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Perchlorate Reduction by Autotrophic Bacteria Attached to Zerovalent Iron in a Flow-Through Reactor

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Biological reduction of perchlorate by autotrophic microorganisms attached to zerovalent iron (ZVI) was studied in flow-through columns. The effects of pH, flow rate, and influent perchlorate and nitrate concentrations on perchlorate reduction were investigated. Excellent perchlorate removal performance (≥99%) was achieved at empty bed residence times (EBRTs) ranging from 0.3 to 63 h and an influent perchlorate concentration of 40−600 μg L^{-1}. At the longest liquid residence times, when the influent pH was above 7.5, a significant increase of the effluent pH was observed (pH > 10.0), which led to a decrease of perchlorate removal. Experiments at short residence times revealed that the ZVI column inoculated with local soil (Colton, CA) containing a mixed culture of denitrifiers exhibited much better performance than the columns inoculated with *Dechloromonas* sp. HZ for reduction of both perchlorate and nitrate. As the flow rate was varied between 2 and 50 mL min^{-1}, corresponding to empty bed contact times of 0.15−3.8 h, a maximum perchlorate elimination capacity of 3.0 ± 0.7 g m^{-3} h^{-1} was obtained in a soil-inoculated column. At an EBRT of 0.3 h and an influent perchlorate concentration of 30 μg L^{-1}, breakthrough (>6 ppb) of perchlorate in the effluent did not occur until the nitrate concentration in the influent was 1500 times (molar) greater than that of perchlorate. The mass of microorganisms attached on the solid ZVI/sand was found to be 3 orders of magnitude greater than that in the pore liquid, indicating that perchlorate was primarily reduced by bacteria attached to ZVI. Overall, the process appears to be a promising alternative for perchlorate remediation.

Introduction

Public health concern has risen in the United States due to the widespread presence of perchlorate in surface water and groundwater. Perchlorate disrupts the production of thyroid hormone by interfering with iodine uptake. Due to its toxicity, the California Department of Health Services has set an action level for perchlorate at 6 ppb (*1*). Contamination has been attributed to perchlorate-laden nitrate fertilizers, releases of ammonium perchlorate, a commonly used oxidizer for solid rocket propellants, fireworks, and automobile air bags, and naturally occurring sources (*2*−*4*). Further, there is speculation that perchlorate contamination may also arise from the use of household bleach, the main component of which is NaOCl. A perchlorate concentration of 8000 μg L^{-1} has been found in a bleach sample stored for 2 years (*5*).

For the treatment of perchlorate-contaminated groundwater, biological remediation is the preferred technology as ClO_4^- is completely transformed into chloride, oxygen, and water, whereas in separation technologies such as ion exchange and reverse osmosis, ClO_4^- is transferred from groundwater to a concentrated brine solution. Perchlorate-reducing microorganisms (PRMs) are ubiquitous in the natural environment, and they can use a variety of electron donors, including organic substrates and inorganics (*6*). Utilization of H_2 to promote autotrophic perchlorate degradation has an advantage in that it minimizes biomass clogging and can be more cost-effective than some organic compounds (*7*). Steady removal of perchlorate by H_2-fed bioreactors has been demonstrated in laboratory studies (*7*−*9*). However, the low solubility of H_2 in water and its hazardous properties during handling and storage may hinder large-scale application.

Zerovalent iron (ZVI) can serve as the ultimate electron donor, supplying H_2 in situ via the iron corrosion process (eq 1). Sanchez et al. (*10*) demonstrated the reduction of

$$Fe^0 + 2H_2O \rightarrow Fe^{2+} + 2OH^- + H_2(g) \qquad (1)$$

perchlorate by combined ZVI−PRMs. Continuous removal of perchlorate at an influent concentration of 1000 ppb was maintained for up to 4000 pore volumes in a column experiment; however, they found that effective treatment required a long contact time and that passivation of ZVI caused a reduction of the treatment efficacy over time.

In our previous study, *Dechloromonas* sp. HZ, an autotrophic H_2-utilizing bacterium was combined with ZVI to degrade perchlorate in successive batch cycles at increasing degradation rates (*11*). The effects of pH, nitrate, cell density, and iron reactivity were also evaluated. Initiation of perchlorate reduction was found to be favored at neutral pH, the reported optimum pH for the growth of sp. HZ (*12*). The presence of nitrate reduced perchlorate degradation rates at concentrations greater than 5 mg L^{-1} as N. An enhancement of the perchlorate removal rate was observed when the initial cell density was increased, whereas the modification of ZVI surface reactivity (to increase the H_2 generation rate) by acid washing or sonication did not improve the perchlorate reduction rate. These suggested that the reduction of perchlorate was limited by the cell density rather than by hydrogen generation or mass transfer.

Successful applications of biological systems to treat perchlorate-contaminated water will require the development of flow-through treatment systems that can handle large flow rates. Therefore, as a follow-up to our previous study, flow-through column experiments of ZVI combined with perchlorate-reducing microorganisms were conducted. The objectives of this study were to evaluate basic operation variables for flow-through systems such as influent composition, flow rate, perchlorate concentration, nitrate concentration, and cell density.

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TABLE 1. Column Operating Conditions[a] and Results (Long Residence Time Experiment)

no. of pore volumes passed	influent[b]	influent pH	influent E_h (mV)	effluent pH	effluent E_h (mV)
1−23	MS	6.2−6.5	23.6−40.5	9.2−9.4	−148 to −133
24−46.5	TW	6.2−6.5	23.6−40.5	9.1−9.4	−148 to −122
47.5−52.5	TW	6.8−7.2	−14.9 to +10.5	9.5−9.7	−156 to −141
53−56	TW	7.5−7.7	−45.3 to −33.4	10.0−10.1	−197 to −186
56.5−60	MS	7.5−7.7	−45.3 to −33.4	10.0−10.1	−197 to −186
61−98	MS	6.5−6.8	5.5−26.4	9.5−9.6	−148 to −141

[a] Flow rate 0.065 mL/min, empty bed residence time 62.8 h, contact time 39 h. [b] MS refers to laboratory-synthesized mineral solution, and TW refers to tap water (Riverside, CA).

Materials and Methods

Bacteria and Solutions. *Dechloromonas* sp. HZ (ATCC BAA-563) was chosen for this study as it was shown to use H_2 as an electron source and CO_2 as a carbon source for perchlorate reduction (*12*). Bacteria were first grown in Luria−Bertani (LB) medium and washed with mineral solution (MS) multiple times prior to inoculation of the bioreactors. Soil obtained from a rapid infiltration tertiary wastewater treatment plant (Colton, CA) was also used in selected experiments as an inoculum. The MS used was modified from Miller and Logan (*12*, *13*) and contained 47 mg L^{-1} K_2HPO_4, 27 mg L^{-1} $NaH_2PO_4{\cdot}H_2O$, 16 mg L^{-1} $NH_4H_2PO_4$, 1000 mg L^{-1} $NaHCO_3$, 3 mg L^{-1} $MgSO_4{\cdot}7H_2O$, 6 mg L^{-1} Na_2EDTA, 1 mg L^{-1} $CaCl_2{\cdot}2H_2O$, 0.2 mg L^{-1} Na_2MoO_4, 0.4 mg L^{-1} $CoCl_2{\cdot}6H_2O$, 0.066 mg L^{-1} Na_2SeO_3, 0.1 mg L^{-1} $NiSO_4{\cdot}6H_2O$, 1.4 mg L^{-1} $ZnSO_4$, 0.2 mg L^{-1} $CuCl_2{\cdot}2H_2O$, 0.85 mg L^{-1} $MnCl_2$ $4H_2O$, and 0.6 mg L^{-1} H_3BO_3.

Experiment Protocol. *Long Residence Time Experiment.* This experiment was set up to simulate a possible in situ treatment such as a reactive barrier. ZVI (570 g, 18/25 mesh, Peerless, Detroit, MI) was packed into a glass column (2.5 cm i.d. × 50 cm), resulting in a bed with a porosity of 60% (±2%). The column was then inoculated with a small amount of biomass (150 mL of an OD_{600} = 0.015 cell suspension, corresponding to approximately 0.6 mg of dry weight, where OD_{600} is the optical density measured at 600 nm). The flow rate was fixed at 0.065 mL min^{-1}, which corresponded to an empty bed residence time of (EBRT) of 63 h and a velocity of 0.19 m d^{-1}, i.e., values similar to those experienced in ZVI permeable reactive barriers used at hazardous waste sites. Mineral solution with a perchlorate concentration of 500 μg L^{-1} ($NaClO_4$, Fisher Scientific) was first used as the influent. The influent was purged with a combination of N_2 and CO_2 gas at a ratio of 90:10 to obtain an initial pH of 6.2 ± 0.05 (the concentration of dissolved oxygen was below 0.2 mg L^{-1}). After 36 d, when a steady state was reached, the influent was switched to Riverside, CA, tap water (TW), which naturally contains nitrate (4.7−5.9 mg L^{-1} as N) (*14*; see the Supporting Information for the detailed composition), and amended with $NaClO_4$. Because the iron corrosion process increases the pH (see eq 1), which affects bacterial activity, the effect of pH was studied by varying the ratio of N_2 and CO_2 gas to steadily increase the influent pH from 6.2 to above 7.5. Each day, 2 mL inlet and outlet samples were collected. The samples were immediately passed through a 0.2 μm filter membrane; the pH/E_h was measured (Orion, 720A), and then the samples were stored at 4 °C for subsequent perchlorate measurement by ion chromatography (IC). The column was operated at room temperature (23 ± 2 °C). Other experimental conditions are summarized in Table 1.

Short Residence Time Experiment. This experiment was set up to mimic ex situ treatment in bioreactors. Four ZVI-filled PVC columns (3.8 cm i.d. × 60 cm), labeled A−D, were run at relatively high flow rates, up to 75 mL/min (approach velocity 95 m d^{-1}) at room temperature. Sampling ports were installed at 10 cm intervals to monitor performance along the flow path. Columns A, B, and D were filled with 50 cm of ZVI (18/25 mesh, Peerless, Detroit, MI) with 10 cm of liquid headspace above the ZVI, i.e., a ZVI bed volume of 570 cm^3. Column C was first filled with 20 cm of soil obtained from a rapid infiltration tertiary wastewater treatment plant (Colton, CA) followed by 40 cm of ZVI. Columns A and B were inoculated with 340 mL of a suspension of *Dechloromonas* sp. HZ at an OD_{600} of 1 and 3 (approximately 90 and 272 mg of dry weight), respectively. The inoculum was recycled; i.e., the effluent was mixed with the influent and pumped through the column at a flow rate of 0.5 mL/min for 1 d prior to the beginning of the experiment. In column C, the local soil was the only source of the inoculum. Column D served as a control to determine whether abiotic reduction of perchlorate by ZVI occurred. Thus, neither bacteria nor soil was introduced in column D.

At the start, all columns were run in an upward continuous flow mode at a rate of 2 mL/min corresponding to an EBRT of 4.8 h (3.8 h in the ZVI section of column C). Column D was operated for only 10 pore volumes merely to confirm that little or no perchlorate degradation occurred, and then the reactor was stopped. Influent MS amended with $NaClO_4$ was maintained at pH 6.5−7.0 (E_h = 10−30 mV) by purging with a 95:5 mixture of N_2/CO_2 at room temperature. Every other day, about 4 mL samples were collected via syringes and filtered through 0.2 μm filters; the pH/E_h was measured (Orion model 720A), and then the samples were stored at 4 °C for subsequent perchlorate measurement by IC.

Three experimental phases were conducted to investigate the effects of the flow rate, influent perchlorate concentration, and influent nitrate concentration. MS amended with $NaClO_4$ was used as the influent. For the flow rate study, influent perchlorate was maintained at 500 μg L^{-1} and the flow rate through each column was increased steadily from 2 to 75 mL min^{-1} (i.e., EBRT ranging from 8 min to 4.8 h in columns A and B and from 6 min to 3.8 h in the ZVI section of column C). For the influent perchlorate concentration study, the flow rates were maintained at 33.5 mL/min and the influent perchlorate concentration was first increased to 1000 μg L^{-1} and then steadily decreased to 20 μg L^{-1}. To determine the effect of nitrate, the flow rates were maintained at 33.5 mL/min and the influent perchlorate concentration was maintained at 30 μg L^{-1}, while the influent nitrate was increased from 0 to 6.3 ± 0.2 mg L^{-1} as N. During the experiment, flow rates were measured daily and adjusted if they deviated by more than 5% of the desired value. Experimental conditions are summarized in Table 2.

Biomass Distribution. At the end of the experiment, columns A−C were disassembled, and near each sampling port, both unfiltered liquid samples (2 mL) and iron samples (about 2 g) were collected for biomass distribution analysis. Liquid samples were immediately stored at −20 °C, while the iron filings were dried under vacuum. For both liquid and iron samples, a modified form of Giblin et al.'s method

TABLE 2. Operating Conditions of Columns A–C[a]

experiment	no. of pore volumes passed	flow rate (mL/min)	run time (h)	influent $[ClO_4^-]$ (μg L^{-1})	influent $[NO_3^-]$ (μg L^{-1})	EBRT (min), columns A and B	EBRT (min), column C
effect of flow rate	1–28	2 ± 0.5	105.7	400 ± 50	0	284	227
	29–97	6 ± 1	86.8	500 ± 50	0	95	76
	98–191	10 ± 1	80.0	500 ± 50	0	57	45
	192–351	20 ± 1	60.4	500 ± 50	0	28	23
	352–703	30 ± 2	88.6	500 ± 50	0	19	15
	704–1423	50 ± 2	108.7	500 ± 50	0	11	9.1
	1424–3207	75 ± 2	180.6	500 ± 50	0	7.4	6.2
effect of perchlorate concentration	3208–4459	33.5 ± 1.5	282.2	800–1000	0	17	13.6
	4460–5398	33.5 ± 1.5	211.6	200–600	0	17	13.6
	5399–5920	33.5 ± 1.5	117.4	20–150	0	17	13.6
effect of nitrate concentration	5921–8633	33.5 ± 1.5	611.4	30 ± 6	0–1500	17	17
	8634–9050	33.5 ± 1.5	94.0	30 ± 6	2000–5000	17	17
	9051–9468	33.5 ± 1.5	94.0	30 ± 6	10000–13000	17	17
	9469–10100	33.5 ± 1.5	142.4	30 ± 6	25000–27000	17	17

[a] The pH in the influent was maintained at 6.9 ± 0.1 by purging with N_2 and CO_2 at a ratio of 95:5. The columns were repacked after 6662 pore volumes of the solutions due to clogging at the entrance; in column C, the soil and ZVI were mixed at that time.

(*9*) was employed to lyse the cells, which were then measured for protein concentration using the BCA protein analysis kit (Pierce, Rockford, IL). Iron corrosion precipitates in the pore spaces at both the entrance and the exit of the columns were collected on 0.45 μm membrane filters, dried under vacuum, and analyzed by X-ray diffractometry.

Definitions and Performance Reporting. System performance was expressed as the perchlorate elimination capacity (EC) as in eq 2, where C_{in} and C_{out} are the influent and effluent perchlorate concentrations, respectively, Q is the influent flow rate, and V is the ZVI bed volume. EC represents the amount of pollutant degraded per unit of reactor bed volume per unit time; it is often reported as a function of the pollutant loading L (eq 3).

$$EC = \frac{(C_{in} - C_{out})Q}{V} \quad (2)$$

$$L = \frac{C_{in}Q}{V} \quad (3)$$

The reactors were considered to have reached a steady state when the concentrations remained unchanged for at least 3–6 pore volumes, although in most cases, steady-state data were collected after a greater number of pore volumes was passed through the reactors.

Analysis. Perchlorate and nitrate were analyzed using ion chromatography (DIONEX 120) with AS16 and AS14 columns, respectively. Detection limits were 4 and 200 μg L^{-1} for perchlorate and nitrate (as N), respectively. Ammonia was analyzed with an autoanalyzer (Alpkem, model RFA 300, Clackamas, OR) with a detection limit of 50 μg L^{-1} as N. The optical density and protein concentration were measured using a Beckman DU 640 spectrometer. The lower detection limit for protein determinations was 2 mg L^{-1}.

Results and Discussion

Long Residence Time Experiment. After an acclimation period of 24 d, during which 15 pore volumes was passed through the column, nearly complete removal of perchlorate was achieved at an influent concentration of 500–700 μg L^{-1}; effluent concentrations were below the detection limit of 4 μg L^{-1} (Figure 1). A slight perturbation occurred when the influent was switched from MS to TW. The effluent perchlorate concentration increased to 20 μg L^{-1} for a short time and was below the detection limit again after 5 pore volumes was passed.

Due to the release of OH^- during the iron corrosion process (reaction I), the pH increased by 2.5–3 units through the column (see Table 1). Complete perchlorate removal was maintained when the influent pH was steadily increased from 6.2 to 7.2. However, breakthrough occurred and the removal efficiency dropped to 60 ± 5% when the influent pH was greater than 7.5 and the effluent pH was greater than 10. After breakthrough was observed the influent feed was changed from TW back to MS, maintaining the same pH, but the perchlorate removal efficiency remained unchanged. Only after 7 pore volumes of MS was passed at an influent pH between 6.5 and 6.8 was complete perchlorate removal resumed.

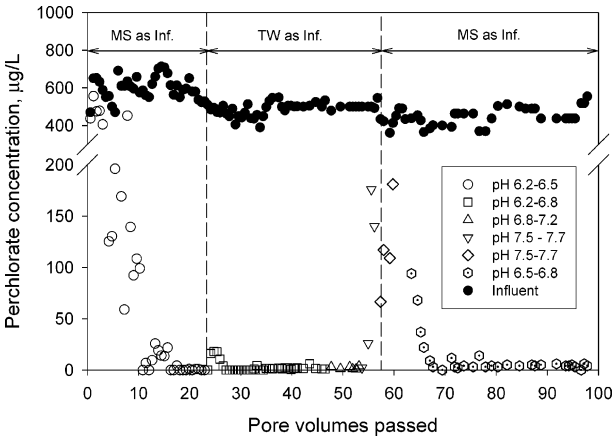

FIGURE 1. Perchlorate reduction performance in the "long residence time" column, EBRT = 63 h. Open data points are effluent values; pH values in the key refer to influent pH.

The municipal water source in Riverside is groundwater containing nitrate, 4.7–5.9 mg L^{-1} as N (*14*). Tap water used in this experiment was used to represent the water quality of common groundwater and the low flow velocity, 17 cm d^{-1}, was used to mimic flow through a typical ZVI permeable reactive barrier. The successful removal of perchlorate from the effluent indicates that the combined ZVI–PRM technology holds potential for in situ remediation of perchlorate-contaminated groundwater with the caveat that the pH must be monitored and possibly controlled. Similar to the results obtained in this research, an increase of the pH to 10.3 along the flow path of a ZVI permeable reactive barrier has been reported in full-scale systems (*15*). The optimum pH for *Dechloromonas* sp. HZ is 6.8 (*12*). In our prior batch experiments conducted in shaken flasks at an initial OD_{600} of 0.015 (*11*), the greatest perchlorate removal rate was observed when the initial pH was neutral and no perchlorate

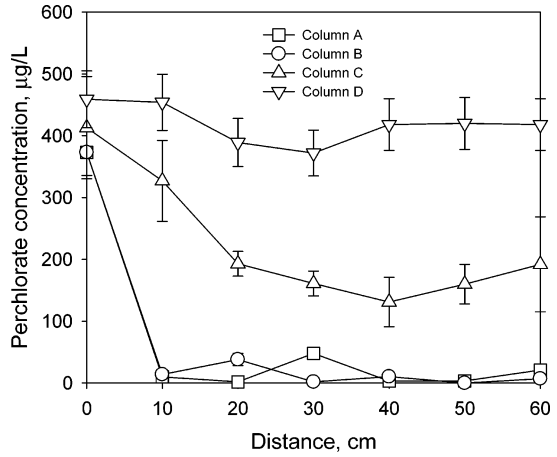

FIGURE 2. Axial concentration of perchlorate during the start-up period. Columns A and B were inoculated with *Dechloromonas* sp. HZ at $OD_{600} = 1$ and 3, respectively, column C was inoculated with 20 cm of native soil obtained from a tertiary wastewater treatment plant, and column D was the ZVI control column without any inoculation (EBRT = 4.7 h (columns A, B, and D), 3.8 h (column C), influent $[ClO_4^-] = 400 \pm 50\ \mu g\ L^{-1}$).

reduction occurred at an initial pH of 9.0. In this study, perchlorate breakthrough occurred when the effluent pH rose to 10, confirming that the process is sensitive to high pH. The most plausible explanation is that perchlorate-reducing microorganisms were inactive at pH greater than 10. This is consistent with our previous flask experiments with the same perchlorate-reducing culture (data not shown) in which hydrogen was sparged. No perchlorate reduction was observed in flasks with pH greater than 9.8. In the reactor experiments, treatment of perchlorate was most likely achieved in the segment of the tubular reactor where pH was lower than about 9. Reduction or cessation of perchlorate biodegradation at elevated pH has also been reported by other researchers (*16*, *17*). Another factor contributing to the decline of the perchlorate degradation rate is that the H_2 generation rate slows at elevated pH (*18*). If ZVI−PRM technology is to be used in a permeable reactive barrier, then pH control may be required for successful in situ bioremediation of perchlorate. The barrier should be designed and operated in such a way to ensure that pH increases within the treatment zone are minimized such as use of a buffer or increasing the flow rate through the reactive zone. In the subsequent set of experiments, the pH increase was minimized (≤1.5 units) when the empty bed residence time was reduced to less than 2 h.

Short Residence Time Experiment. *Start-Up.* The perchlorate degradation profiles along the flow paths during the first 3 pore volumes are shown in Figure 2. At an influent perchlorate concentration of 400 μg L^{-1}, significant perchlorate reduction (≥90%) occurred within the first 10 cm in columns A and B, which were inoculated with *Dechloromonas* sp. HZ. Thus, little or no acclimation was needed to initiate perchlorate reduction in systems that were adequately inoculated. No difference was observed between columns A and B, although column B was inoculated with a 3-fold greater amount of microorganisms, indicating that, at the conditions of the experiment, the system was not limited by biomass density. In contrast, in column C, which was filled with 20 cm of soil followed by 40 cm of ZVI, perchlorate removal in the ZVI zone was initially minimal. Perchlorate reduction transiently took place in the soil layer, most likely due to the ubiquitous presence of perchlorate-reducing microorganisms (*19*) and residual organic matter (0.5% by mass) within the collected soil matrix. Where neither *Dechloromonas* sp. nor soil was introduced, removal of perchlorate was negligible (column D). This result is consistent with the very slow kinetics between ZVI and perchlorate reported by others (*20*, *21*).

Compared to the long residence time experiment in which a 15 pore volume acclimation period was needed before nearly complete perchlorate removal was achieved, only 3 pore volumes of solution was required in columns A and B. This shortening of the acclimation period was most probably due to the greater cell density added to the columns. This result is also consistent with the findings from our previous batch experiments (*11*). In column C, 7 pore volumes of influent was required before complete (>99%) perchlorate removal was observed.

Within columns A, B, and D, the pH jumped quickly from below 6.5 to about 9 in the first 20 cm and then steadily increased to about 9.5 by the end of the column. In column C, the pH increased slightly, 0.2 unit, in the first 20 cm of the soil layer and rose to above pH 9 within the ZVI layer. As expected, E_h in all columns decreased from 30 ± 2 mV at the inlet to -140 ± 10 mV at the outlet (Table 2).

Effects of Flow Rate. Flow rate was increased in steps from 6 to 75 mL min^{-1}, corresponding to EBRTs ranging from 95 to 7 min. Steady-state perchlorate profiles were obtained and are shown in Figure 3. The profile for column C includes the 20 cm of soil at the beginning of the column. In all columns, reaction fronts migrated toward the end of the column as the flow rates increased.

At an influent perchlorate concentration of 500 μg L^{-1}, nearly complete degradation was achieved in all columns at EBRTs longer than about 10 min. Breakthrough was observed at shorter EBRTs. Thus, complete perchlorate removal was achieved at liquid contact times similar to those required for ion exchange treatment systems. Hence, the potential size of a full-scale ZVI−PRM perchlorate treatment unit would be similar to that of an ion exchange system.

In our previous batch study in shaken flasks (*11*), an increase in the OD_{600} from 0.025 to 0.08 led to a 4-fold increase of the perchlorate reduction rate. In this experiment, although the initial loading of biomass in column B was 3 times greater than in column A, the overall performance of these two columns was comparable, suggesting that, in this experiment, the initial cell density was not a rate-limiting factor in the degradation of perchlorate in both columns.

Excluding the data for the shortest EBRT (7.6 min), the maximum perchlorate ECs in columns A and B were found to be 2.7 ± 0.8 g m^{-3} h^{-1} (Figure 4), comparable to that determined by Sanchez et al. (*10*), where a maximum reduction rate of 2.3 g m^{-3} h^{-1} was observed. The elimination capacities are also within the range (1−14 g m^{-3} h^{-1}) of the published reports on perchlorate removal rates in flow-through systems using H_2 as the electron donor (*7*−*9*). Thus, ZVI can adequately supply the H_2 needed by the perchlorate-reducing microorganisms. However, calculations based on the rate of corrosion of ZVI in abiotic systems (~1.85 $mmol_{H_2}$ released kg_{ZVI}^{-1} d^{-1} at pH 6−9, X. Yu, unpublished results) indicate that the maximum rate of perchlorate reduction would be about 1.6 g m^{-3} h^{-1}. Thus, the fact that bacteria attached to the ZVI and consumed hydrogen appears to have increased the rate of iron corrosion.

Although soil provided the only inoculum for column C, continuous perchlorate degradation was observed in the ZVI zone in column C. This result suggests that the soil initially contained bacteria capable of perchlorate reduction and that these were able to transport from the soil and colonize the ZVI, utilizing H_2 from the iron corrosion process to reduce perchlorate. Excluding the data from the shortest EBRT condition, the perchlorate removal rate in column C was found to be 3.0 ± 0.7 g m^{-3} h^{-1}, i.e., slightly greater than that of columns A and B.

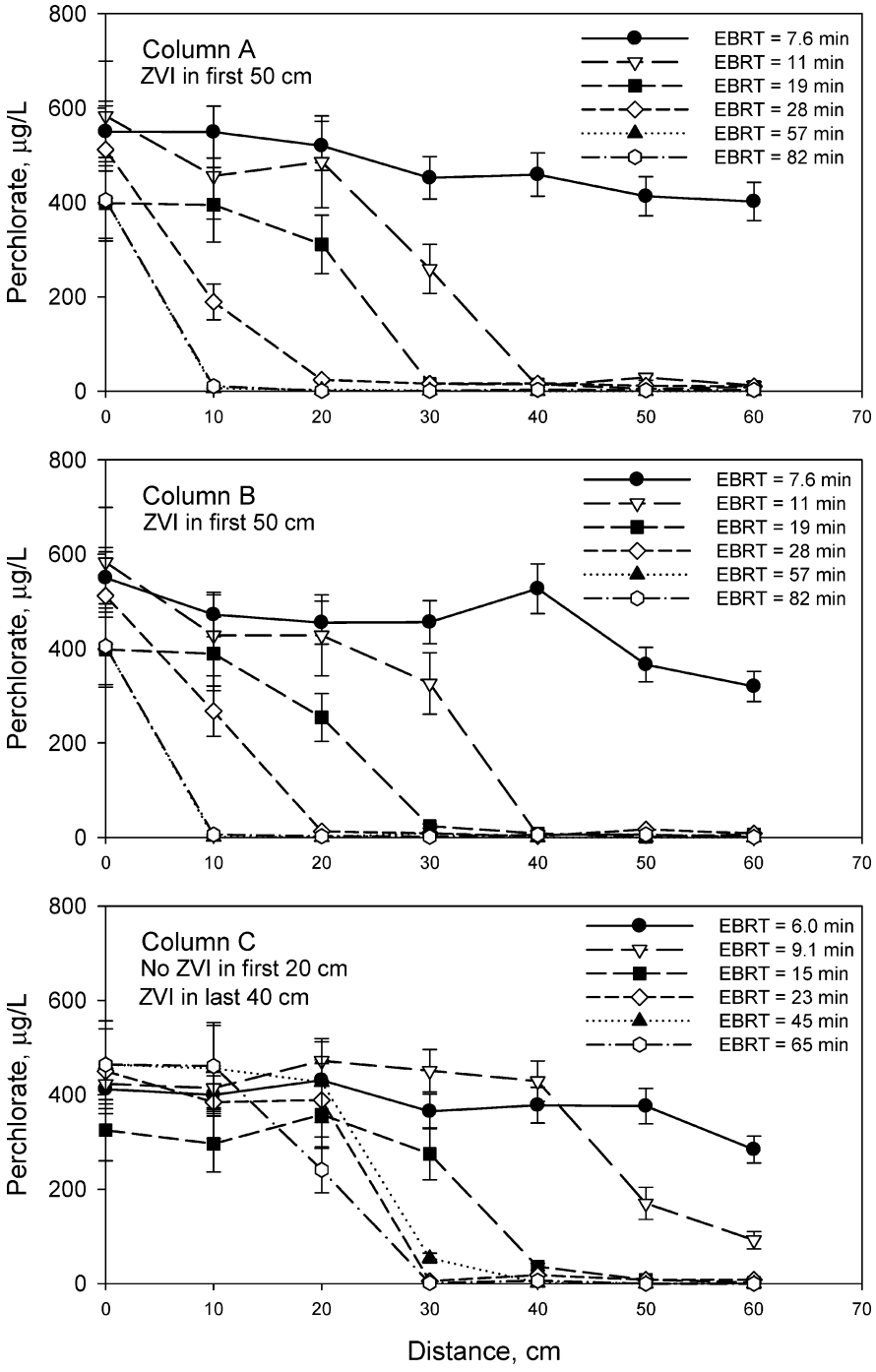

FIGURE 3. Effect of flow rate on axial perchlorate concentrations (influent [ClO_4^-] = 400−600 μg L^{-1}).

During experimentation, it was evident that the perchlorate removal performance was deteriorating at the highest influent flow rates. After 690 pore volumes of solution was passed at an EBRT of 6 or 7 min, the perchlorate removal rates decreased to 0.6, 1.2, and 1.8 g m^{-3} h^{-1} in columns A, B, and C, respectively. After another 1000 pore volumes of solution was passed, greater than 90% breakthrough was observed in the effluent of columns A and B, while breakthrough in column C also occurred, but to a lesser extent. This detrimental effect caused the low ECs at the highest loadings shown in Figure 4. One possible reason for this decrease of EC was probably preferential flows in the columns, the impact of which was more pronounced at high flow rates. Small channels with apparent water flowing through them were observed in the vicinity of many sampling ports, indicating the existence of preferential flow in the columns.

Effects of Perchlorate Concentration. Flow rates were fixed at 33.5 mL min^{-1} (EBRT = 17 min in columns A and B and 15 min in column C), and influent perchlorate concentrations were decreased stepwise from about 900 to 30 μg L^{-1}. Each influent concentration change was maintained for a minimum of 400 pore volumes. Recall that this flow rate is near the highest level in which nearly complete removal was achieved with an influent concentration of 500 μg L^{-1} (see Figure 4). The elimination capacity and removal efficiency are shown in Figure 5.

As the influent perchlorate concentration was decreased, the removal efficiency in all columns improved, with nearly complete removal in column C (≥90%). When the influent

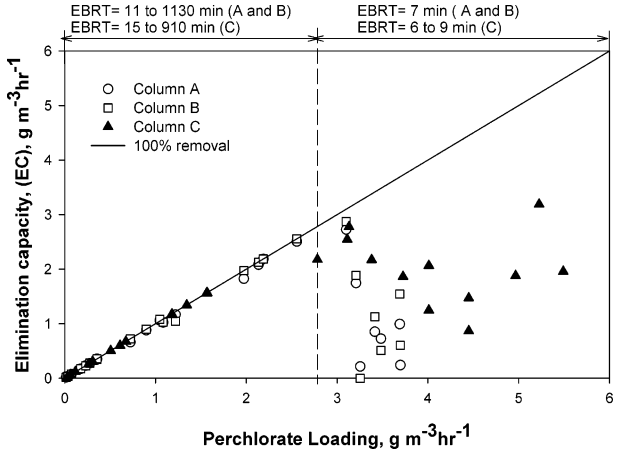

FIGURE 4. Perchlorate elimination capacity as a function of perchlorate loading (flow rate 6−75 mL min^{-1}, influent [ClO_4^-] = 400−600 μg L^{-1}.

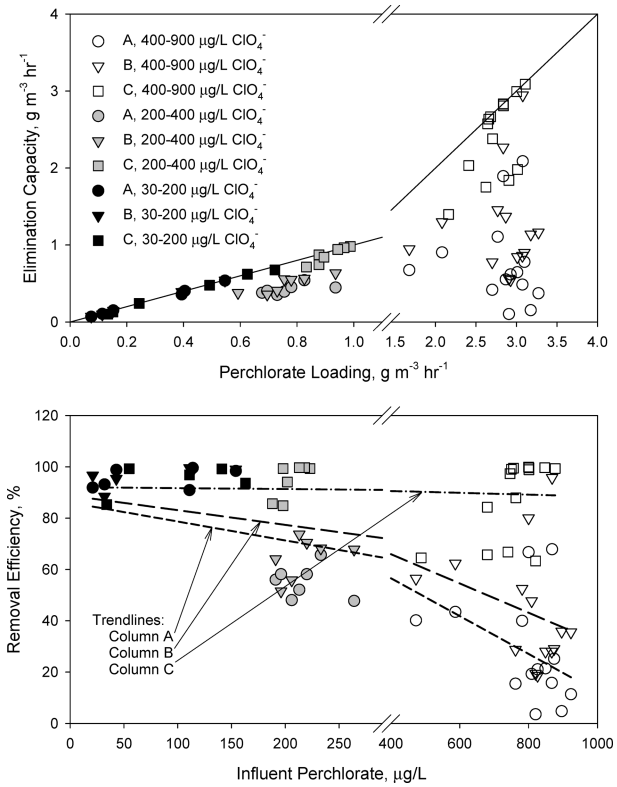

FIGURE 5. Perchlorate elimination capacity (top) and removal (bottom) as a function of the influent perchlorate concentration (flow rate 33.5 mL min^{-1}). The solid line in the upper chart shows 100% removal.

concentration was less than about 150 μg L^{-1}, nearly complete removal was experienced in all three test columns. While performance dropped in both columns A and B above 150 μg L^{-1} perchlorate, the removal efficiency remained relatively steady with nearly complete removal in column C; at concentrations above 400 μg L^{-1}, however, perturbations in the column C performance were observed.

Lower removal efficiencies at the highest influent perchlorate concentrations were probably due to hydrogen limitation. When the influent perchlorate concentration ranged between 400 and 900 μg L^{-1}, the elimination capacities of columns A and B were only 0.5−1.4 g m^{-3} h^{-1}, i.e., lower than the 2.7 g m^{-3} h^{-1} obtained in the previous experiment, but very similar to the elimination capacity corresponding to the maximum abiotic iron corrosion rate. It is possible that iron passivation limited hydrogen production. Sanchez et al. (*10*) observed that the decreasing perchlorate removal efficiency was concurrent with a decrease of the H_2 generation rate resulting from passivation of the iron surface by iron precipitates over time. When external H_2 gas was supplied in their experiments, perchlorate treatment immediately improved. Addition of gaseous hydrogen to test this hypothesis was not attempted here.

In this experiment, iron near the inlet ends of both columns A and B was covered by vivianite (XRD data in the Supporting Information), a type of iron(II) phosphate ($Fe_2(PO_4)_3{\cdot}8H_2O$). Meanwhile, little perchlorate removal was observed in the inlet segment of columns A and B, suggesting that vivianite passivates the ZVI surface. It also has been reported that iron minerals can precipitate on the bacterial surface and inhibit the growth of bacteria (*17*, *22*). Further research is needed to confirm and clarify the mechanism as well as understand the possible role of microorganisms that can oxidize iron(II). Along the flow path, magnetite was observed to become the dominant iron precipitates (XRD data in the Supporting Information).

In column C, vivianite was also observed in the iron layer close to the interface between iron and soil. The amount of vivianite generated was much lower in column C compared to the other two columns. Sorption of phosphate within the soil (the phosphate adsorption capacity ranged between 12 and 70 mg kg^{-1} as P at phosphate concentrations of 10−50 mg L^{-1} as P) preceding the iron zone reduced the amount of available phosphate for precipitation formation and the resultant passivation within the iron zone. The extent of perchlorate reduction in column C was significantly greater than that of the other two columns, especially at the higher concentration range of 800−900 μg L^{-1}.

Effects of Nitrate Concentration. High concentrations of nitrate have been shown to repress (per)chlorate reductase production (*23*). In addition, competition between nitrate and perchlorate for the available supply of H_2 has been reported (*11*). To ascertain whether nitrate reduction occurred abiotically, a ZVI batch experiment was conducted without biomass addition. A first-order reaction rate constant of 0.11 h^{-1} for abiotic nitrate reduction was obtained with the Peerless iron used in this research, which is comparable to published results (*24*). On the basis of this value and the short contact time in the ZVI column in these experiments, the effect of abiotic nitrate reduction was considered to be negligible. This was confirmed by the absence of an increase of ammonia, the major end product of abiotic nitrate reduction, in any of the columns.

Influent nitrate concentrations were increased in columns A−C in stages from 0 to 6 mg L^{-1} NO_3 as N, while the influent perchlorate concentration was maintained at 30 μg L^{-1} at a flow rate of 33.5 mL/min. The perchlorate removal efficiency as a function of the influent nitrate concentration is shown in Figure 6. Examination of Figure 6 reveals that a high degree of perchlorate removal was maintained at low concentrations of nitrate in all columns. However, breakthrough ([ClO_4^-] > 6 μg L^{-1}) occurred in columns A, B, and C when the influent nitrate concentrations reached 0.35, 0.84, and 6.3 mg L^{-1} as N, respectively. Corresponding molar ratios of nitrate to perchlorate were 84:1, 200:1, and 1500:1. The decrease of the perchlorate removal efficiency with an increase of the nitrate concentration is consistent with other studies, including those on H_2-fed bioreactors (*8*, *25*). Although the perchlorate reduction rate decreased, it did not stop completely, however. At a nitrate concentration of 5.9 mg L^{-1} as N, perchlorate removals for columns A, B, and C were 8%, 20%, and 50%, respectively. When high concentrations of nitrate are present (e.g., greater than 5.9 mg L^{-1} as N), longer retention times will be needed to achieve high levels of perchlorate removal. As presented earlier (see Figure 1) in the column experiment

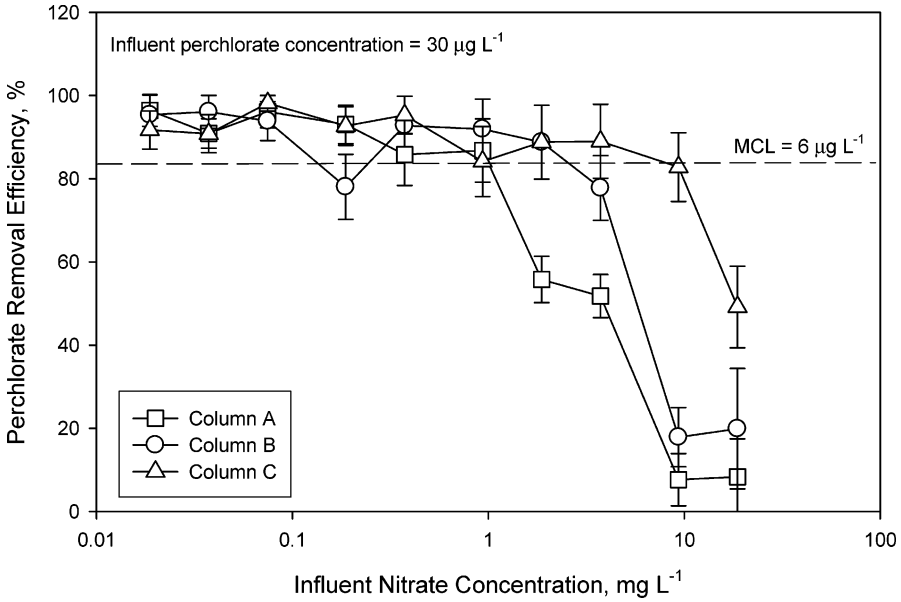

FIGURE 6. Effect of nitrate on perchlorate removal (flow rate 33.5 mL min^{-1}, influent [ClO_4^-] = 30 μg L^{-1}).

simulating in situ perchlorate treatment, complete perchlorate removal (at an influent perchlorate concentration of 500 μg L^{-1}) to a nondetectable level was maintained when tap water containing nitrate in the concentration range of 4.7−5.9 mg L^{-1} as N was used as the influent.

Biomass Distribution At the end of the experiments, the columns were disassembled and the biomass concentrations in the pore liquid and associated with the iron particles were analyzed. A figure with the results is shown in the Supporting Information (Figure B). Bacteria were evenly distributed axially within the solid phase, and all bioreactors had protein concentrations between 1 and 1.5 mg g^{-1} of solid. In the liquid phase, the concentrations of cells were in the range of 4−10 mg of protein L^{-1} in all columns. The porosity of the packed beds was about 0.62, and the solid to liquid ratio was around 1100 g:500 mL. On the basis of a cell protein content of 35% by weight, the amount of biomass associated with the solid phase ranged from 3.01 to 4.56 g of dry weight (g_{dw}), while within the liquid phase cell mass was only 0.006−0.013 g_{dw}. Thus, the mass of cells associated with the ZVI was almost 3 orders of magnitude greater than in the liquid phase. This suggests that perchlorate reduction in the bioreactors was essentially mediated by attached cells.

Before the start of the column experiments, 3 times more biomass of *Dechloromonas* sp. HZ was introduced into column B than into column A. However, by the end of the experiments (6 months of operation processing more than 10000 pore volumes of solution), the biomass contents in the two columns were similar, suggesting that growth and detachment of the bacteria reached a steady state and that the bacterial capacity of the ZVI is around 3−5 mg_{dw} g^{-1}.

In this research, results of long-term column experiments demonstrating the feasibility of using perchlorate-reducing bacteria combined with ZVI for perchlorate removal are reported. Successful perchlorate reduction was achieved in laboratory-scale columns simulating both long residence time and short residence time perchlorate remediation conditions, suggesting that this technology holds potential for both in situ and ex situ large-scale application. System performance was affected by several factors including the source of perchlorate-reducing microorganisms, pH, and nitrate concentration. The best performance of perchlorate reduction was observed in the system inoculated with soil containing a native mixed culture of denitrifiers. A maximum perchlorate reduction rate of about 3 g m^{-3} h^{-1} was observed. High pH, greater than 10, is toxic to most bacteria but is commonly found in ZVI-filled permeable reactive barriers (*26*). Therefore, pH control may be required to ensure its success of such perchlorate treatment systems with long residence time applications. Nitrate tends to compete with perchlorate for reduction by perchlorate-reducing microorganisms, so that a longer retention time in the reactive zone is necessary to overcome the competing effect of nitrate when its concentration is significantly greater (e.g., 1000 times) than that of perchlorate.

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Supporting Information Available

A table showing the composition of Riverside tap water, a figure that shows the XRD data of iron precipitates, and a figure detailing biomass distribution. This material is available free of charge via the Internet at http://pubs.acs.org.

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SUPPORTING INFORMATION

Composition of Riverside, California tap water (TW):

Ion	Concentration (mg/L)
Chloride	20-25
Sulfate	51-78
Nitrate	15-26
Floride	0.5-0.8
Sodium	28-41
Calcium	56-84
Potassium	1-4
Magnesium	5-14
Hardness ($CaCO_3$)	170-240

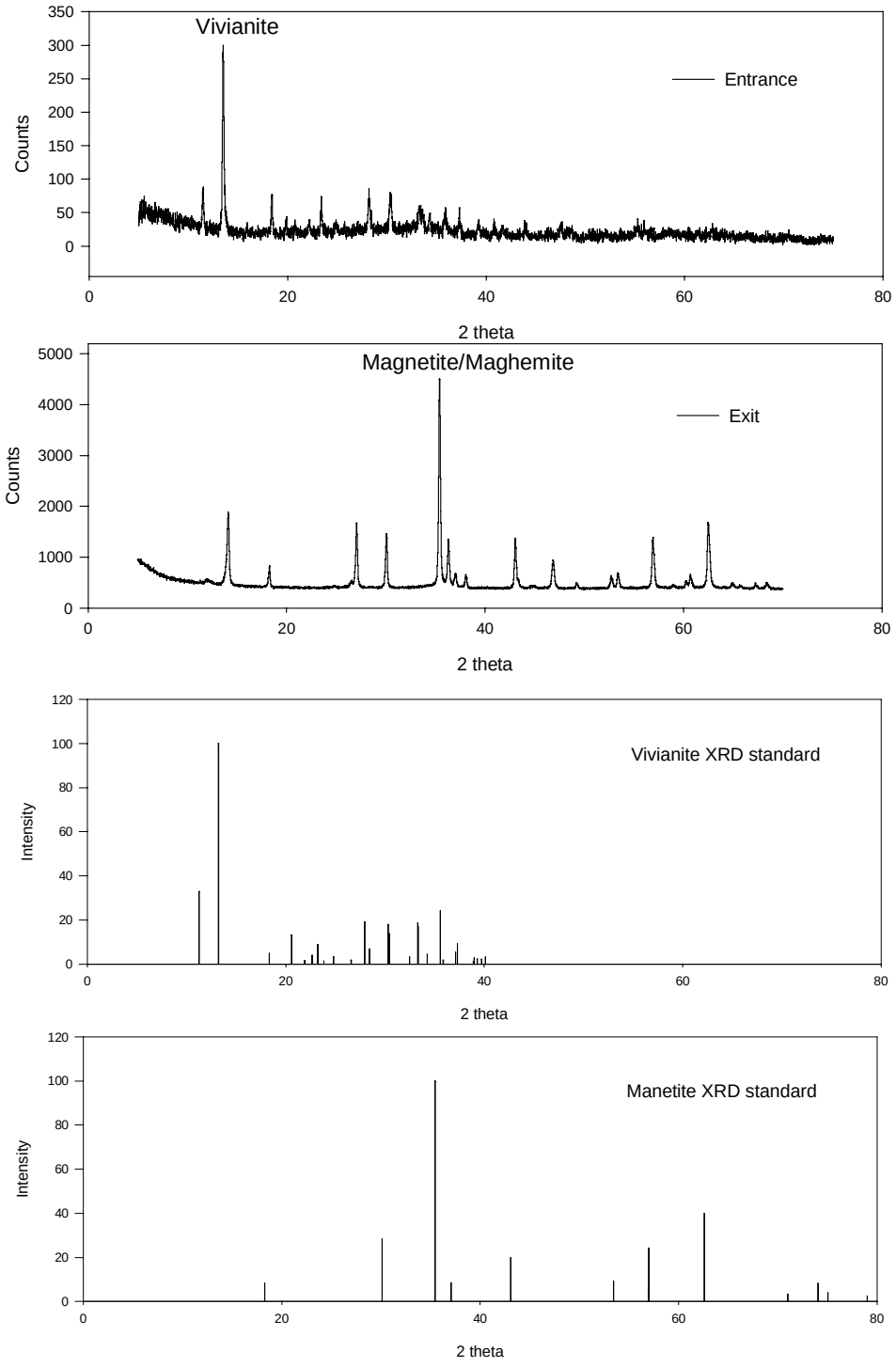

Figure A. XRD data of iron precipitates at the entrance and the exit of column B.

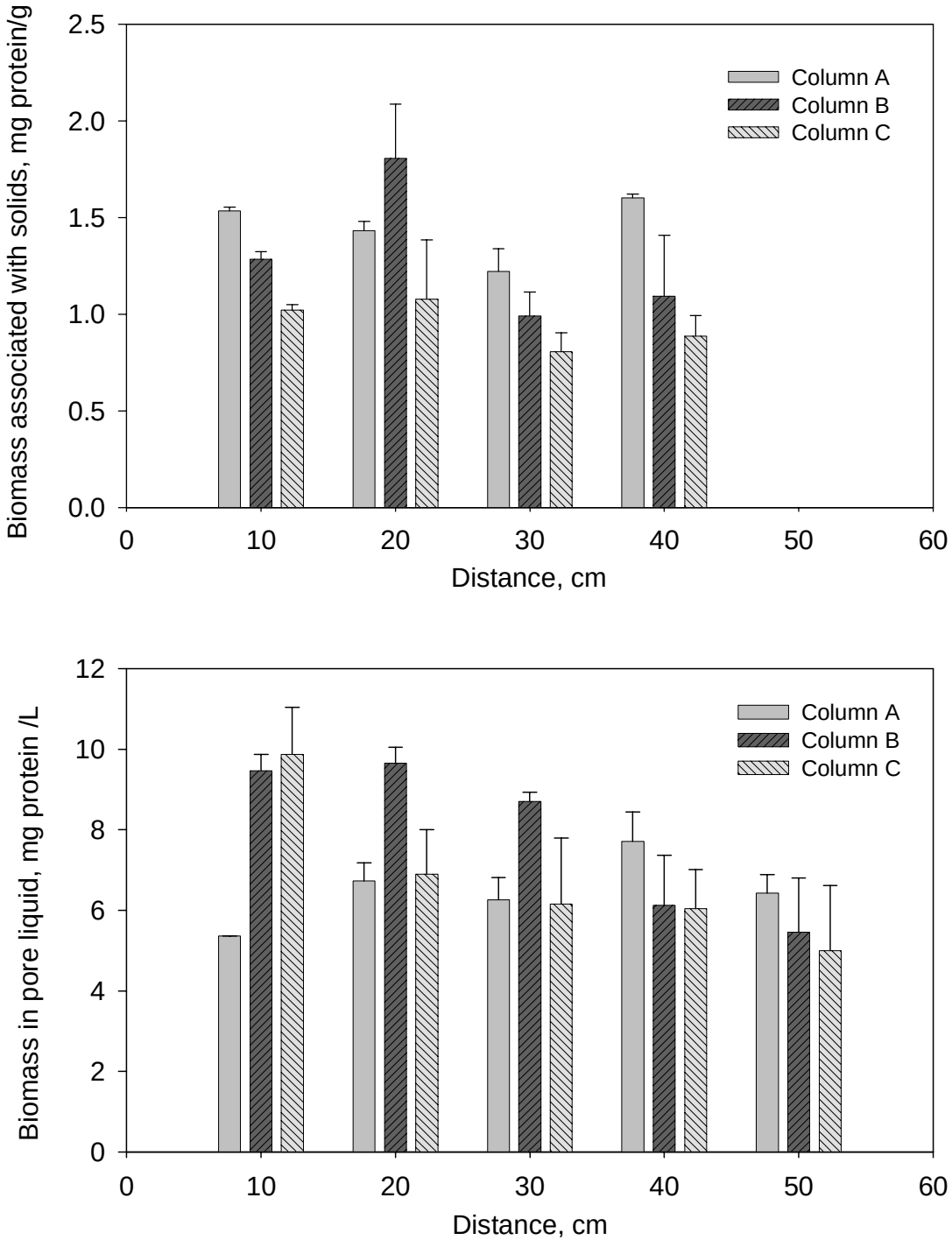

Figure B. Biomass distribution attached on ZVI (top) and suspended in the liquid (bottom) along the columns.