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Co-reduction of nitrate and perchlorate in a pressurized hydrogenotrophic reactor with complete H_2 utilization

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HIGHLIGHTS

- A two-stage hydrogenotrophic process to remove NO_3^- and ClO_4^- is presented.
- NO_3^- is removed in a first unsaturated-flow pressurized reactor stage.
- The residual H_2 is coupled to ClO_4^- reduction in a second polishing stage.
- Large presence of *Dechloromonas* was detected before and after ClO_4^- addition.
- Effluent ClO_4^- concentration of 2 μg/L and ~100% H_2 utilization were achieved.

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ABSTRACT

A novel pressurized hydrogenotrophic reactor operating at high rates was recently developed specifically for the removal of nitrate (NO_3^-) from drinking water. The reactor is characterized by safe and economical operation since hydrogen (H_2) purging intrinsic to conventional H_2-based denitrifying systems is not required and H_2 loss occurs only through the effluent, resulting in H_2 utilization efficiency above 90%. In this research, a new treatment scheme to remove NO_3^- and perchlorate (ClO_4^-) combining the pressurized reactor with a following open-to-atmosphere polishing unit is presented. In the pressurized reactor, NO_3^- and ClO_4^- are simultaneously removed. In the polishing unit, the residual dissolved H_2 from the pressurized reactor serves to further reduce ClO_4^- to trace concentrations below recommended levels.

First, ClO_4^- reduction together with denitrification was demonstrated in the pressurized reactor without special inoculation and a maximal ClO_4^- volumetric removal rate of 1.83 g/($L_{reactor}$·d) was achieved. Microbial population analyses before and after the addition of ClO_4^- were similar with a large fraction of the genus *Dechloromonas*. Results show that the combined treatment scheme consisting of the pressurized reactor and the polishing unit allowed for the reduction of ClO_4^- concentration down to a minimal value of 2 μg/L with a simultaneous increase of the H_2 utilization efficiency from 95% up to almost 100%.

1. Introduction

Intensive use of nitrogen-based fertilizers and wastes from rockets facilities are the main sources for groundwater contamination by nitrate (NO_3^-) and perchlorate (ClO_4^-), respectively [1,2]. Despite the different pollution source, co-occurrence of both ions is common, especially in groundwater close to military bases that house rockets [2]. In some cases, the high ClO_4^- concentration in the discharge point can lead to migration of ClO_4^- in groundwater far away from the focus of pollution and mixing with NO_3^--contaminated groundwater, as reported in the Ramat Hasharon area in Israel [3]. High ClO_4^- levels were also detected in groundwater throughout the U.S., mainly in California, Nevada, Utah, Arizona and other states where rocket and missile production occurs [4]. Also, ammonium perchlorate (NH_3ClO_4) occurs naturally in NO_3^- deposits that are used in some fertilizers [5]. In California, for example, drinking water sources that contain ClO_4^- was found to have much higher concentrations of NO_3^- than wells with no measurable ClO_4^- [6].

The World Health Organization (WHO) standard for NO_3^--N is 11.3 mg/L (as nitrogen) [7]. As for ClO_4^-, standards are more variable and location-dependent. The Environmental Protection Agency (EPA), for example, established an advisory standard of 15 μg/L, but numerous states in the U.S. promulgated enforceable standards for ClO_4^- in drinking water of only 2 μg/L [2]. Therefore, a comprehensive solution for meeting the drinking water standards determined by the health organizations is required. In the

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case of ClO_4^-, reduction to trace concentrations is needed and therefore much harder to achieve by any treatment technique [8].

Biological denitrification and biological ClO_4^- reduction are two processes proved to efficiently reduce NO_3^- and ClO_4^- concentrations to the permitted thresholds without the production of waste brine [7,9]. Moreover, many perchlorate-reducing bacteria can grow also on NO_3^- and therefore a biological denitrification system may be (but not necessarily) effective for ClO_4^- reduction [10]. Using H_2 gas as the electron donor for bacterial growth in both processes is advantageous over the common organic donors (mostly methanol, ethanol and acetate) for drinking water treatment, mainly due to the lower cell yield of autotrophic bacteria reducing reactor clogging, sludge production and post-treatment costs [7,11]. However, supplying H_2 economically and safely at high transfer rates remains one of the major challenges for the successful application of H_2-based systems [7].

Several hydrogenotrophic systems for removal of NO_3^- [12–16], ClO_4^- [10,17] and both ions together (i.e. simultaneously removal of NO_3^- and ClO_4^-) [5,18–22] were proposed in the last 15 years for the treatment of potable water. Among these technologies, the membrane biofilm reactor (MBfR) has gained the most attention due to its safe and economical gas delivery system with close to 100% utilization efficiency of H_2 gas, and is implemented in full-scale in various groundwater treatment plants in California, USA since 2012 [23]. Membrane fouling and scaling together with difficulties of biomass control are possible drawbacks of a typical MBfR [7].

Recently, a novel unsaturated-flow pressurized reactor for hydrogenotrophic denitrification of groundwater operating at high denitrification rates together with minimal H_2 loss and low risk was presented. A detailed explanation and description of the reactor was given by Epsztein et al. [24]. Briefly, the reactor is based on the simple concept suggesting that N_2 gas build-up in a closed-headspace denitrifying system will not occur due to the fact that at steady state a gas-liquid equilibrium is maintained within the reactor according to Henry's law and effluent water carries the excess of N_2 gas out of the reactor. Since N_2 reaches equilibrium in the reactor and does not accumulate over time, there is no need for gas purging and the risky and uneconomical H_2 loss to atmosphere is eliminated. Hydrogen loss is therefore limited only to the dissolved H_2 in the effluent and H_2 utilization efficiencies above 92% were achieved [24]. Except for the MBfR, the biofilm-electrode reactor [14,17] and a system proposed by Rezania et al. [13], there are no reports on such high utilization efficiencies of H_2 in hydrogenotrophic systems. In the pressurized reactor, high denitrification rates of up to 7.5 g NO_3^--N/($L_{reactor}$·d) are ensured by operating the reactor under an unsaturated flow regime where water is recirculated through the H_2 gas-enriched headspace and trickled over high surface area biofilm carriers [25].

Despite the promising results of the pressurized reactor, achievement of 100% H_2 utilization efficiency is essential for improving the safety and the economic viability of the process and to prevent a possible regrowth of biomass in the distribution system and ensure a safe drinking product. In the following research work, the simultaneous removal of ClO_4^- and NO_3^- was investigated in a modified version of the above reactor, i.e., a combined treatment scheme. The combined treatment scheme (Fig. 1) combines the unsaturated-flow pressurized reactor with an upflow submerged open-to-atmosphere polishing unit. The polishing unit aims to increase H_2 utilization (~100%) by the consumption of the residual dissolved H_2 from the pressurized reactor and further reduction of ClO_4^- to trace concentrations below recommended levels (between 1 and 15 μg/L).

2. Materials and methods

2.1. Experimental setup

A schematic diagram of the combined treatment scheme is illustrated in Fig. 1. The combined treatment scheme included the unsaturated-flow pressurized reactor, i.e., the main reactor unit, combined with a submerged open-to-atmosphere polishing unit to reduce ClO_4^- by the residual dissolved H_2 in the effluent of the main reactor unit. A detailed description of the main reactor unit was given in an earlier publication [24]. Briefly, it comprised of a clear PVC cylindrical reactor 70 cm in height and 10.5 cm in diameter divided into three unequal parts. The top part of the reactor (height 20 cm) served as an empty headspace, the middle part (height 30 cm) was filled with plastic biofilm carriers (total surface area of 900 m^2/m^3, *Aqwise*) and separated by a metal screen from the bottom part (height 20 cm) of the reactor where recirculating

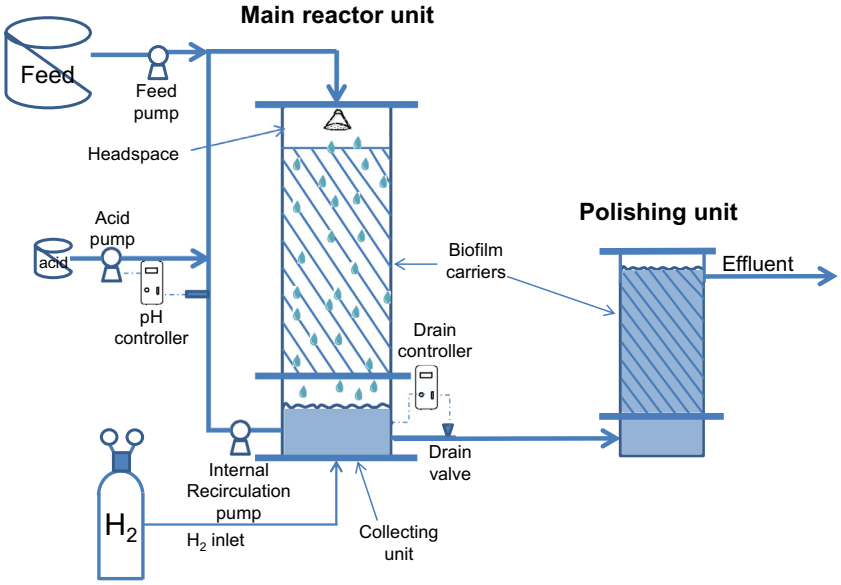

Fig. 1. Schematic diagram of the combined treatment scheme consisting of the unsaturated-flow pressurized reactor, i.e., the main reactor unit, and a following submerged open-to-atmosphere polishing unit.

water collected. Hydrogen gas was supplied continuously from H_2 cylinder. The reactor was connected to a feed pump (Diaphragm pump model 7090-42, Cole-Palmer, USA), recirculation pump (FL-2403, ProPumps, China) and pH controlling unit (standard pH electrode, pH controller – Alpha 190, Eutech, Singapore; hydrochloric acid tank and acid pump – gamma/L, ProMinent, Germany). The main reactor unit was operated as a trickling filter with water recirculation. It was continuously fed with simulated NO_3^- and ClO_4^- contaminated groundwater. An automatic drain valve discharged accumulated water to the polishing unit.

The polishing unit comprised of a PVC cylindrical polishing unit 25 cm in height and 10.5 cm in diameter, filled with the same plastic biofilm carriers as in the main reactor unit. The effluent water from the main reactor unit was introduced at the bottom of the polishing unit and released at the top part. The polishing unit was operated under a saturated-flow mode (i.e. submerged unit) and its discharge was open to the atmosphere.

Reactor start-up and initial investigation of ClO_4^- reduction (Sections 3.1) were performed in the main reactor unit only, using the same biomass carriers from previous denitrification experiments [24]. Start-up of the polishing unit in the following trials was performed by filling the polishing unit with additional clean carriers mixed with biomass carriers from the main reactor unit (the biomass carriers taken from the main reactor unit were replaced with new carriers). Tap water enriched with $NaNO_3$, $NaClO_4$ and KH_2PO_4 (influent concentration of 1 mg P/L) was used as feed solution for all experiments. Carbon source for bacterial growth was not added and based on the inherent carbon content of the water (alkalinity of ~140 mg/L as $CaCO_3$ at pH 7.5–8). The recirculation flow rate was 6600 mL/min in the first experiment when only the main reactor unit was used (Section 3.1). In all other trials, the recirculation flow rate in the main reactor unit was 3800 mL/min. Water temperature was kept at 30 ± 1 °C. The pH in the main reactor unit was maintained at 7–7.1 to by dosing hydrochloric acid. The relatively low pH was aimed to prevent an extreme pH increase within the biofilm, which leads to NO_2^--N accumulation [26,27]. Samples of influent, effluent from the main reactor unit and effluent from the polishing unit were collected for further water analyses.

Rate calculations in this work were based on the packing volume of the carriers in the main reactor unit (2.5 L) and the polishing unit (1.9 L). In all experiments, excess biomass growth was removed every few days by washing of carriers, column and pipes with tap water (the polishing unit never had to be cleaned).

2.2. Water and gas analyses

Nitrate, perchlorate and sulfate were determined using a Metrohm 761 ion chromatograph (IC) equipped with a 150 mm Metrosep A Supp 5 column with column guard and suppressor using a CO_3^{-2}/HCO_3^- eluent. Nitrite-N and alkalinity were measured according to Standard Methods (Method 4500 and Method 2320, respectively). Total Organic Carbon (TOC) concentration was determined by a TOC-VCPH analyzer (Shimadzu, Kyoto, Japan). DOC concentration was determined by performing TOC analysis on samples filtered through 0.22 mm syringe filter. Hydrogen concentration in gas phase was measured by gas chromatography (TCD detector; column: HP-PLOT-Q 30 m; 0.53 mm. 40u, Agilent 7890A). Gas samples were injected directly from the reactor headspace into a 20 mL sealed serum bottle for 1 min with gas flow rate of 250 mL/min to ensure exchange of the entire gas volume in the bottle. Dissolved H_2 concentration was measured by headspace analysis of effluent samples injected to a sealed serum bottle using the same gas chromatograph.

2.3. Microbial population analysis using high-throughput sequencing and PCR-DGGE

Biofilm samples for microbial population analysis were taken from the pressurized hydrogenotrophic denitrifying reactor (i.e. the main reactor unit) before (t = 0) and after (t = 25 days) the addition of ClO_4^-. Total genomic DNA was extracted using FastDNA SPIN Kit for Soil (MP Biomedicals) following the manufacturer's protocol. Pellets of 0.5-mL from suspensions of the reactor's biofilm were used as samples. The DNA concentrations of the extracts were measured with the NanoDrop 1000 Spectrophotometer (Thermo Scientific), adjusted for polymerase chain reaction (PCR) amplification and stored at −20 °C until further use.

High throughput sequencing analysis was performed by using Illumina Miseq (Hy laboratories Ltd, Israel). Samples of DNA were subjected to two rounds of PCR to prepare the libraries for sequencing. The first PCR reaction was performed to amplify the V4 region of the 16 s rDNA gene, with primers that included the CS1 and CS2 sequences from Fluidigm. The second PCR was done using the Access Array Barcode Library for Illumina Sequencers from Fluidigm. The sample data were analyzed using the 16 s metagenomic application on BaseSpace (Illumina). The high quality reads that passed quality filtration were used for the identification of microbial population. Only predominant microbial populations are given; the remaining microbial population is shown as 'Others'.

For DGGE, approximately 600 base pairs (bp) of the 5′ end of the variable region V3–V5 of the bacterial 16S rDNA were amplified using the primer pair consisting of 341F (5′-CCTACGGGAGGCAG CAG-3′) with a GC clamp (5′-CGCCCGCCGCGCCCCGCGCCCGTCCCG CCGCCCCCGCCCG-3′) and 907 R (5′-CCGTCAATTCCTTTRAGTTT-3′) [28]. The PCR reactions were performed in a thermocycler (TProfessional Basic Gradient Thermocycler; Biometra) with Apex RED Taq Master Mix (Genesee Scientific Corp.). The PCR conditions, denaturing gradient gel electrophoresis (DGGE) and sequencing procedures were used as mentioned by Desitti et al. [29]. Four dominant bands from DGGE were sequenced and compared with the partial sequence of 16s rDNA bacterial names obtained by high throughput sequencing method. Sequence alignment and phylogenetic tree were performed using MEGA6 [30].

3. Results and discussion

3.1. Perchlorate removal in the pressurized hydrogenotrophic denitrifying reactor

An initial investigation of ClO_4^- removal in the pressurized hydrogenotrophic denitrifying reactor using biofilm carriers from former denitrification experiments was first carried out for 25 days. During the experimental period, the inlet NO_3^--N and ClO_4^- concentrations were 15 and 20 mg/L, respectively. The flow rate was increased gradually over time from 20 to 200 mL/min. The reactor's total pressure was 2 bar and the recirculation flow rate was 6600 mL/min. The results for volumetric ClO_4^- removal rate over time are shown in Fig. 2. Fig. 2 shows that ClO_4^- reduction started immediately after ClO_4^- addition, i.e., during the first day of operation. The immediate acclimation of bacteria from the former denitrification reactor to reduce ClO_4^- demonstrates that no specialized inoculation was required. A maximal ClO_4^- volumetric removal rate of 1.83 g/($L_{reactor}$·d) was observed after 25 days of operation. For comparison, Logan et al. reported a slightly lower removal rate of 1.16 g/($L_{reactor}$·d) in a non-pressurized unsaturated-flow hydrogenotrophic reactor at a lower temperature of 23 °C, similar pH (7) and influent ClO_4^- concentration (18 mg/L) without NO_3^- [5]. Sharp fluctuations in the ClO_4^- removal

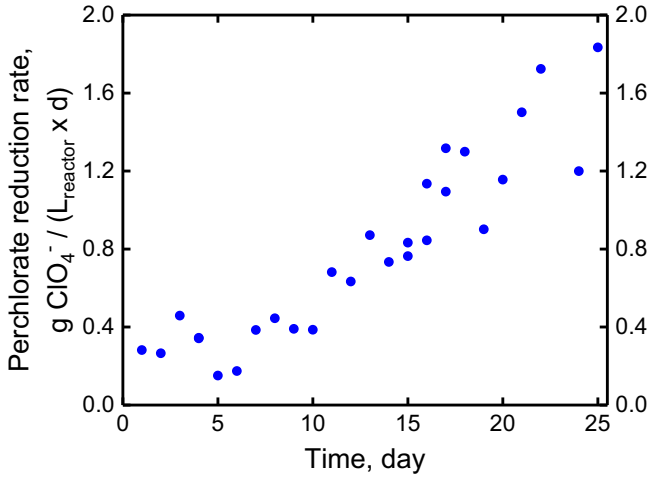

Fig. 2. Volumetric ClO_4^- removal rate in the pressurized reactor as a function of time. At t = 0, ClO_4^- was introduced at the reactor for the first time.

rates (e.g. day 19 and 24) can be attributed to reactor cleaning accompanied with loss of biomass and change of conditions. Effluent ClO_4^- concentrations were generally below 1 mg/L (i.e. removal efficiency above 95%), except on days when the loading rate was increased where the effluent concentrations reached 3–4 mg/L ClO_4^-. The effluent NO_3^--N concentration from the pressurized reactor was always below 1 mg/L (i.e. removal efficiency above 93%). Effluent NO_2^- concentrations were always below detection levels.

3.2. Microbial population analysis with high-throughput sequencing and PCR-DGGE

The microbial population was examined before and after the addition of ClO_4^- to the pressurized hydrogenotrophic denitrifying reactor (at t = 0 and t = 25 days). Results from high throughput sequencing of the two sampling dates were similar, giving evidence to the presence of bacteria with the ability to degrade ClO_4^- immediately upon its addition (Section 3.1). Two main phyla, *Proteobacteria* and *Bacteroidetes*, were found before ClO_4^- addition to the reactor with relative amounts of 75.5% and 22.8%, respectively, and 60.5% and 28.2%, respectively, after the addition of ClO_4^-. Within the phylum *Proteobacteria*, only *Betaproteobacteria* was present, while the second phylum, *Bacteroidetes*, consisted of only *Flavobacteriia*.

Fig. 3 shows the relative amounts of the dominant genera before and after the addition of ClO_4^-. *Zoogloea* was the dominant genus in the reactor both before and after the addition of ClO_4^-, accounting for 38.3% and 31.0%, respectively, followed by *Dechloromonas* (28.7% before, 23.4% after), *Flavobacterium* (13.7% before, 20.3% after), *Chryseobacterium* (10.5% before, 11.0% after), and *Vogesella* (2.4% before, 2.9% after). Less significant genera are listed as 'Others' (6.4% before, 11.0% after). All five genera have species that can carry out denitrification, but only *Dechloromonas* has been associated with denitrification and ClO_4^- reduction [31].

PCR-DGGE analysis of the pressurized hydrogenotrophic denitrifying reactor gave similar results with seven nearly identical bands observed before and after the addition of ClO_4^- (Fig. 4). Four of the bands from the DGGE were sequenced (Fig. 4, Lane 1) and compared to the closest phylogenetic relatives found in the NCBI gene bank of the predominant bacteria recovered from high throughput sequencing (*Zoogloea ramigera*, *Zoogloea resiniphila*, *Dechloromonas hortensis*, *Dechloromonas agitate*, *Vogesella perlucida*, *Flavobacterium cheniae*, and *Chryseobacterium soli*). The aligned sequences from high throughput sequencing and PCR-DGGE are presented in Fig. 5.

The phylogenetic tree shows a divergence of only 5%, with DGGE bands-1,2 showing a near 100% similarity to *Dechloromonas* sp., while DGGE bands-3,4 have a close similarity with *Flavobacterium* and *Chryseobacterium*. The reactor's ability to metabolize ClO_4^- almost immediately from the outset of ClO_4^- addition was due to the large presence of *Dechloromonas* as confirmed by high throughput sequencing and PCR-DGGE analysis. Strains of *Dechloromonas* have been shown to grow on ClO_4^- and NO_3^-, while its ability to use H_2 as an electron donor has also been shown [32]. Significant changes were not observed in the microbial population after 25 days of concurrent ClO_4^- and NO_3^- reduction, primarily due to the much greater electron accepting capacity (EAC) of NO_3^- (15 mg/L or 5.4 mequiv EAC) as opposed to ClO_4^- (20 mg/L or 1.6 mequiv EAC) during the experimental period.

3.3. Reduction of different electron acceptors in the combined treatment scheme

Following the initial investigation of ClO_4^- reduction using only the pressurized hydrogenotrophic reactor, the removal of different electron acceptors (NO_3^--N, NO_2^--N, ClO_4^- and SO_4^{2-}-S) was studied in the combined treatment scheme at different flow rates for two months. The inlet concentrations of NO_3^--N and ClO_4^- were adjusted to 25 and 10 mg/L, respectively, while SO_4^{2-}-S concentrations in tap water ranged between 7 and 9 mg/L. The results are summarized in

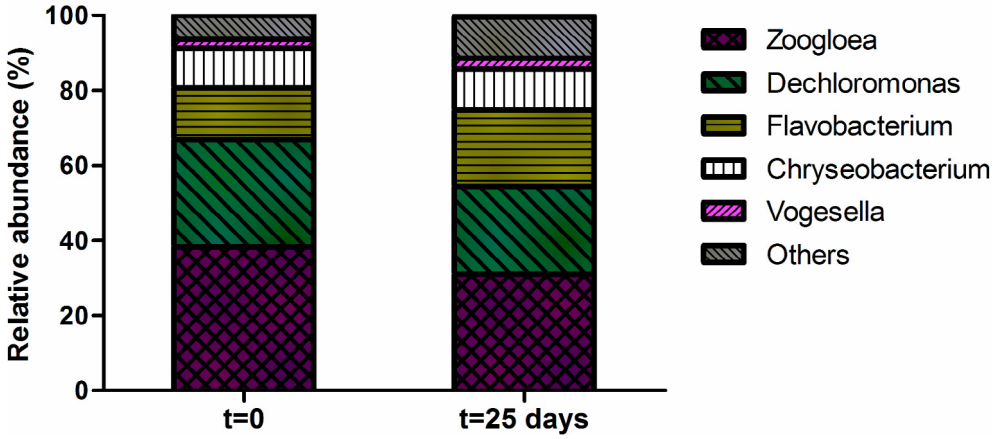

Fig. 3. Relative abundance of dominant genera in the pressurized hydrogenotrophic denitrifying reactor, before (t = 0) and after (t = 25 days) the addition of ClO_4^-.

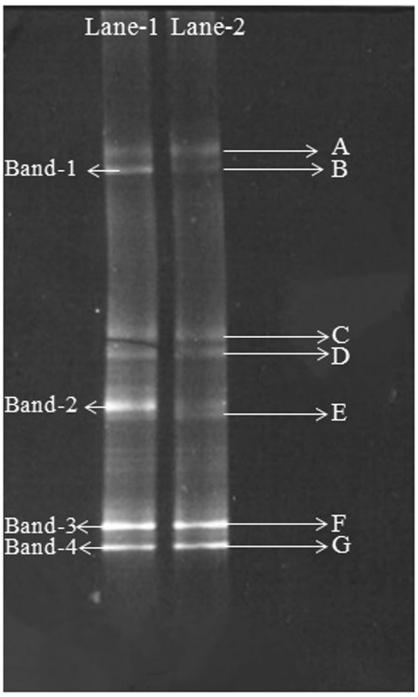

Fig. 4. PCR-DGGE analysis, Lane-1: pressurized hydrogenotrophic denitrifying reactor before the addition of ClO_4^-; Lane-2: pressurized hydrogenotrophic denitrifying reactor after the addition of ClO_4^-. Dominant bands are labeled A to G. Bands 1, 2, 3 and 4 were sequenced.

Fig. 6. All measurements in Fig. 6 were repeated five times, each in a different day.

As expected, higher NO_3^--N removal was observed in the main reactor unit at lower flow rates due to the higher retention time. Significant ClO_4^- removal was observed in the main reactor unit (from 10 to 2 mg/L) only when the lowest flow rate was applied; probably due to the higher retention time with the correspondent lower NO_3^--N concentration (CSTR conditions, i.e., NO_3^--N concentration of about 1 mg/L). At higher flow rates, the effluent NO_3^- concentration in the main reactor increased and the average ClO_4^- removal rates calculated in the main reactor unit decreased (0.73, 0.3 and 0.26 g/($L_{reactor}$·d) for the operation with 150, 225 and 300 mL/min, respectively), suggesting that simultaneous removal of NO_3^- and ClO_4^- occurred with inhibition of ClO_4^- reduction due to the competition for electrons by NO_3^- [33].

In the polishing unit denitrification always occurred, while significant ClO_4^- reduction (>1 mg/L) occurred only in the presence of very low NO_3^--N concentrations. Sulfate reduction was observed only in the presence of very low NO_3^--N and ClO_4^- concentrations. These results can be explained by the combination of low concentration of dissolved H_2 in the polishing unit (Section 3.4), higher denitrifying population than perchlorate-reducing population in the system, NO_3^- reduction by some perchlorate-reducing bacteria (Section 3.2) and the thermodynamics-based priority of NO_3^- and ClO_4^- reduction over SO_4^{2-} reduction. Therefore, further reduction of ClO_4^- to concentration close to zero occurred in the polishing unit only under the lowest flow rate when the NO_3^--N concentration was already very low. The average ClO_4^- removal rates calculated in the polishing unit were much lower compared to the main reactor unit (0.12, 0.02 and 0 g/($L_{reactor}$·d) for the operation with 150, 225 and 300 mL/min, respectively). This observation

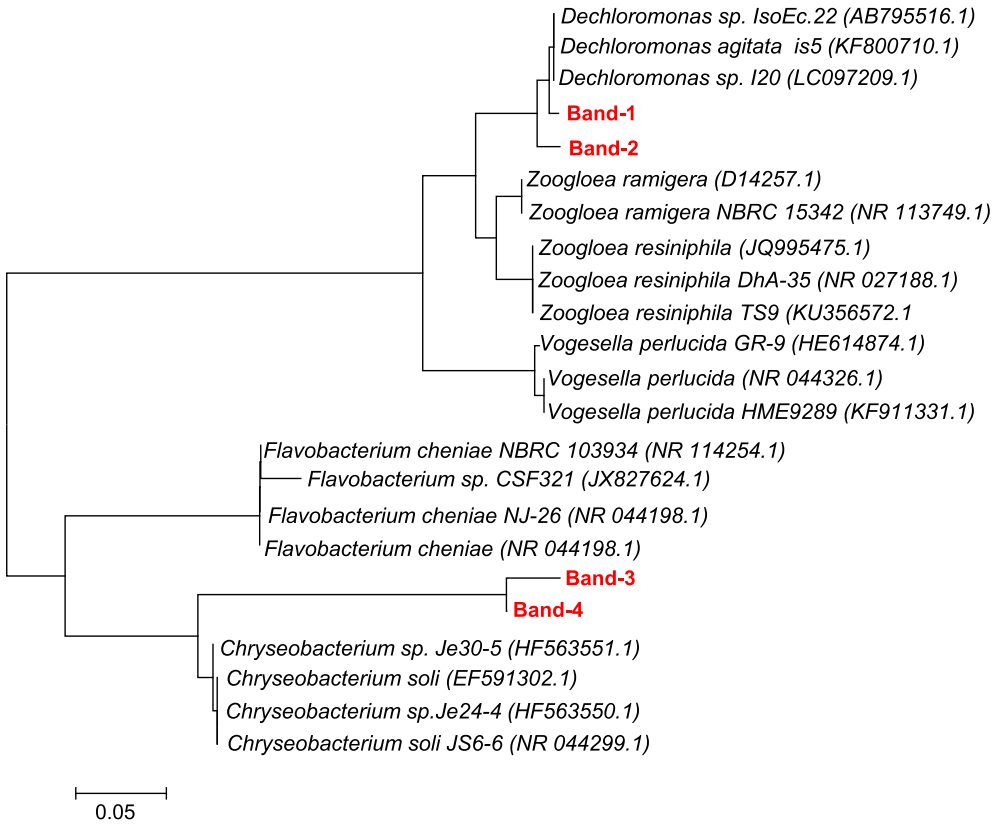

Fig. 5. Phylogenetic tree comparison of bacteria determined by high throughput sequencing and PCR-DGGE sequences (bands-1, 2, 3, 4) from the pressurized hydrogenotrophic denitrifying reactor.

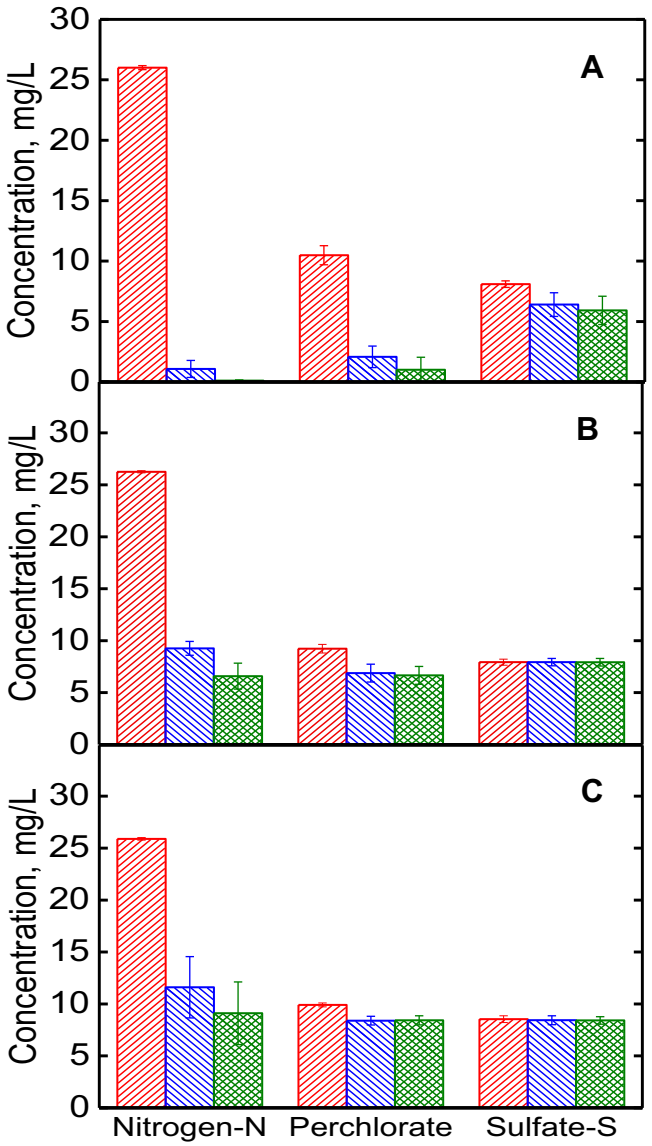

Fig. 6. Concentration of the inorganic nitrogen (NO_3^--N + NO_2^--N), ClO_4^- and SO_4^{2-}-S in the influent (red), effluent of the main reactor unit (blue) and effluent of the polishing unit (green) at different flow rates of (A) 150 mL/min; (B) 225 mL/min; and (C) 300 mL/min. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

can be attributed to the lower ClO_4^- concentration in the polishing unit. Also, the lower NO_3^- concentration in the polishing unit may not support a significant growth of ClO_4^- reducing bacteria as in the main reactor unit [8]. In the case where NO_3^- or ClO_4^- are further reduced in the polishing unit, an improved H_2 utilization is achieved. Sulfate reduction is not one of the treatment goals and therefore does not improve H_2 utilization in terms of financial aspects. However, it minimizes the amount of H_2 released to atmosphere and therefore may contribute to the safety of the process. Detailed calculations and measurements for H_2 utilization efficiencies are described in the next section. DOC analysis showed a minor increase of 0.15 mg/L after the polishing unit as compared to the inlet of the polishing unit.

3.4. Hydrogen utilization and effluent quality using the combined system for the treatment of typical polluted groundwater

Following the experiments with relatively high influent ClO_4^- concentrations, the removal of a lower inlet ClO_4^- concentration of 1.5 mg/L (the NO_3^--N concentration remained 25 mg/L) was studied in order to simulate typical conditions and to check the ability of the polishing unit to decrease ClO_4^- levels to trace concentrations, below 15 μg/L. The flow rate in this experiment was adjusted to 155 mL/min.

The removal of ClO_4^-, together with that of NO_x^--N and SO_4^{2-}-S, over the different treatment stages at steady state are shown in Fig. 7. Fig. 7 shows that ClO_4^- concentration was reduced to an average trace level of lower than 7 μg/L in the polishing unit. The lowest value observed during steady state was 2 μg/L. Together with reduction of NO_3^- and NO_2^- concentrations to below 0.1 mg/L, without any accumulation of chlorate (ClO_3^-) and chlorite (ClO_2^-), and with minimal increase in DOC concentration after the biological process (maximum DOC measured in effluent water was ~2 mg/L compared to 0.6 mg/L in feed water), the combined treatment scheme is suitable for drinking water production. The plug-flow character of the polishing unit is advantageous for reducing ClO_4^- concentrations to such low trace levels for two main reasons: (1) in CSTRs, reaching such low trace concentrations is harder due to mixing with the inlet stream having much higher concentrations; (2) better performance of ClO_4^- reduction can be achieved downstream after depletion of NO_3^-.

The submerged-flow regime in the polishing unit minimizes H_2 discharge to the atmosphere and allows for its further consumption. In the polishing unit where ClO_4^- concentration is very low, NO_3^- can also support growth of ClO_4^- reducing bacteria and thus maintain this bacterial population [8]. Fig. 7 also shows that no

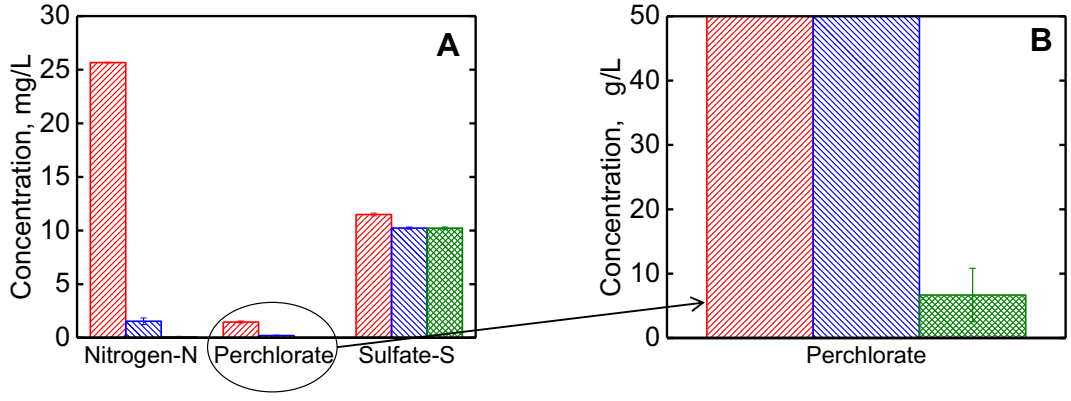

Fig. 7. Left (A): Concentration of the inorganic nitrogen (NO_3^--N + NO_2^--N), ClO_4^- and SO_4^{2-}-S in the influent (red), effluent of main reactor unit (blue) and effluent of polishing unit (green) at a flow rate of 155 mL/min. Right (B): zoom-in of the ClO_4^- column in units of μg/L. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Results achieved using the combined treatment scheme for the treatment of typical polluted groundwater (1.5 mg ClO_4^-/L and 25 mg NO_3^--N/L).

Denitrification rate in main reactor unit [g N/($L_{reactor}$·d)]	2.154 ± 0.028
Total pressure in main reactor unit [bar]	2.5
*Theoretical H_2 pressure at steady-state in the main reactor unit [bar]	0.36
Measured H_2 pressure at steady-state in the main reactor unit [bar]	0.39 ± 0.01
**Dissolved H_2 concentration in the effluent of the main reactor unit at saturation [mg/L]	0.59 ± 0.01
Measured dissolved H_2 concentration in the effluent of the main reactor unit [mg/L]	0.52 ± 0.04
H_2 utilization efficiency after the main reactor unit [%]	95.4 ± 0.3
Theoretical consumption of H_2 in the polishing unit [mg/L]	0.56
Measured dissolved H_2 concentration in the effluent of the polishing unit [mg/L]	0.002 ± 0.004
H_2 utilization efficiency after the polishing unit [%]	99.979

* Based on Epsztein et al. [24].
** Using Henry's constant of 1.5 mg H_2/(L·bar).

SO_4^{2-} reduction was observed in the polishing unit due to the low concentrations of H_2 (Table 1).

Table 1 summarizes the main results and calculations at steady state, including GC analyses for H_2 concentration in gas and liquid phase. Table 1 shows the high denitrification rates obtained in the pressurized reactor as compared to other technologies, even at low effluent NO_3^--N concentrations. The rate can be further increased by applying higher recirculation rates [25]. A good correlation was found between measured and theoretical H_2 pressure in the closed-headspace reactor, indicating steady-state conditions. As expected, the measured dissolved H_2 concentration was a bit lower than its value at saturation due to H_2 consumption by biomass. The H_2 utilization efficiencies of the main pressurized reactor unit or the combined treatment scheme were calculated by Eq. (1).

$$H_2\ utilization\ efficiency = \frac{H_C}{H_C + H_e} \times 100\% \quad (1)$$

where H_e is the measured dissolved H_2 in the effluent of the main reactor unit or the polishing unit; and H_C is the H_2 consumption (in units of mg/l) in the main reactor unit or overall process. In order to calculate the H_2 consumption, a previously suggested metabolic stoichiometry for hydrogenotrophic denitrification [34] (Eq. (2)) and SO_4^{2-} reduction [35] were used. For ClO_4^- reduction, the metabolic stoichiometry (Eq. (3)) was built applying the same yield coefficient used for hydrogenotrophic denitrification due to the similar thermodynamics of the processes [10].

$$NO_3^- + 3H_2 + H^+ + 0.22CO_2 \rightarrow 0.48N_2 + 3.35H_2O + 0.04C_5H_7O_2N \quad (2)$$

$$ClO_4^- + 4.61H_2 + 0.31CO_2 \rightarrow Cl^- + 4.48H_2O + 0.06C_5H_7O_2N \quad (3)$$

The H_2 utilization efficiency calculated after the main reactor unit was similar to the previous findings in the pressurized reactor [24]. The theoretical consumption of H_2 in the polishing unit was based on the assumption that all three electron acceptors were reduced by H_2 consuming bacteria. The result (0.56 mg/L) was very close to the measured dissolved H_2 after the main reactor unit (0.52 mg/L), albeit a bit higher. The difference can be attributed to minor heterotrophic activity. The almost zero residual of H_2 in the polishing unit effluent correlates well with the fact that SO_4^{2-} was not reduced in the second unit due to lack of H_2. The results of the combined treatment scheme show almost complete H_2 utilization with a total consumption of 10.9 mg H_2 per liter of water treated. To the best of our knowledge, our results of 100% utilization of H_2 gas together with reduction of perchlorate concentration to low trace concentrations of ~2 μg/L were previously reported only for the MBfR [10].

4. Conclusion

A new treatment scheme for removal of NO_3^- and ClO_4^- from drinking water, based on an unsaturated-flow pressurized hydrogenotrophic reactor combined with an up-flow submerged-bed open-to-atmosphere polishing unit was investigated. Degradation of ClO_4^- started immediately after the addition of ClO_4^- to the pressurized denitrification reactor, indicating that no special inoculum was needed for adjusting the reactor for ClO_4^- reduction. This finding was supported by the large presence of the genus *Dechloromonas* in the reactor prior to the introduction of ClO_4. Co-reduction of NO_3^- and ClO_4^- was observed in the pressurized reactor with significant inhibition in the ClO_4^- reduction rate at higher NO_3^- concentrations. The combination of submerged and plug-flow conditions in the polishing unit minimizes the discharge to atmosphere of the residual dissolved H_2 from the pressurized reactor and allows for the decrease of ClO_4^- concentration to trace levels of 2 μg/L. The further consumption of H_2 in the polishing unit resulted in an increase in H_2 utilization efficiency from 95% to almost 100%.

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