Degradation of tetrachloroethylene by zero valent iron nanoparticles in the presence of a natural groundwater bacterial biofilm in a sandy porous media

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Research article

Degradation of tetrachloroethylene by zero valent iron nanoparticles in the presence of a natural groundwater bacterial biofilm in a sandy porous media

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ABSTRACT

Biofilms are naturally present in aquifers and can interact with zero valent iron nanoparticles (nZVI) used as remediation agents in contaminated groundwater; thereby they may alter nZVI reactivity towards targeted contaminants in porous media. Laboratory scale experiments using columns filled with sand (50 cm long and 5.2 cm in diameter) were performed to investigate the impact of natural biofilms on nZVI reactivity towards tetrachloroethylene (PCE) in conditions simulating an unconsolidated sandy aquifer. Solutions containing PCE were injected through the sand columns in the presence or absence of biofilm and nZVI. Concentrations in PCE and its metabolites were monitored during 45 days in dissolved and gas phases. PCE concentrations decreased at the column outlets due both to its reductive dechlorination by nZVI (~30% of injected PCE) and its sorption or deposition (as PCE-DNAPL) on sand (~35% of injected PCE). No significant differences in PCE concentrations were found in presence or absence of biofilm. However, biofilm presence affected the nature of PCE metabolites. A higher release of ethene in the column containing biofilm was observed, whereas ethane was dominant in the absence of biofilm. Microbes consumed H_2 released by the corrosion of nZVI limiting the hydrogenation of ethene to ethane. The consequences of biofilm development in porous media should be taken into account when considering treatment with nZVI, as it may affect the nature of produced metabolites.

1. Introduction

Aliphatic chlorinated hydrocarbons, such as tetrachloroethene (PCE), have been extensively used as solvents worldwide. Hydrophobic and poorly biodegradable, they are highly persistent in the environment (half-life > 30 days).

Remediating groundwater polluted with aliphatic chlorinated hydrocarbons is technically challenging. Passive (permeable reactive barriers PRB (Obiri-Nyarko et al., 2014)) or active (e.g., soil vapor extraction, bioremediation, nanoremediation) remediation approaches have been tested. ZVI is commonly described as efficient in PRB as a reducing agent (Obiri-Nyarko et al., 2014), although other materials have also been described for groundwater in situ remediation of various organic contaminants (halogenated or phenolic compounds, pharmaceuticals, nitroaromatics, etc. (Cai et al., 2020)), such as zeolite (Vignola et al., 2011), calcite (Turner et al., 2008), pyrite (Wang et al., 2020) or combined materials (Liu et al., 2019). Advantages of nanoparticles as a remediation agent are their high reactivity due to their higher specific surface area (Cundy et al., 2008; Zhang, 2003) and their ability to be directly injected into the aquifer to treat both source and plume areas. As

for PRB, ZVI is often used in remediation using NPs, but other stabilized nanoparticles have been developed, such as Fe_3O_4, FeS, bimetallic Fe–Mn binary oxides or $Fe_3(PO_4)_2$ (Cai et al., 2020). Amongst the methods tested in the past decades (Kim et al., 2017; Stroo et al., 2012), zero-valent iron (ZVI) injected as nanoparticles (nZVI) has be proven to be efficient for dechlorinating PCE through chemical reduction (Dong et al., 2019; Fu et al., 2014; Gillham and O'Hannesin, 1994; Han et al., 2019; Henderson and Demond, 2007; Stefaniuk et al., 2016; Thiruvenkatachari et al., 2008). The reactivity of nZVI towards the contaminant(s) depends on the chemical and physical properties of the nZVI particles (e.g., solubility, pH_{IEP}, crystallinity, size and eventual coating) (Cornelis et al., 2014; Petosa et al., 2010) and the targeted contaminant(s), but it may also be affected by the environmental conditions (e.g., physical and chemical composition of both groundwater and porous media) (Johnson et al., 2009; Kim et al., 2012; Kocur et al., 2014; Saleh et al., 2008).

Microorganisms are present in natural aquifers and could impact both the reactivity of nZVI toward the targeted contaminant and the nature of metabolites produced by the reductive dechlorination. Indeed, the number of viable bacterial cells in saturated aquifers is of the order of 10^7 colonies forming units per gram dry weight (CFU/g dw) of solid (Balkwill

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and Ghiorse, 1985; Bone and Balkwill, 1988; Webster et al., 1985; Wilson et al., 1983). In groundwater, bacteria are present both as individual cells (planktonic), and as microbial communities in biofilms, i.e., complex aggregations of bacterial cells and secreted extracellular polymeric substances (EPS) (Watnick and Kolter, 2000). Biofilms are resistant to many contaminants (due mainly to EPS), resilient, and may be useful for decontaminating groundwater (Antizar-Ladislao and Galil, 2010; Weaver et al., 2015; Zhao et al., 2016). Their presence can modify the water flow and pathways in the porous media (Crampon et al., 2018; Peszynska et al., 2015). They can also alter the roughness, electrical charge, hydrophobicity and chemistry of the aquifer grain surfaces on which they have grown (Donlan, 2002) and thereby modify the ways contaminants interact with the porous media. They can interact with other components present in the porous media such as nZVI used as remediation agents and potentially modify their reactivity and performance. To our knowledge there is nothing published on the impact of biofilms on both the reactivity of nZVI towards PCE contaminants and the nature of produced metabolites.

Few studies or field remediation address the impact of the presence of a biofilm when considering a groundwater remediation strategy. However, biofilms are omnipresent in these kinds of environment NP, and the impact of this presence on the results of the remediation should be carefully considered. Consequently, the aim of this study is to evaluate the impact of the presence of a biofilm grown from a natural groundwater bacterial community on the reactivity of nZVI and the nature of metabolites produced, by performing column experiments at the lab-scale with a natural sandy groundwater material and by monitoring parameters related to the efficiency of PCE dechlorination by nZVI. Although working at laboratory scale with columns is a limitation of this study, the information acquired is of interest for extrapolation to real field-scale conditions.

2. Material and methods

2.1. nZVI, synthetic water and PCE addition

Zero-valent iron nanoparticles (nZVI), NANOFER 25S NP (modified nZVI particles containing organic and inorganic stabilizers (surfactants, 3% in the commercial suspension)), were provided by NanoIron s.r.o. (Czech Republic). Moderately hard synthetic water (FIm) as described by US EPA (EPA, 2002) was used for the column experiments. It was prepared with Millipore ultrapure water (Millipore, France), $MgSO_4$, $NaHCO_3$, KCl and $CaSO_4$ (Sigma-Aldrich) of reagent grade (purity $\geq$99%). PCE solution was prepared from a PCE stock solution ($\geq$99% purity; provided by Sigma-Aldrich) diluted with the synthetic water (FIm) leading to a PCE concentration of 2 mg.L^{-1} (C$_0$) at the columns inlets.

2.2. Experimental setup

2.2.1. Sand characteristics

Sand used both for biofilm growth and column experiments came from an aquifer in Stuttgart, Germany (MII sand; NanoRem FP7 European project, http://www.nanorem.eu). Granulometric classes go from fine sand to fine gravel, with values of D10 = 0.23 mm, D50 = 0.625 mm, D60 = 0.9 mm and D90 = 3.3 mm. The bulk density of the initial sand is 1.87 kg L^{-1}. Its organic matter content is below the detection limit (<0.008 mg/g sand).

2.2.2. Step 1: biofilm growth

Bacteria used for biofilm growth were collected from natural uncontaminated groundwater. There was no evidence of the presence of organohalide respiring bacteria or other dechlorinating bacteria in the bacterial community, as shown via cloning and sequencing of the 16S rRNA gene in a previous study (Crampon et al., 2019). Biofilm was grown following the same protocol as described in Crampon et al. (2018) in two

separate columns by circulating a suspension of substrate (sodium nitrate, yeast extract and sodium acetate) and bacteria. After two months (time based on previous assays), sand from the bottom of the columns, where biomass was highest, was recovered for the experiment using biofilm, and mixed with nZVI before filling column R3.

2.2.3. Step 2: column set up

Three glass columns (50 cm * 5.2 cm ID) were prepared: (i) R1 is the control column without biofilm and without nZVI, (ii) R2 is the column containing 25S nZVI and no biofilm and (iii) R3 is the column containing both 25S nZVI and biofilm (Figure 1). Each column consists of two "buffer" zones (sand only), one at the inlet (5cm-long) and one at the outlet (10cm-long), to simulate a demarcated reactive zone in the middle. Inox tubing was used between the column outlets and gas traps to avoid gas losses.

nZVI was mixed either with sand containing biofilm (for R3) or without biofilm (for R2), in an anaerobic atmosphere (glove box). In detail, 6.25 mL of the commercial nZVI suspension were diluted in 100 mL FIm water (O$_2$-free) and then mixed with sand to reach a total amount of 1g of Fe (i.e., 1.79E^{-02} mol of Fe) in each column, corresponding to a concentration of 0.5706 g Fe per kg^{-1} sand. This concentration was chosen to ensure an excess of Fe0 considering the reaction between PCE and nZVI. To avoid solution loss when filling the columns, the remaining water in mixing vessel was added to columns after the filling.

Columns were flushed with CO$_2$ (5 pore volumes) to evacuate O$_2$ and obtain anaerobic conditions, then filled with O$_2$ free FIm water. Pore volume, evaluated by weighing the column before and after water filling, was ~326 mL, corresponding to a porosity of ~31%.

2.2.4. Step 3: column monitoring

All column experiments were monitored simultaneously, to ensure homogeneity of the experimental conditions (e.g., room temperature) between the three columns.

Flow rate was set to ~110 mL.d^{-1} corresponding to an apparent pore water velocity of 16.7 cm.d^{-1} corresponding to typical values in sand (from 0.0864 to 864 cm.d^{-1}). Four injection cycles (H$_2$O FIm only and H$_2$O FIm + PCE) were carried out in order to monitor nZVI reactivity over time and follow PCE metabolites, starting with the PCE water solution for 2.5 PV, then water alone for 2.5 PV, followed again by the PCE water solution and finally the water alone, both during ~5 PV. Thus, 15 PVs of water (PCE spiked or not) passed through the column during the experiment which was carried out for 45 days, with a total of 24 days PCE injection. The total amount of PCE injected in each column was 3.14 × 10^{-5} mol (Table 1). Concentrations at the inlet of the columns (2mg.L^{-1}) corresponded overall to concentrations comparable to those found in the most downstream part of a contaminant plume. Retardation coefficient for PCE was evaluated in R1, allowing to characterize PCE sorption. It was calculated following the equation:

$$R = 1 + [B_d/\varphi] * Kd,$$

where B$_d$ is the bulk density of the porous media, φ is the porosity of the porous media and Kd the adsorption coefficient of the studied contaminant (Benker et al., 1998).

2.3. Influent and effluent analyses

Concentrations of tetrachloroethylene (PCE), trichloroethylene (TCE), *Cis*- and *Trans*-dichloroethylene (DCE), were analyzed in the liquid phase at the column's inputs and outputs using GC-FID (Varian CP3800 equipped with an Agilent DB-624 column (30m*0.32mm*1.8μm)) using the headspace method by heating the samples at 80 °C for 30 min prior to injection of the gaseous phase (Combipal CTC Analytics injector). The limit of quantification (LOQ) for these molecules was 50 μg.L^{-1}.

Production of dihydrogen, ethene and ethane were measured in the gas phase by GC-TCD/FID (Thermo trace GC Ultra) after collection of gas
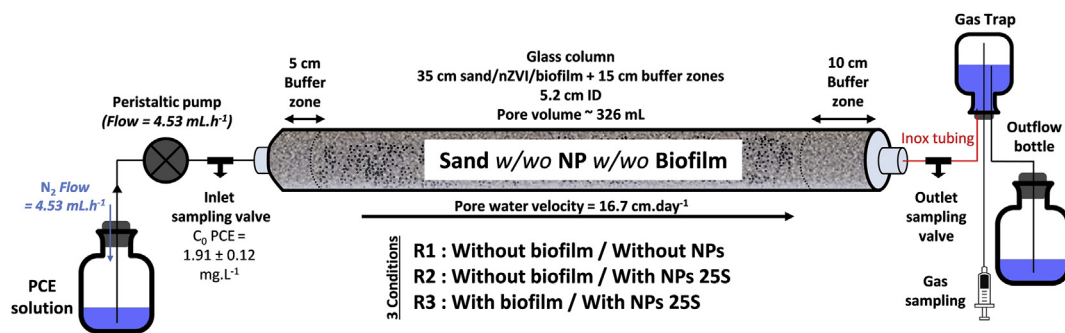
Figure 1. Experimental setup. Here is presented the experiment system for one column. The three columns (R1, R2 and R3) were set-up and monitored simultaneously.

Table 1. Results of cumulative products at the columns inlets and outlets presented as total moles during the whole experiment, injected PCE corresponds to the mean value between the three columns (n = 3); PCE and Cl- quantities (moles) were calculated as the sum of analyzed concentrations at the column's outlets during the whole experiment.

		R1	R2	R3
Experimental conditions	Introduced nZVI (moles Fe)	0	1.79E-02	1.79E-02
	Injected PCE (moles)	3.14E-05	3.14E-05	3.14E-05
Cumulative PCE at the column outlet	PCE (moles)	2.04E-05	1.12E-05	1.13E-05
	Percent of injected	*64.7%*	*35.5%*	*36.0%*
Chlorides	Cl$^-$ (moles)	0	1.40E-04	1.47E-04
	Degraded PCE (mass balance) according to Cl$^-$ (%)	0	111.30	117.02

samples by pumping 5 mL with a gas-syringe in the gas trap. Results were expressed as percent of gas in the gas traps, corrected according to the pressure in the samples. Calibration curves were performed with standards of the gas of interest. LOQ were 0.005% and 0.0002% for H_2 and ethene/ethane, respectively. Because the gas traps could not be flushed between samplings, these compounds accumulated in the traps.
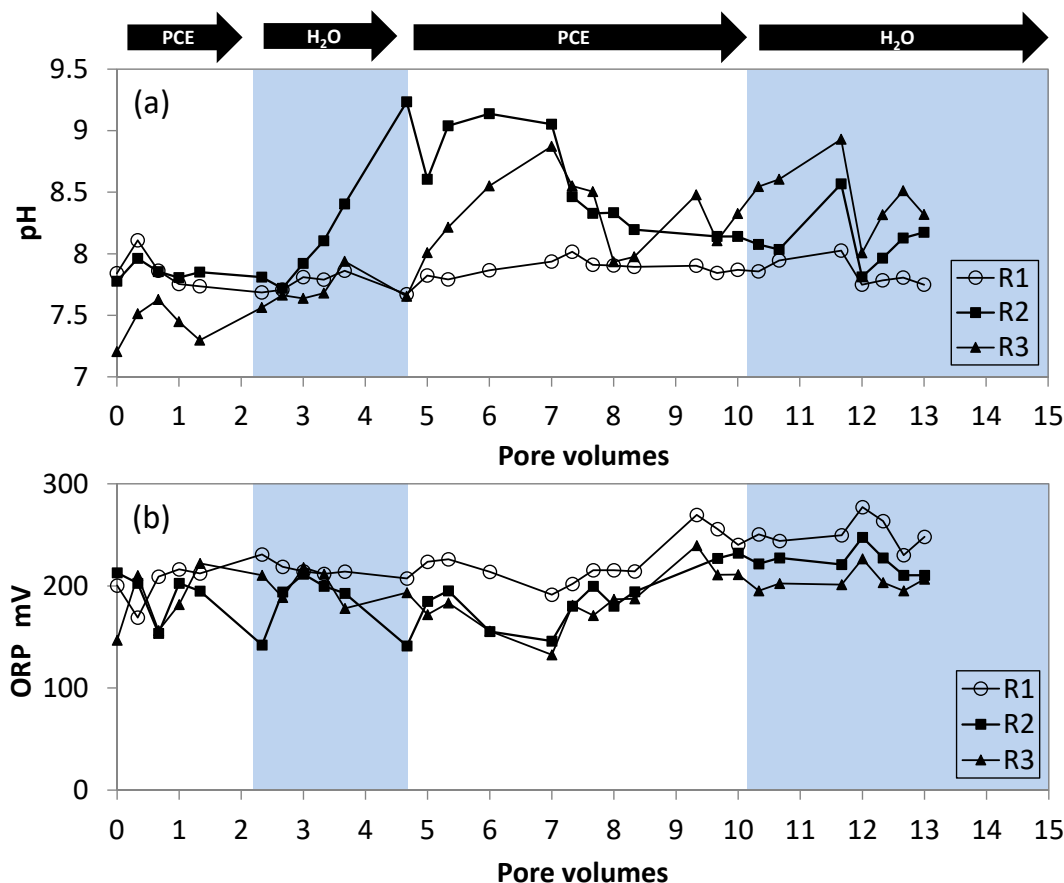
Figure 2. (a) pH and (b) ORP monitoring for the three columns R1, R2 and R3. Injection cycles (H2O FIm/PCE solution) are mentioned at the top of the figure.

Consequently, the concentrations of byproducts measured in the gas traps were not used to evaluate the mass balance of the reaction between nZVI and PCE.

The formation of chlorides from PCE and its metabolites dechlorination was monitored by ionic chromatography analysis of Cl^- concentrations (LOQ 0.5 mg.L^{-1}) in the solutions injected into the columns (C_0 used as the blank value, through the inlet sampling valve) and in outflow bottles (the bottles were washed after each sampling). C_0 values were 1.99 ± 0.12 mg.L^{-1}, 1.92 ± 0.13 mg.L^{-1} and 1.93 ± 0.12 mg.L^{-1} for R1, R2 and R3 columns, respectively.

The RedOx potential (ORP) and pH of the effluent were monitored in outflow bottles. Because some contacts with air occurred before the measurements, leading to a potential increase in ORP values, they will need to be interpreted with precaution.

3. Results and discussion

3.1. Evolution of pH and ORP

In the absence of nZVI and biofilm (column R1), pH was between 7.8 and 8.1 throughout the monitoring period (Figure 2). In the presence of nZVI however, the pH at the column outlets increased (up to ~9 for R2) after 3 and 5 PV, for columns R2 and R3, respectively. This can be due either to a consumption of H^+ ions during the PCE dechlorination and/or a release of OH^- in water *via* nZVI anaerobic corrosion. The lag phase before this increase suggests an initial buffer effect of the sand, enhanced by the presence of biofilm in R3. After this increase, pH in columns R2 and R3 decreased after 7–8 PV and then oscillated around 8.5, thus remaining globally higher than in column R1.

ORP values changed slightly when solutions passed through the column but were globally constant over time in outflow solutions at around 200–250 mV (Figure 2). Unsurprisingly, the lowest ORP values were measured in the water samples from the R2 and R3 columns that contain sand with nZVI (reducing agent).

3.2. Impact of nZVI on PCE dechlorination

3.2.1. PCE breakthrough curve

PCE concentrations at the column inlets (C_0) were checked regularly. They were 1.98 ± 0.02 mg.L^{-1} (n = 5 dates * 3 columns = 15). Additionally, H_2 released from the column was measured to quantify anaerobic corrosion, which leads to the inactivation of nZVI, and consequently a decrease in its reactivity towards PCE (Liu and Lowry, 2006).

PCE concentrations increased more rapidly in the outflow waters of the R1 column (after 0.5 PV) than in the outflow waters of columns R2 and R3 (after 1 PV; Figure 3) suggesting a stronger interaction between PCE and porous media in columns R2 and R3 compared to R1. C/C_0 ratios

remained, however, <0.2 in outflow waters of the three columns over the experiment showing that PCE also interacts with sand (Figure 4a). After 15 pore volumes, the cumulative PCE measured at the R1 column outlet was 2.04×10^{-5} mol, corresponding to only 64.7% of injected PCE (Table 1, Figure 5a).

In R1, no PCE metabolites or Cl^- production were detected, corroborating the absence of PCE dechlorination in this column, as expected (Table 1 and Figure 4b, d, e). The C/C_0 values for chlorides in column R1 are >1 at the beginning of the experiment probably due to equilibration time and flushing of the first pore volume, as this phenomenon disappeared after PV1.

As no degradation was observed in R1, the 35.3% of PCE injected not recovered in the outlet water samples were sorbed onto the porous media. Sorption of PCE mainly depends on the organic carbon content, grain size distribution and chemical composition (clay and silt contents) of the aquifer material, and on groundwater flow rate (Cwiertny and Scherer, 2010; Lerner et al., 2012). According to Mackay et al. (2006), log Koc for PCE in various solid media with 0.0105–3.7 mg.g^{-1} organic carbon is 2.4 ± 0.3 (n = 19), indicating a quite mobile to a slightly mobile compound. In sandy aquifers with low OM contents, PCE sorption on porous media is low, with Kd values ranging from 0.0051 to 0.0069, depending on the sand grain size, Kd values being lower for higher grain sizes (Kret et al., 2015). Here, TOC concentration in sand was below the detection limit (<0.008 mg.g^{-1} sand), the sorption of PCE is consequently expected to be very low. Retardation coefficients were calculated and considering the porosity of 0.307 observed in our columns, the bulk density for the studied sand of 1.87 and a Kd for PCE on sand (with negligible organic matter content) around 0.006 (Kret et al., 2015), the retardation coefficient for PCE in our porous media without biofilm was 1.036, highlighting a very limited retardation, and therefore a low interaction of PCE with sand. Sorption on sand alone cannot explain the 35.3% decrease in PCE in the porous medium found in R1. Sorption on finer particles, even if present at very low concentrations (see material and methods section 3.2), could be responsible for the observed PCE sorption (Mazari-Hiriart et al., 2000).

In the presence of nZVI (R2 and R3), PCE concentrations (Figure 3) and cumulative PCE quantities at the column outlets (Figure 5a) were significantly lower than those measured at the outlet of the column without nZVI (R1). The total cumulative amount of PCE at the R2 and R3 columns outlets were ~35.5% and ~36.0% of injected PCE, respectively, versus ~64.7% for R1 (Table 1).

Biological dechlorination of PCE and TCE leads to the production of intermediate byproducts (DCE, VC), while Fe0 reduces them directly to acetylene, ethene, and ethane, with only trace production of *cis*-DCE and VC (Liu et al., 2005; Taghavy et al., 2010). In our study, the reaction between PCE and nZVI did not lead to intermediate byproducts (TCE, DCE and VC were not detected by GC-FID analyses), but led to the
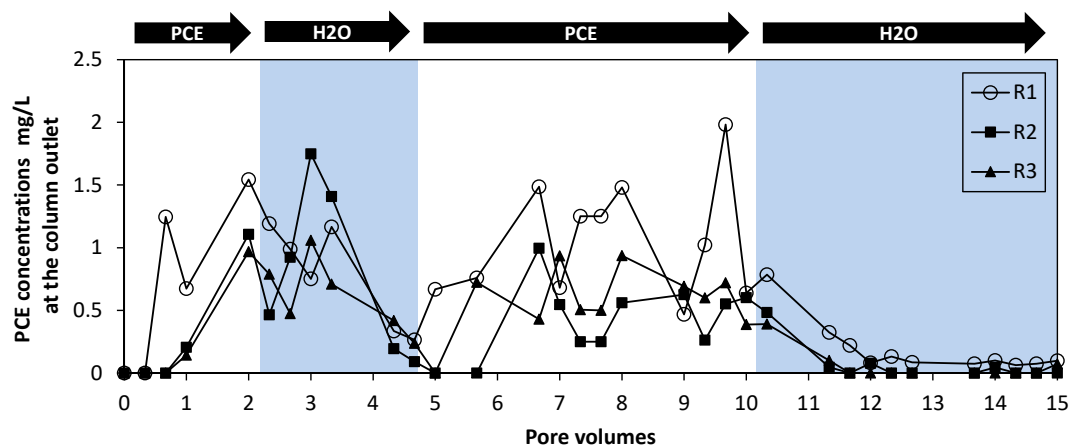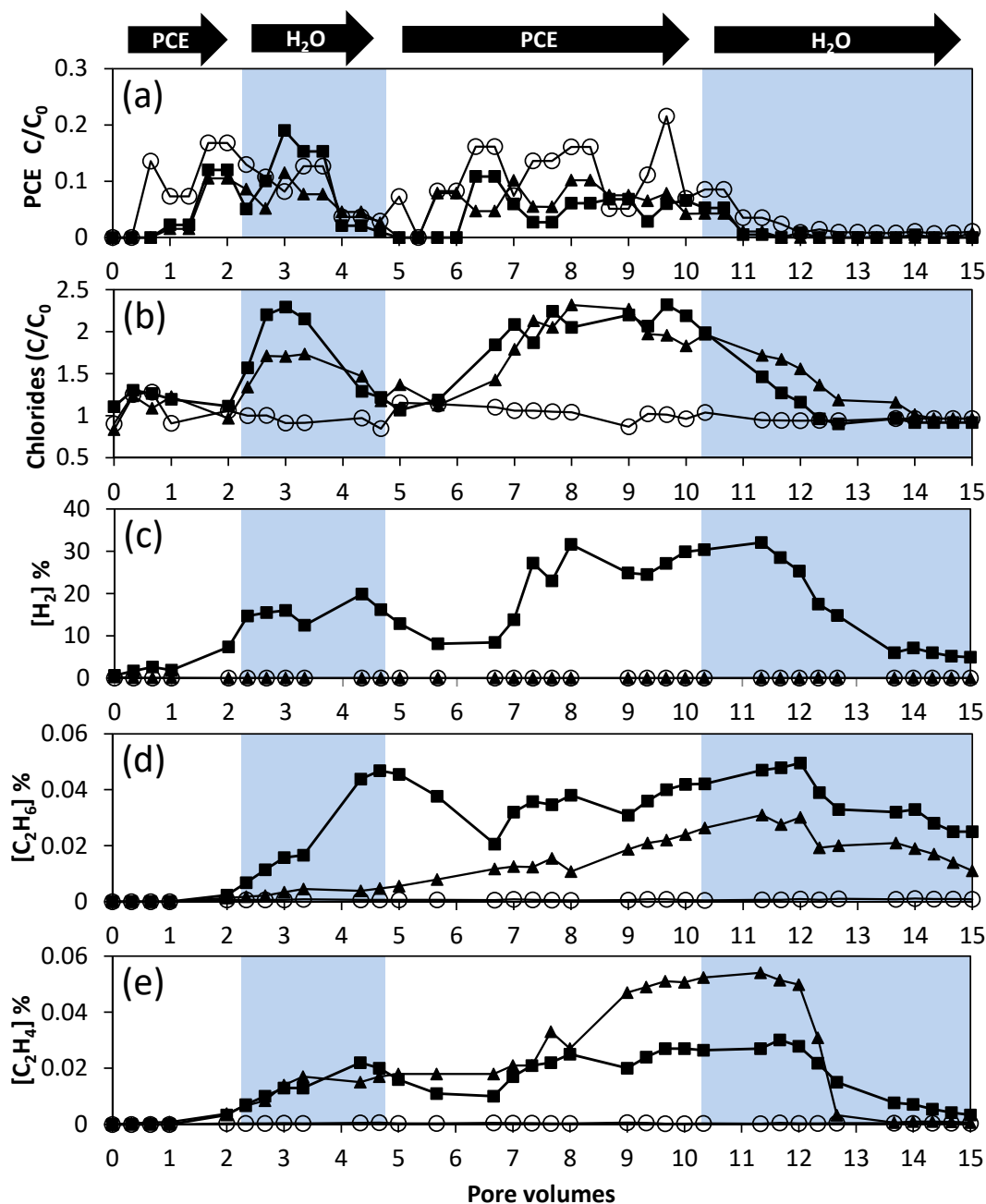

Figure 3. Raw PCE concentrations at the column outlets during the whole experiment for R1 (–○–), R2 (–■–) and R3 (–▲–) columns.

Figure 4. Monitoring of (a) PCE concentration and (b) Chlorides concentrations at the column outlets as C/C0 (concentrations at the outlets divided by concentrations at the inlets), and of (c) H2, (d) C2H6 and (e) C2H4 concentrations in the gas traps for R1 (–○–), R2 (–■–) and R3 (–▲–) columns.

production of gaseous byproducts (C_2H_4 and C_2H_6 for dechlorination, and H_2 from anaerobic corrosion of nZVI). The bacterial community producing the biofilm was characterized in a previous study; it did not contain species known to dechlorinate chloroethenes (Crampon et al., 2018). Regarding (i) the absence of intermediate byproducts (TCE, DCE, VC), (ii) the production of gaseous metabolites, and (iii) the absence of any production of byproducts in R1, we can conclude that PCE dechlorination in R2 and R3 columns was only due to nZVI. The lower concentrations compared to the inlet are both attributable to (i) sorption (35.3% according to the R1 column) and (ii) degradation by nZVI (biodegradation not being possible due to the absence of species able to dechlorinate PCE). Considering that 35.3% of injected PCE was sorbed, about 29.2% and 28.7% of PCE was thus degraded by nZVI in R2 and R3 columns, respectively. Li et al. (2006) showed a PCE removal rate of 12–25% with microscale ZVI, which is slightly lower, but comparable to

non-micrometric ZVI in our study. With nanometric ZVI, Taghavy et al. (2010) showed 30% PCE dechlorination up to ethene in a column previously filled with water and contaminated with 20 mL of PCE-DNAPL (mass balance evaluated after injection of 2 PVs of water). The experimental conditions however differ from our study, even if ZVI efficiency was comparable. Our values are consequently comparable with the literature.

3.2.2. Impact of nZVI on PCE dechlorination

Lower PCE concentrations in the outflow waters of the columns containing nZVI (R2 and R3) demonstrated PCE degradation. The fact that TCE, DCE (*Cis*-1,2-DCE, *Trans*-1,2-DCE and 1,1-DCE) and VC (vinyl chloride) were detected only at traces levels (<LOQ = 10 µg.L^{-1}) indicates that PCE was directly reduced to ethane (Taghavy et al., 2010). Considering that K_{oc} values of PCE metabolites are lower than the mother
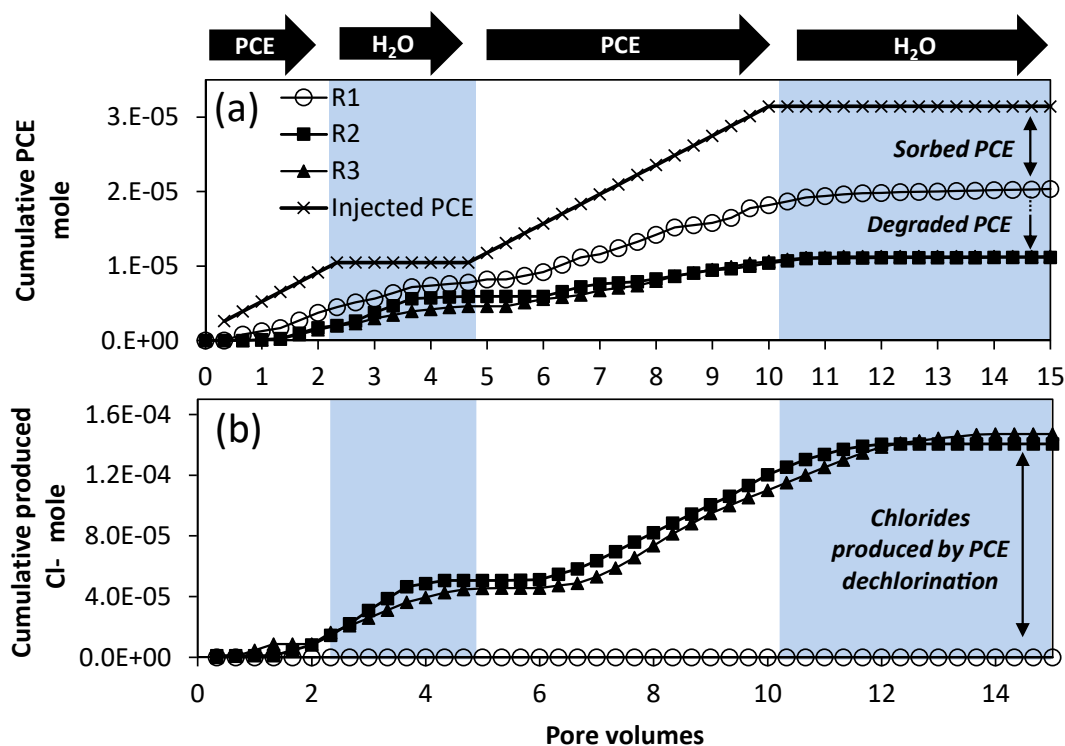
Figure 5. (a) Cumulative PCE concentrations at the columns outlets and theoretical PCE injected in the columns calculated as the cumulative PCE concentrations at the column inlets, (b) cumulative produced Cl- as moles of produced metabolites, for R1 (–○–), R2 (–■–) and R3 (–▲–) columns.

molecule (Koc (L/kg) = 247/111/35.5/38/8 to 98 for PCE/TCE/Cis-1, 2-DCE/trans-1,2-DCE and VC, respectively (Mackay et al., 2006)), their sorption on the solids in the columns must therefore be extremely limited. This is confirmed by the PCE gaseous degradation products being quantified in R2 and R3 gas traps (Figures 4d and 4e).

Chloride production (C/C0, Figure 4b) is another approach to measure PCE dechlorination by nZVI. At the end of the experiment, the total amount of Cl^- produced in R2 was $1.40.10^{-4}$ mol (Figure 5b), and chloride production followed the PCE injection phases, confirming the origin of Cl^- from PCE dechlorination. Looking at the stoichiometry (equation *(1)*), the produced Cl^- corresponds to a PCE degradation of around 111%. Considering that about 35% of PCE was sorbed into column R1, this result suggests that the impact of nZVI on PCE dechlorination was not limited by PCE sorption. Indeed, the presence of nZVI induces changes in ORP in the water all around zones where ZVI is present (Lefevre et al., 2016; Shi et al., 2015). It is thus possible that dechlorination by nZVI occurred also for sorbed PCE, which is generally not the case for bioremediation of PCE with bacteria, as sorption of PCE limits bioaccessibility (Kret et al., 2015; Reid et al., 2000; Tick et al., 2003). However, chloride mass balance overestimates PCE degradation as the later was still detected at the column outlet.

H_2 was detected in the gas trap of R2 but not R1 (Figure 4c). As indicated in equation *(2)*, nZVI oxidation releases H_2. The presence of H_2 thus shows that anaerobic nZVI corrosion occurred in our experimental setup. Note that the H_2 measured in the presence of biofilm (column R3) was much lower than without biofilm (R2); it was detected in R3 column only at trace levels, and not detected at all in R1 column. This is discussed in the next section.

3.3. Impact of biofilm on nZVI reactivity towards PCE

PCE breakthrough was not significantly affected by the presence of biofilm in column R3. Indeed, PCE concentrations over time and the total amount of PCE degraded at the end of the experiment were very similar between R2 (no biofilm) and R3 (biofilm) (Figures 4a and 5a).

Cumulative PCE dechlorination or sorption was slightly higher in the presence of biofilm at the beginning of the experiment (triangles in Figure 5a). Biofilms may change the interactions between nZVI and PCE as well as the water flow in the porous media due to the presence of preferential pathways (Peszynska et al., 2015). The flow may be higher in these preferential pathways compared to the flow in the parts of the porous matrix without biofilm. Furthermore, Kim et al. (2017) showed that increasing water flow (pore water velocities of 7, 14, 53 and 113 $cm.d^{-1}$) in glass columns filled with sand + nZVI (polymer modified) and fed with PCE solution led to an increase in PCE dechlorination. This could explain the slightly lower cumulative outlet PCE concentrations in R3 (Figure 4). However, a higher PCE sorption in the presence of biofilm is more prone to explain the differences than an increase in velocity in the presence of biofilm. Increased interactions in the presence of biofilm have been documented (Xie et al., 2017).

In the presence of biofilm, Cl^- concentrations in R3 outflow waters were slightly lower than those measured in R2 (Figure 4b), reinforcing the idea that PCE sorption was higher with biofilm, limiting the impact of nZVI.

The bacterial community used to grow the biofilm was previously characterized (Crampon et al., 2018). It was dominated by a strain closely affiliated to *Pseudomonas Stutzeri*. Some strains of *P. Stutzeri* have been shown to oxidize some PCE metabolites *via* Toluene/o-Xylene monooxygenase (ToMO) enzymatic activity (Chauhan et al., 1998), but are not known to carry out organohalide respiration. It must be noted that if *Dehalococcoides* strains, especially *Dehalococcoides mccartyi*, had been detected, the results on the degradation of PCE would have been different in the presence of biofilm. In spite of having no direct impact on PCE, it seems that bacteria present in the biofilm had an impact (direct or indirect) on the fate of PCE metabolites after PCE dechlorination by nZVI. Indeed, in R3, more C_2H_4 was detected compared to R2, and the opposite result was observed for C_2H_6 with higher concentrations in R2 compared to R3 (Figures 4d and 4e). C_2H_6 is produced from C_2H_4 in the reaction chain of PCE with nZVI, through ethane hydrogenation. Moreover, a very good correlation between the H_2 curve for R2 (Figure 4c) and C_2H_6

production (Figure 4d) was observed. H$_2$ from nZVI anaerobic corrosion reaches up to 30% in R2 compared to less than 1% in R3. Bacteria consuming H$_2$ as an electron donor for metabolic reactions could explain this difference. The H$_2$ would be a limiting factor for the transformation of C$_2$H$_4$ into C$_2$H$_6$ leading to C$_2$H$_6$ values in R3 up to ~1000 times lower than in R2. Taghavy et al. (2010) showed, in column experiments using Ottawa sand without adding biofilm, that ethene was the dominant byproduct from the reaction between PCE and nZVI but they did not monitor ethane concentrations.

H$_2$ from the reaction between ZVI and water has previously been shown to favor organohalide respiring bacteria (Summer et al., 2020). However, this was observed for microscale ZVI and not for nZVI due to the higher toxicity of nanoparticles. In future applications, the promotion of synergistic effects between biotic and abiotic mechanisms could reinforce the overall efficiency of remediation. Summer et al. (2020) suggest this could be obtained by using lower concentrations of nZVI, also diminishing the In Situ Chemical Reduction treatment costs.

4. Conclusions

Consequences of the presence of biofilms regarding the reactivity of nZVI were studied. Biofilm did not significantly affect the amount of PCE dechlorinated by nZVI but led to differences in the dechlorination byproducts. H$_2$ consumption decreased the potential of hydrogenation reactions, leading to the preferential release of C$_2$H$_4$ compared to C$_2$H$_6$, the latter being much more persistent in the environment. A suggested future research direction would be to re-inforce our knowledge on the synergistic effects due to H$_2$ release, *via* anaerobic corrosion of nZVI, on bacterial community activities regarding a range of reactions using H$_2$ as electron donor in aerobic or anaerobic conditions, linked to the fate of organic (dehalorespiration of polyhalogenated hydrocarbons or anaerobic reduction of azo groups for example) and inorganic contaminants (denitrification or sulfato- and ferri-reduction for example). Indeed, in the case of real scale nZVI remediation on a contaminated site, the released H$_2$ could be considered as stimulant for the biodegradation of targeted contaminants, and even of untargeted ones. This synergistic effect could lead to consider that remediation using nZVI also leads to a biostimulation of organohalide respiring bacteria, present in biofilm or as planktonic bacteria, and consequently to a more complete remediation. The diversity of organohalide respiring bacteria may be considered when implementing a groundwater remediation process to increase the remediation efficiency.

Declarations

Author contribution statement

Crampon Marc: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hellal Jennifer, Mouvet Christophe & Ollivier Patrick: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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