Removal of As from groundwater by \textit{in situ} bioprecipitation and zero-valent iron

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ABSTRACT

The aim of this study was to evaluate the \textit{in situ} bioprecipitation (ISBP) and zero-valent iron (ZVI) for removal of arsenic from groundwater. Batch experiments were set up to test the metal/oxyanion removal efficiency using the processes induced by ZVI, by ISBP and by combining both technologies. For the construction of microcosms in anaerobic condition, aquifer material was mixed with synthetic groundwater in serum bottles. Solutions that contained commercial iron (powder, filings), iron from foundry (chips) and lactate with the carbon concentration of 0.02\% (w/v) were amended. After 57 days the pH increased to 7.9 in the microcosm with lactate + iron chips. In the microcosms amended with lactate + commercial iron (powder and filings), pH was between 6.9 and 7.5. The highest decrease in sulfate concentrations was observed in the microcosm supplied with lactate (from 1,976 to 630 mg/L), and with lactate + iron filings (from 1,985 to 1,280 mg/L). The results showed that ISBP and ZVI can be efficient in the removal of As from groundwater; however, it strongly depends on the type and particle size of ZVI. The most effective was commercial ZVI. The highest decrease in As concentration was observed in the microcosm supplied with ethanol and iron powder (from 4,832 to 131 $\mu$g/L).

Key words | groundwater, heavy metals, \textit{in situ} bioprecipitation, sulfate reduction, zero-valent iron (ZVI)

INTRODUCTION

Heavy metal contamination of soil and groundwater due to anthropogenic sources, such as non-ferrous industrial sectors, is a worldwide problem. In general, more than 60\% of contaminated sites in the world have problems associated with the presence of toxic metals, such as Cd, Pb, Cu, Zn, Hg, Co and Ni (Diels et al. 2005). The leaching of metals by infiltrating rainwater from the polluted soil eventually contaminates the groundwater (Diels & Vanbroekhoven 2008; Satyawali et al. 2010). Heavy metal contaminated groundwater is typically treated by ‘pump-and-treat’, but this remediation approach can be costly, last for years and still not meet the required standards in the end (Diels et al. 2005, 2006). A sustainable and economical technology that has been developed for the removal of metals from the groundwater is \textit{in situ} bioprecipitation (ISBP) (Diels et al. 2005, 2006; Szewczyk et al. 2008; Vanbroekhoven et al. 2008).

ISBP consists of stimulating sulfate-reducing bacteria (SRB) that reduce sulfate into sulfides with subsequent precipitation of metals to form insoluble metalsulfide precipitates according to the reaction (Diels et al. 2005; Szewczyk et al. 2008):

\[
\begin{align*}
\text{CH}_3\text{COOH} + \text{SO}_4^{2-} &= 2\text{HCO}_3^- + \text{HS}^- + \text{H}^+ \\
\text{H}_2\text{S} + \text{Me}^{2+} &= \text{MeS} + 2\text{H}^+
\end{align*}
\]

Parameters such as pH, redox potential, carbon source and sulfate concentration seem to play a very important role in the induction of the sulfate reduction process. On the other hand, the selection of the electron donor seemed to be important for keeping the process going. Presumably combinations of electron donors must be selected in order to induce and to keep the process going (Diels et al. 2006; Satyawali et al. 2010; Vanbroekhoven et al. 2007). Stimulation of SRB is often realized by injection of substrates like lactate, acetate, molasses, etc. that provide the e-donors for reduction of sulfate and result in the reduced conditions necessary for the sulfate reducing activity (redox potential (Eh) \sim 200 mV) (Jong & Parry 2005; Diels et al. 2006).
The biological process of sulfate reduction has the additional advantage of decreasing the sulfate concentration and neutralizing the groundwater’s acidity by means of the bacterially produced bicarbonate (HCO$_3^-$). The ISBP can be achieved by injecting agents and/or nutrients in a series of wells, so that a reactive zone is created in the flow path of the groundwater, where metals are intercepted and immobilized (Vanbroekhoven et al. 2008). The technology is proven for heavy metals like Zn and Cd but oxyanions like arsenate As(V), often present as co-contaminants at these metal contaminated sites (also geogenically), do get reduced as well into mobile and the toxic arsenite As(III) and represent a high risk for nearby receptors like drinking water wells (Kumar et al. 2009). The feasibility of inducing As(III) precipitation through bacterial reduction of sulfate was evaluated by Rittle et al. (1995) in laboratory microcosms established under strictly anaerobic conditions. As(III), Fe(II), and sulfate concentrations were routinely monitored in the aqueous phase as sulfate was reduced to sulfide. Both As(III) and Fe(II) concentrations in the sediment microcosms decreased as sulfide was made available.

Zero-valent iron (ZVI) is considered as a potential remediation agent for the elimination of numerous heavy metals from contaminated groundwater. Extensive laboratory studies have demonstrated that nanoscale iron particles are effective for the treatment of a wide array of common groundwater contaminants such as various inorganic compounds. Several field tests have also demonstrated the promising prospective for in situ remediation (Zhang & Elliott 2006).

The ZVI technology is very effective for the removal of heavy metals including oxyanions with different chemical characteristics due to multiple ZVI-metal interactions such as surface complexation, reduction, (co)precipitation and cementation. The basis for these reactions is the anaerobic corrosion of ZVI. The first corrosion product is amorphous ferrous hydroxide, which is predicted thermodynamically to convert to magnetite (Fe$_3$O$_4$). Mixed-valent iron salts known as green rusts may also form. Their subsequent oxidation can lead to the formation of magnetite, goethite and lepidocrocite. As result of these reactions, the ZVI surface is coated by a layer of iron oxides and oxyhydroxides, similar to natural oxide solid phases (Kumar et al. 2009, 2000a, b; Rajan 2011). In the presence of water, the surfaces of iron oxides are generally covered with surface hydroxyl groups (Fernandez-Sanchez et al. 2004). As Fe$^0$ dissolves, Fe$^{2+}$ is released and protons are reduced to elemental H$_2$:  

$$Fe^0 + 2H^+ = H_2 + Fe^{2+}$$  \hspace{1cm} (3)  

Electrons released by Fe$^0$ dissolution cause the oxidation state to decrease and a subsequent formation of hydrogen. The reduced conditions are beneficial for the activity of SRB. The formed H$_2$ is a potential e-donor for SRB, and might therefore become interesting as a stimulation for microbial activity. Reduction of sulfate by ZVI has been reported for the following SRB: Desulfovibrio desulfuricans, Desulfovibrio vulgaris, Desulfovibrio multispirans, Desulfovibrio salexigens, and Desulfobacterium sp. strain IS4. Furthermore, the reduction of protons into hydrogen results in increased alkalinity, which obviously prevents metals from remaining in solution. ZVI is utilized as a slow-release electron donor for sulfate reduction in anaerobic sludge and, therefore, has a promising potential in bioremediation applications (Karri et al. 2005). There is little knowledge available as to how the quantity of Fe$^0$ added, as well as the corresponding available surface area and contact time, may affect desirable biogeochemical interactions and process performance (Johnson et al. 1996; Fernandez-Sanchez et al. 2004; Kumar et al. 2000a, b). Three different particle sizes of ZVI (granular, micro and nano size) were used for heavy metal removal in a low pH sediment and groundwater via stimulation of sulfate reduction and precipitation of metal sulfides (Kumar et al. 2000a, b). Their laboratory experiments strongly supported that ZVI can be a very effective tool for inorganic contaminant removal and speeding up microbial-mediated redox processes. Granular and micro iron showed abiotic metal removal as well as stimulation of sulfate reduction in parallel. With nano iron, sulfate reduction could not be induced despite a higher amount of H$_2$ production, though it is highly reactive and fast in a biotic metal precipitation.

The aim of this study was to evaluate ISBP and ZVI for removal of As from groundwater.

**Materials and Methods**

Aquifer soil samples were taken from a heavy metals contaminated saturated zone located at an industrial site in Poland (Piekary Slaskie). The aquifer material used in this study was sampled between 5 and 6 m below ground surface. The samples were collected with hand-operated equipment for soil research (Eijkelkamp Agrisearch Equipment, The Netherlands). The aquifer was characterized by pH = 7.7 and total organic carbon = 0.3% of C. The concentration of Zn, Ni, Cu, Cd, Pb, As, Fe and sulfate was: 280, 6, 12, 120, 55, 32, 6,400 and 36 mg/kg$_{dm}$, respectively. A synthetic
groundwater (0.5 mM CaCl₂·2H₂O, 0.5 mM MgCl₂·6H₂O) containing Zn, Cd and As was used in all tests. No carbonates were used in the synthetic groundwater because of their high precipitation potential for metals. Two types of metal iron were used in this study. Pure ZVI powder (Fe > 99%) and pure ZVI filings (Fe > 99.9%, <0.25 mm) were purchased from Sigma-Aldrich. Iron chips were obtained as industrial waste and screened to less than 3 mm. The iron content in chips was larger than 75%.

The pH and Eh was measured with a Hamilton glass combination electrode and Cyberscan Eutech pH meter. The concentration of sulfate was quantified using a DR/4000 Hach spectrophotometer and SulfaVer® Sulfate reagent. Groundwater samples for metal analyses were filtered over a 0.45-mm filter, acidified by pure HNO₃ (2% (v/v)), and analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

**Experimental set-up**

For the construction of microcosms in an anaerobic condition, 40 g of aquifer material was mixed with 100 mL of synthetic groundwater in 250 mL serum bottles. Solutions that contained commercial iron (1 g of powder, 1 g of filings), iron from foundry (1 g of chips) and lactate with the carbon concentration of 0.02% (w/v) were amended. Two control experiments were set up: an abiotic control containing 0.0175% (w/v) of formaldehyde and a natural attenuation control, which was not amended with an extra C-source. At the start of the experiment the groundwater was contaminated with 5 mg Zn/L (in form of ZnCl₂), 5 mg Cd/L (in form of CdCl₂), 5 mg As/L (in form of Na₂HAsO₄) and 2,000 mg SO₄²⁻/L (as K₂SO₄). Serum bottles were sealed using gas-tight butyl rubber stoppers, incubated statically at room temperature and under a N₂ atmosphere.

Samples were visually examined for blackening, indicative for sulfide precipitation, every 2 weeks. pH was measured every week while the redox potential, sulfate content and metal concentrations (Zn, Cd, As) were analyzed every 2 weeks. Sulfate analysis indicated the immobilization of heavy metals as sulfides.

**RESULTS AND DISCUSSION**

**pH and the redox potential**

The efficiency of As removal by bioprecipitation of arsenic sulfide depends mainly on the physical–chemical parameters as pH governs the formation of soluble thioarsenic species (Battaglia-Brunet et al. 2012). SRB can be induced at a pH between 4 and 8. At neutral pH, SRBs from different origins can be detected (Diels et al. 2005, 2006). Teclu et al. (2008) used a mixed culture of sulfate-reducing bacteria to study the bioremoval of arsenic species (As(III) or As(V)) from groundwater. They observed a removal of about 77 and 55% of As(V) and As(III), respectively for pH = 6.9.

As shown in Figure 1 the pH dropped in conditions with C-source after the addition of lactate.

After 57 days the pH increased to 7.9 in microcosms with lactate + iron chips. In the microcosms amended with ethanol and commercial iron (powder and filings), the pH was between 7.4 and 7.5 at the end of the experiment. In the microcosms amended only with lactate, pH = 7.1 was observed. The pH dependence of arsenite removal by ZVI has been observed by Yu et al. (2006) who reported greater removal of arsenite at pH 7 and 8 compared to removal at pH 6.3 and 9.

Signes-Pastor et al. (2007) noticed that under moderately reduced conditions (+50 mV, −150 mV), As solubility seemed to be controlled by the dissolution of iron oxy-hydroxides; however, at −200 mV, As chemistry was dominated by the formation of insoluble sulfides. SRB require a redox potential below −200 mV (Diels et al. 2006). In all samples amended with lactate and iron the redox potential was between −348 and −367 mV, i.e. lower as compared to an abiotic control containing formaldehyde and a natural attenuation control, which was not amended with an extra C-source (Figure 2). A simultaneous decrease in Eh was observed in a
natural attenuation control amended with lactate. Wilopo et al. (2013) used ZVI and two kinds of organic materials, compost and wood chips, as carbon sources for removal of As from groundwater in a column test. They observed that initially pH increased from 6.25 in the influent to around 7 in the effluent, then decreased after pv 33 (pore volume (pv) was defined as the required time for the influent to travel from the bottom to the top of the column). Dissolution of the ZVI caused a pH increase in the effluent. Eh decreased throughout the experiment from 420 mV in the influent to −100 mV in the effluent, indicating that reduction occurred.

**Sulfate concentration**

After 57 days, the sulfate concentration decreased in the microcosms with lactate and lactate + iron filings (Figure 3).

The highest decrease in sulfate concentrations was observed in the microcosm supplied with lactate (from 1,976 to 630 mg/L). In the microcosm amended with lactate + iron filings the sulfate concentration dropped from 1,985 to 1,280 mg/L. The lowest decrease in sulfate concentration was observed in the case of lactate + iron powder and lactate + iron chips. No sulfate removal was noted in the abiotic control and in the microcosm which was not amended with any additional C-source.

Karri et al. (2005) tested ZVI as an electron-donating substrate for sulfate in the anaerobic sludge. ZVI of 325 mesh was supplied at 46.6 g/L to the anaerobic culture containing 1.5 g of volatile suspended solids per litre of sludge and incubated for approximately 15.5 weeks. They observed that some sulfate was eliminated slowly from the two controls; however, the loss in sulfate concentration was distinctly greater and more rapid in the complete treatment. The results clearly indicate that ZVI was utilized by SRB. At the end of the experiment, the complete treatment removed all of the sulfate supplied to the medium. Based on the net removal of sulfate (corrected for endogenous sulfate reduction), the ZVI contributed to 72.5% of the sulfate reduced, which was equivalent to 7.56% of the electron equivalents supplied with 46.6 g/L of ZVI. The rate of sulfate removal in the complete treatment was 2.9- to 3.5-fold faster than in the controls. The occurrence of sulfate reduction in the uninoculated ZVI control would suggest some growth of sulfate reducers after prolonged incubations.

**As removal**

The results showed that the highest removal of As was obtained in microcosm with lactate + iron powder (Table 1). The removal of As was observed for the

<table>
<thead>
<tr>
<th>Samples</th>
<th>C\text{initial} (μg/L)</th>
<th>C\text{end} (μg/L)</th>
<th>Removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG</td>
<td>4,500</td>
<td>4,110</td>
<td>9</td>
</tr>
<tr>
<td>Abiotic</td>
<td>4,960</td>
<td>3,890</td>
<td>22</td>
</tr>
<tr>
<td>AG + lactate</td>
<td>4,321</td>
<td>1,100</td>
<td>73</td>
</tr>
<tr>
<td>AG + lactate + iron powder</td>
<td>4,832</td>
<td>131</td>
<td>97</td>
</tr>
<tr>
<td>AG + lactate + iron filings</td>
<td>4,890</td>
<td>647</td>
<td>87</td>
</tr>
<tr>
<td>AG + lactate + iron chips</td>
<td>4,321</td>
<td>697</td>
<td>84</td>
</tr>
</tbody>
</table>

\(^{\text{C\text{initial}} - \text{Initial concentration in groundwater, } C\text{end} - \text{concentration in groundwater after termination of the experiment.}}\)
microcosms with lactate + iron filings and lactate + iron chips: 87% of As and 84% of As was removed from groundwater after 57 days. The lowest removal of As was observed in the microcosm with lactate. Teclu et al. (2008) observed that arsenic species at concentrations of 5 mg/L or less can be removed by precipitating the metalloid out of solution as the metal sulfide by reacting with the H₂S produced by SRB.

Kumar et al. (2009) tested ZVI to stimulate sulfate reduction for subsequent removal of metals such as As, Cd and Zn in groundwater and sediments at low pH (~3). In their experiment, they found that ZVI is very efficient in stimulating sulfate reduction, fast removal of contaminants and more stable precipitates. Wilopo et al. (2008) also observed that As was removed effectively by being immobilized in the ZVI and by being incorporated into precipitates that formed because of bacterial reduction of sulfate.

CONCLUSIONS

The results showed that ZVI is very efficient in stimulating sulfate reduction and removal of As from groundwater; however, it strongly depends on the type and particle size of ZVI. In the case of commercial ZVI the most effective was iron powder. In this case, removal of As was 97%; however, the sulfate removal was the least in comparison to other samples amended with iron. In the microcosm amended with iron filings the sulfate removal of 35% was obtained while the removal of As was 87%. ZVI from the foundry was also an effective iron source. In the microcosm amended with iron chips the sulfate removal of 27% was obtained while the removal of As was 84%.

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