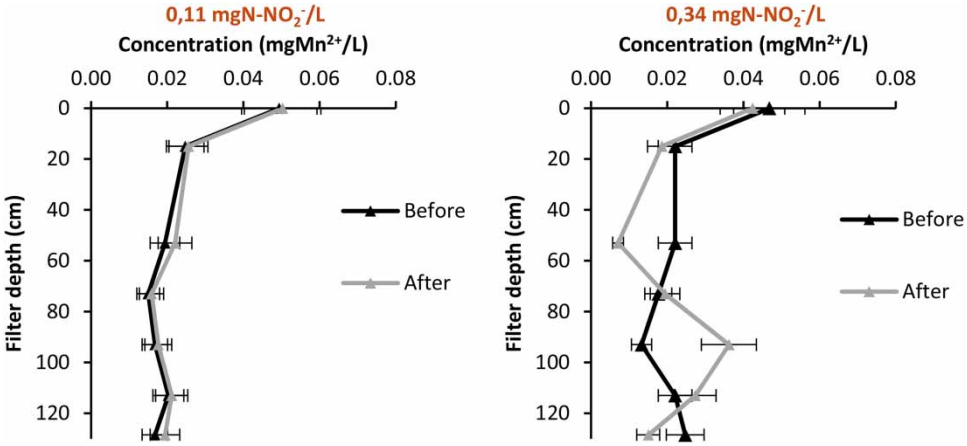


Figure 7 | Manganese profiles before and 45 min after each nitrite dosing, during experiment SUA3 at days 119 and 122 of the experiment.

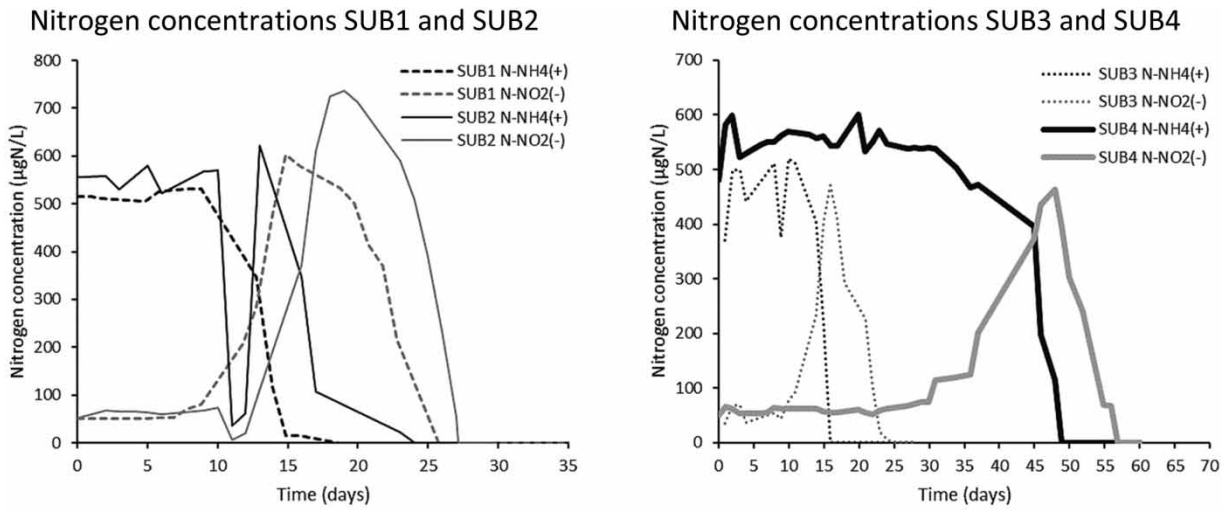


Figure 8 | Ammonium and nitrite concentrations at the outlet of the filter during the start-up experiments on the borehole B (SUB1, SUB2, SUB3 and SUB4).

oxidation under 0.9 mg/L of ammonia (Tang *et al.* 2021), which brings into question the role of nitrogen compounds on the sequence of nitrification followed by manganese oxidation during the start-up phase, in these experimental conditions.

Borehole B

Experiments on borehole A showed no clear gain of the chosen start-up strategy on manganese oxidation but showed interesting trends on the nitrification activity. Thus, to improve the start-up strategy and challenge it, the pilot was used on another borehole, in another industrial location (borehole B), using MnO_2 reactive sand to focus on nitrification. The experimental plan was then completed with other temperatures (with or without substrates dosing, and with a new parameter for the start-up strategy: partial recirculation of the filtered water (Table 2)). Experiments SUB1 and SUB2 were set without recirculation of the filtered water, and experiments SUB3 and SUB4 were set with 75% of filtered water recirculation. Figure 6 presents ammonium and nitrite time series obtained for the four experiments. Firstly, for all experiments, nitrification was achieved, with transitional nitrite accumulation (indicating, like for borehole A, a nitrification in two steps during the start-up periods). Regarding experiments SUB1 and SUB2 operated in the same conditions, nitritation was achieved in 15 and 20 days respectively, and nitratation in 26 and 27 days respectively, indicating a good repeatability of the start-up strategy. Compared with experiments SUA1, SUA2 and SUA3, differences are only the composition of the source water, and the temperature used for the accelerated start-up strategy (~30 °C for SUA experiments and ~20 °C for SUB experiments). For SUA experiments,

nitritation was achieved in 11.3 days compared to 17.5 days for SUB experiments (mean values), and nitratation was achieved for SUA in 15.7 days compared to 26.5 days for SUB (mean values), indicating a higher nitrification rate at 30 °C than at 20 °C, as expected for common nitrifiers (Antoniou *et al.* 1990; Kaelin *et al.* 2009). This suggests the possibility of different start-up strategies adapted on different sites. However, as water is lost, thermal energy is consumed, and chemicals are used during this start-up, two experiments were operated to try to reduce the costs of the start-up strategy, while maintaining the reduced durations. Experiment SUB3 is carried out in the same conditions as SUB1 and SUB2 but with 75% recirculation of outlet water, and experiment SUB4, with the same recirculation rate, but with no temperature increase (with the aim to limit energy consumption). For experiment SUB3, nitritation was achieved in 15 days and nitratation in 25 days, corresponding to the values recorded without recirculation. Henceforth, 75% of the water could be saved in this configuration with no impact on the time gained. Regarding experiment SUB4, nitritation was achieved in 48 days and nitratation in 57 days, indicating that recirculation without temperature was not an interesting strategy for start-up acceleration despite the nutrient supplementation. Thus, temperature remains the key parameter to accelerate nitrification.

The results obtained in this study have showed that for specific groundwaters where no exogenous biomass inoculation is possible, different start-up strategies are feasible. The time to obtain complete biological activities was 63 days in experiment RefA2, leading to 151.2 m^3 water consumed before production. Different strategies have been investigated, (1) with no substrates dosing and no temperature increase, the recirculation of 75% of the waters enabled completing the start-up phase in 63 days but with only 37.8 m^3 of water consumed, (2) with temperature and substrates dosing and without water recirculation, complete start-up phase was reached in 40 days, i.e. 96 m^3 of water consumed, (3) combining recirculation of 75% of the filtered water, temperature increase and substrates dosing, the production period was obtained in 40 days, but with only 24 m^3 of water consumed, thus 84% of water saved.

CONCLUSIONS

This study investigated start-up strategies to produce drinking water from groundwaters with temperature increase, substrates dosing, and without any addition of exogenous biomass. Focus on nitrification and manganese oxidation highlighted a different impact of the chosen parameters on both activities, with an acceleration of nitrification and no influence on manganese oxidation. In all start-up experiments, manganese oxidation always occurred after nitritation. However, nitrite pulses have shown no influence of nitrite on manganese oxidation in short and long periods after the start-up, in the experimental conditions of this study.

The start-up strategy for nitrification was challenged on another borehole, in another location. Table 3 summarizes the results obtain for the operated experimental conditions. Combining recirculation, ammonium, nitrite and phosphorus dosing and increased temperature could save up to 90% of water for an installation of efficient biological activities for nitrogen and manganese removal. In addition to these savings, the start-up time reduction is also paramount from the business

Table 3 | Times to observe complete nitritation, complete nitratation and complete manganese oxidation for start-up experiments on the boreholes A and B compared to reference experiments (with the operating conditions, at a 0.1 m^3/h flow rate)

	Sand	T°C	[N-NH_4^+] (μgN/L)	[N-NO_2^-] (μgN/L)	[Mn^{2+}] (μg/L)	Recirc (%)	Time nitritation (d)	Time nitratation (d)	Time Mn^{2+} oxidation (d)	Water loss (m^3)
RefA1	SiO_2	12	85	5	120	0	40	92	41	221
RefA2	SiO_2	12.2	65	0	55	0	32	63	25	151
SUA1	SiO_2	30	500	50	100	0	5	10	20	48
SUA2	SiO_2	29.7	450	55	60	0	17	20	30	72
SUA3	SiO_2	29.3	500	40	55	0	12	17	40	96
SUA4	SiO_2	29.5	85	5	450	0	17	35	110	264
SUB1	MnO_2	21.2	550	70	/	0	15	26	/	16
SUB2	MnO_2	22.2	550	70	/	0	17	27	/	16
SUB3	MnO_2	17	500	0	/	75%	15	25	/	15
SUB4	MnO_2	11	500	0	/	75%	48	57	/	34

aspect, as each day of start-up phase is a day without production. This study proposed a strategy to limit these constraints in industrial situation where exogenous biomass could not be added to start a new biofilter, such as natural mineral waters.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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