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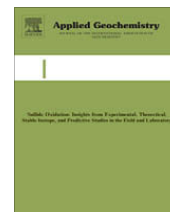
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## Geochemistry and mineralogy of arsenic in (natural) anaerobic groundwaters

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### ABSTRACT

Here new data from field bioremediation experiments and geochemical modeling are reported to illustrate the principal geochemical behavior of As in anaerobic groundwaters. In the field bioremediation experiments, groundwater in Holocene alluvial aquifers in Bangladesh was amended with labile water-soluble organic C (molasses) and MgSO<sub>4</sub> to stimulate metabolism of indigenous SO<sub>4</sub>-reducing bacteria (SRB). In the USA, the groundwater was contaminated by Zn, Cd and SO<sub>4</sub>, and contained <10 µg/L As under oxidized conditions, and a mixture of sucrose and methanol were injected to stimulate SRB metabolism. In Bangladesh, groundwater was under moderately reducing conditions and contained ~10 mg/L Fe and ~100 µg/L As. In the USA experiment, groundwater rapidly became anaerobic, and dissolved Fe and As increased dramatically (As > 1000 µg/L) under geochemical conditions consistent with bacterial Fe-reducing conditions. With time, groundwater became more reducing and biogenic SO<sub>4</sub> reduction began, and Cd and Zn were virtually completely removed due to precipitation of sphalerite (ZnS) and other metal sulfide mineral(s). Following precipitation of chalcophile elements Zn and Cd, the concentrations of Fe and As both began to decrease in groundwater, presumably due to formation of As-bearing FeS/FeS<sub>2</sub>. By the end of the six-month experiment, dissolved As had returned to below background levels. In the initial Bangladesh experiment, As decreased to virtually zero once biogenic SO<sub>4</sub> reduction commenced but increased to pre-experiment level once SO<sub>4</sub> reduction ended. In the ongoing experiment, both SO<sub>4</sub> and Fe(II) were amended to groundwater to evaluate if FeS/FeS<sub>2</sub> formation causes longer-lived As removal. Because As-bearing pyrite is the common product of SRB metabolism in Holocene alluvial aquifers in both the USA and Southeast Asia, it was endeavored to derive thermodynamic data for arsenian pyrite to better predict geochemical processes in naturally reducing groundwaters. Including the new data for arsenian pyrite into Geochemist's Workbench, its stability field completely dominates in reducing Eh–pH space and “displaces” other As-sulfides (orpiment, realgar) that have been implied to be important in previous modeling exercises and reported in rare field conditions.

In summary, when anaerobic bacterial metabolism is optimized by providing both electron donors and acceptors, As is mobile under Fe-reducing conditions, immobile under SO<sub>4</sub>-reducing conditions, and arsenian pyrite is the likely stable mineral phase formed under SO<sub>4</sub>-reducing conditions, instead of pure As–S phases such as realgar or orpiment.

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### 1. Introduction

Natural As enrichment of groundwater occurs around the world by a variety of geochemical processes

(e.g., Welch et al., 2000; Smedley and Kinniburgh, 2002). In the last few decades, pollution of major rivers throughout the world, principally by human and livestock wastes, has led to increased usage of groundwater in adjacent river floodplain deposits. This shift in water usage has occurred particularly in developing nations. In the early 1990s, it became apparent that As enrichment of groundwater in young (Holocene) alluvial floodplain aquifers was an important health problem, particularly in Southeast Asia. Korte (1991) first proposed that the As enrichment of alluvial aquifer groundwater was caused by the co-deposition of hydrous ferric oxides (HFO) containing sorbed As and natural organic matter in river floodplain alluvium, and that the organic matter caused reductive dissolution of HFO, releasing both Fe(II) and As to groundwater. The scale of As enrichment of groundwater started to be recognized in Southeast Asia in the mid to late 1990s (Chatterjee et al., 1995; Nickson et al., 1998; Acharyya et al., 1999, 2000). Saunders et al. (1997), Penny et al. (2003) and Lee et al. (2007) extended geochemical model of Korte (1991) to include the metabolic effects of Fe-reducing bacteria (FeRB) and Mn-reducing bacteria for releasing Fe and As (and other trace elements such as Mn, Co, Ni, Ba, V, REEs, etc.) in alluvial aquifers in the USA. Further, Saunders et al. (1997) showed that  $\text{SO}_4$ -reducing bacteria removed As, Fe, Co and Ni by coprecipitating them in biogenic pyrite. Not long after, other research showed that FeRB were apparently responsible for causing the As enrichment of Holocene alluvial aquifers in Bangladesh and India (e.g., Nickson et al., 2000; McArthur et al., 2001; Dowling et al., 2002). Laboratory investigations by Islam et al. (2004) on sediment cores from Southeast Asia showed that FeRB such as *Geobacter* could liberate As from minerals. Field investigations by Saunders et al. (2005a) at Korte's (1991) discovery location (Kansas City, MO, USA) showed that FeRB (of the genus *Geobacter*) were abundant in groundwaters containing elevated As, and absent in groundwaters without As. SRB (principally *Desulfovibrio desulfuricans*) were also present in As-enriched groundwater. Saunders et al. (2005a) proposed that such bacteria were important in As geochemical cycling in Southeast Asia as well.

Saunders et al. (1997, 2005a) and Lee et al. (2005) proposed that As was mobile under Fe-reducing conditions and immobile under  $\text{SO}_4$ -reducing conditions, given ample supplies of necessary electron donors and acceptors. Kirk et al. (2004) reached a similar conclusion for As-rich groundwater in Illinois. Further, Saunders et al. (1997, 2005a) proposed that As-bearing pyrite should be the most important solid As phase formed under  $\text{SO}_4$ -reducing conditions in natural systems. In contrast, O'Day et al. (2004) and O'Day (2006) reported pure As–S phases including realgar (AsS) and orpiment ( $\text{As}_2\text{S}_3$ ) from an industrial As-contaminated site, and proposed that those As solid phases would form under reducing conditions. More recently, much As-bearing pyrite has been found in alluvial sediments in Bangladesh (Lowers et al., 2007) and West Bengal, India (Acharyya and Shah, 2007) in addition to the USA (Huerta-Diaz and Morse, 1992; Saunders et al., 1997; Southam and Saunders, 2005). Based on interpretations of geo-microbiological controls on the geochemistry of As and

trace metals in an alluvial aquifer in the USA (Saunders et al., 1997), patented bioremediation processes were developed (Saunders, 1998; Lee et al., 2008) to exploit both the geochemical effects of SRB metabolism to remove metals, radionuclides and metalloids such as As and Se from groundwater. Initial results have been promising (Lee and Saunders, 2003; Saunders et al., 2005b) and the authors are in the process of trying to optimize the technique to remove As from groundwater. Here results of two bioremediation experiments are presented to illustrate in real time As geochemical behavior. Further, the conclusions are supported by observations from a landfill in Maine where leaking organic C stimulated biogenic Fe-reduction in groundwater and significant As release (Keimowitz et al., 2005). Similarly, more recent laboratory experiments by Keimowitz et al. (2007) demonstrate that As is released under Fe-reducing conditions, and removed during biogenic  $\text{SO}_4$  reduction. This study is the first documentation that SRB can remove As under field conditions, although the exact mechanism(s) are still not well understood. Here it is proposed that adsorption of As on surfaces of sulfide minerals (particularly Fe-sulfides) is important for As removal by biogenic  $\text{SO}_4$  reduction, and bioremediation of As might be possible by “engineering” SRB to make appropriate high-surface area “biominerals” of Fe-sulfide.

## 2. Materials and methods

### 2.1. Field bioremediation experiments

Two field bioremediation pilot experiments have been conducted that have implications for As geochemistry under reducing/anaerobic conditions in alluvial aquifers. The first experiment was conducted at a site in Oklahoma, USA where shallow oxidizing groundwater is contaminated by Cd, Zn and  $\text{SO}_4$  from an old Zn smelter (Fig. 1). A test of using indigenous SRB to remediate metal-contaminated groundwater was performed. A mixture of methanol (84 mg/L) and sucrose (108 mg/L) were pumped into injection well PTIW-2 (Fig. 1) at a rate of 114 L/min for two days in the approximate center of the contaminated groundwater plume. Bromide was also added as a tracer to the injected solution. Seven multiport monitoring wells were installed to intercept the plume of amended groundwater along the flow path. Water samples were collected using a peristaltic pump connected with Teflon tubes that connected to the various multiports of the monitoring wells. Water samples were collected from the monitoring wells for approximately six months after injection and analyzed for Cd, Zn, Fe, As and  $\text{SO}_4$ . Only data (Fig. 2) from the middle multiport (depth = 5.2 m) of monitoring well PTMW-2 (see location, Fig. 1) is shown here.

In Bangladesh, existing water-supply tube wells were used to characterize groundwater geochemistry in the Manikganj region (Fig. 3), and those sampling and analytical procedures are detailed in Shamsudduha (2007) and Shamsudduha et al. (in press). At the bioremediation experiment site in Bangladesh, a hand pump was used to extract water from a former tube well. Water samples for major cations and trace elements were filtered and acidi-

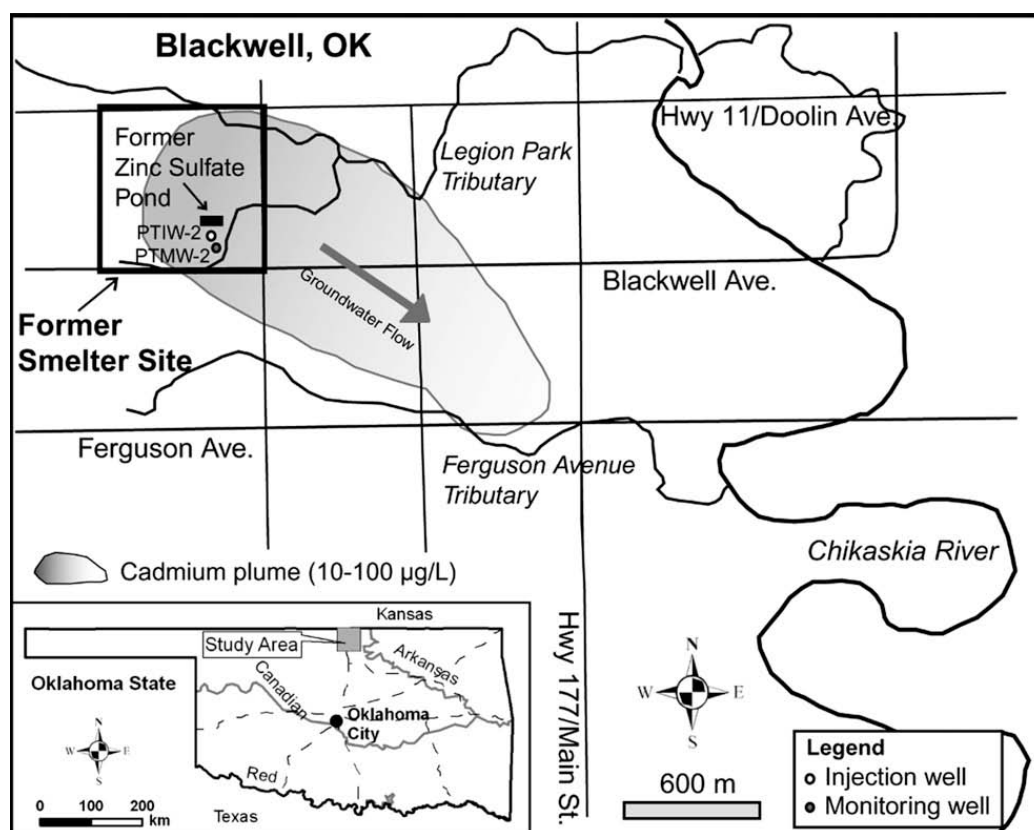


Fig. 1. Location map for the bioremediation demonstration (pilot) project at Blackwell, Oklahoma, USA at the site of an old Zn smelter.

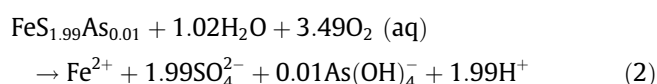
fied using US EPA standard procedures at both sites. Water samples were analyzed by ICP-OES and ICP-MS in the laboratory, and, at the Bangladesh field site, a colorimetric technique was also used for As which yielded similar results to ICP analyses.

### 2.2. Geochemical modeling

In general, a reaction path model traces how a fluid's chemistry evolves and which minerals precipitate or dissolve over the course of geochemical processes. Construction of geochemical models for As mobility and reactivity under  $SO_4$ -reducing conditions requires the inclusion of arsenian pyrite ( $FeS_{1.99}As_{0.01}$ – $FeS_{1.90}As_{0.10}$ ) solid solution. The equilibrium constant for chemical reactions can be calculated directly from the standard free energy change by the equation

$$\log K = \frac{-\Delta G_R^0}{2.303RT_K} \quad (1)$$

Here  $R$  is the gas constant and  $T_K$  is absolute temperature. Consider the reaction



The Gibbs free energy  $\Delta G_R$  of the reaction is the difference between the standard Gibbs free energy of the products and reactants. The standard Gibbs free energies  $\Delta G_f$  for  $H_2O$ ,  $O_2 (aq)$ ,  $H^+$ , and  $As(OH)_3$  are  $-237.14$ ,  $0$ ,  $0$ , and  $-639.77$  kJ/mol, respectively (Drever, 1997; Pokrovski

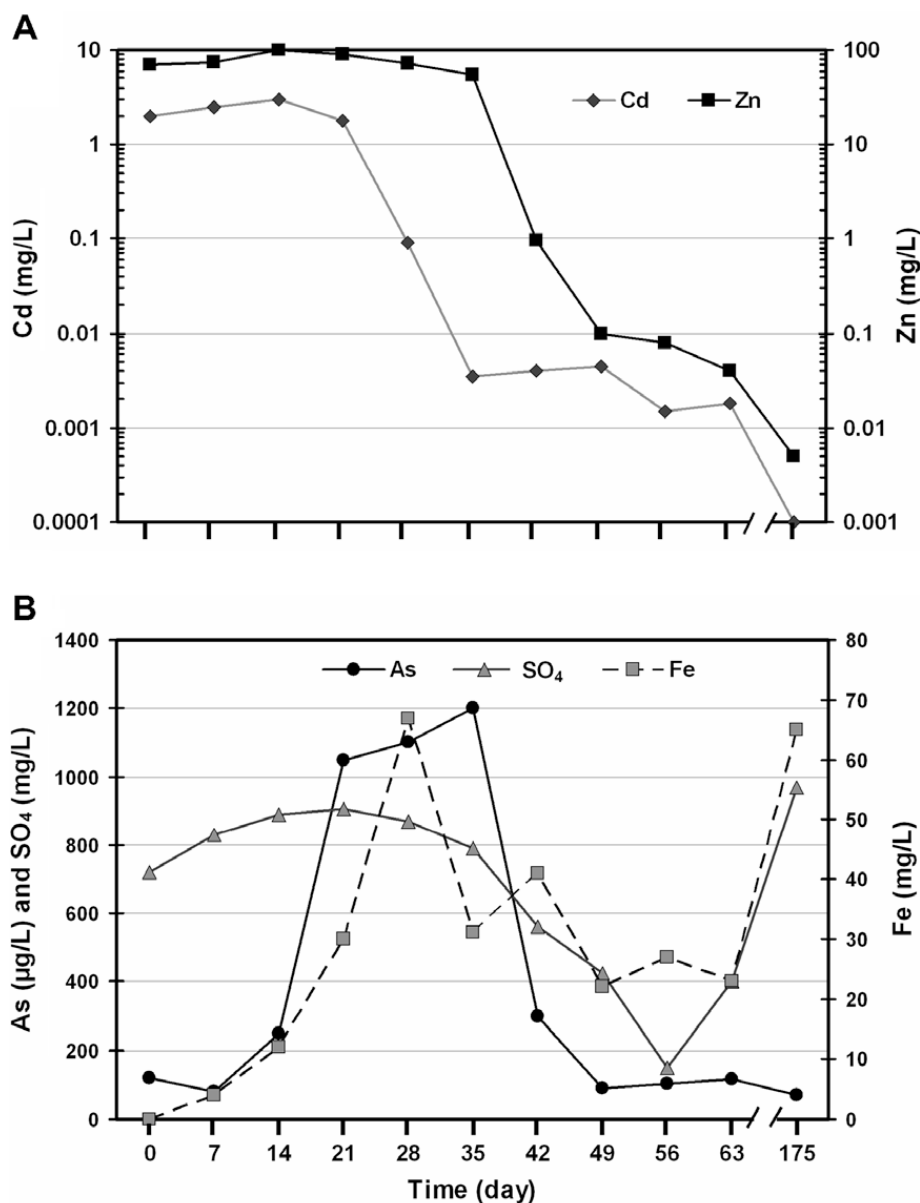
et al., 2002). The  $\Delta G_f$  for  $As(OH)_4^-$  can be calculated accordingly as  $-859.87$  kJ/mol from the mass action equation. The free energy for arsenian pyrite solid solution  $FeS_xAs_y$  can be calculated from that of  $FeSAs$  by an equation presented by Pokrovski et al. (2002)

$$\Delta G^0 (FeS_xAs_y) = \Delta G^0 (FeSAs) + 2.303RT(\log x + \log y) \quad (3)$$

Table 1 summarizes calculated  $\Delta G_f$  and  $\log K$  values for various arsenian pyrite solid solution containing 1–10 mol% of arsenic (i.e.,  $FeS_{1.99}As_{0.01}$ – $FeS_{1.90}As_{0.10}$ ). The thermodynamic data for arsenian pyrite, thioarsenite species and amorphous As and Fe sulfide phases were compiled into a revised Geochemist's Workbench (GWB, Bethke, 1996) database *Thermo08-As* which can be obtained from Auburn University. This new thermodynamic database is more realistic in characterizing and predicting the As behavior in reducing Fe-bearing groundwater conditions. GWB calculations were carried out using use new thermodynamic data to characterize the speciation of As in Fe–S–As– $H_2O$  systems. Arsenic reactivity and precipitation is also modeled under  $SO_4$ -reducing conditions.

### 3. Field bioremediation experiments: results and discussion

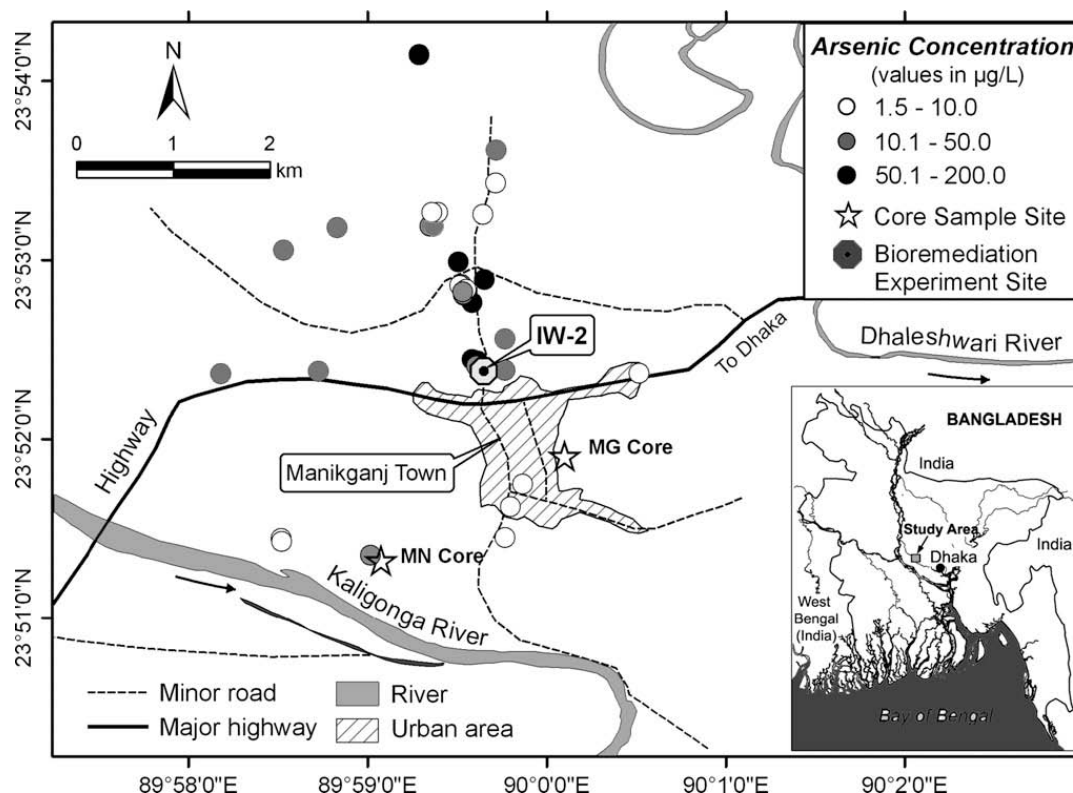
Fig. 2 shows the water chemistry changes in the monitoring well (PTMW-2, Fig. 1) closest to the injection wells at the Oklahoma site. The results show that Cd and Zn decreased dramatically as they were removed as a consequence of biogenic  $SO_4$  reduction. Lee and Saunders



**Fig. 2.** (A) Plot of dissolved Zn and Cd concentrations in the monitoring well closest to the injection well (PTMW-2, Fig. 1), as a function of time after injection of the organic C source (mixture of methanol and molasses) into shallow, oxidizing (i.e., aerobic) groundwater in a Holocene river floodplain. Zinc and Cd concentrations drop dramatically simultaneously with onset of biogenic SO<sub>4</sub> reduction, apparently precipitating as Cd-bearing sphalerite. (B) Plot of dissolved Fe, As and SO<sub>4</sub> concentrations observed in monitoring well PTMW-2. Initially Fe and As concentrations are low under oxidizing conditions, both increase dramatically when anaerobic biogenic Fe reduction begins (14–28 days), both decrease substantially (along with SO<sub>4</sub>) between 28–49 days when biogenic SO<sub>4</sub> reduction occurs, and Fe and SO<sub>4</sub> increase significantly after 56 days. That increase may parallel a return to Fe-reducing conditions. Importantly, As remains below pre-injection levels from the 49<sup>th</sup> day until end of the experiment at 175 days. That behavior is interpreted here as the result of As sorption onto the “biominerals” (in particular Fe-sulfide phases) produced during biogenic SO<sub>4</sub> reduction.

(2003) and Saunders et al. (2005b) showed that Zn and Cd were removed from groundwater by SRB metabolism due to the precipitation of Cd-bearing sphalerite at an Alabama site. However, the geochemical behavior of Fe, As and S are the thrust of this paper, and Fig. 2 shows that initially dissolved As and Fe increase dramatically in about two weeks after injection of the labile C into the groundwater. The concurrent increase in As and Fe parallels the onset of biogenic Fe reduction. As can be seen in Fig. 2, dissolved SO<sub>4</sub> remains relatively constant at about 900 mg/L as dissolved Fe and As increase. At about one month, As and Fe reach their maximum concentrations about where dissolved

SO<sub>4</sub> begins to decline and biogenic SO<sub>4</sub> reduction begins, causing Zn and Cd precipitation (Fig. 2). Once biogenic SO<sub>4</sub> reduction begins, the concentration of dissolved Fe also begins to drop, due to the formation of Fe-sulfides. Arsenic generally follows the transition metals as they decrease in concentration, and by about two months, dissolved As had returned to pre-experiment concentrations. It is interesting to note that at the end of the experiment (175 days, Fig. 2) the dissolved SO<sub>4</sub> had returned to approximate pre-injection levels as the “remediated” plume had passed by the monitoring well. Dissolved Fe at 175 days reached similar concentrations to earlier in



**Fig. 3.** Map showing tube well locations sampled for this study in the Manikganj district of Bangladesh and their relative dissolved As concentrations. Location of the injection well (IW-2) for the Bangladesh bioremediation experiment is also shown.

**Table 1**

Calculated Gibb's free energy  $\Delta G^\circ$  for various compositions of Fe–S–As solid solution ( $\text{Fe}_{1.99}\text{As}_{0.01}$ – $\text{Fe}_{1.90}\text{As}_{0.10}$ )

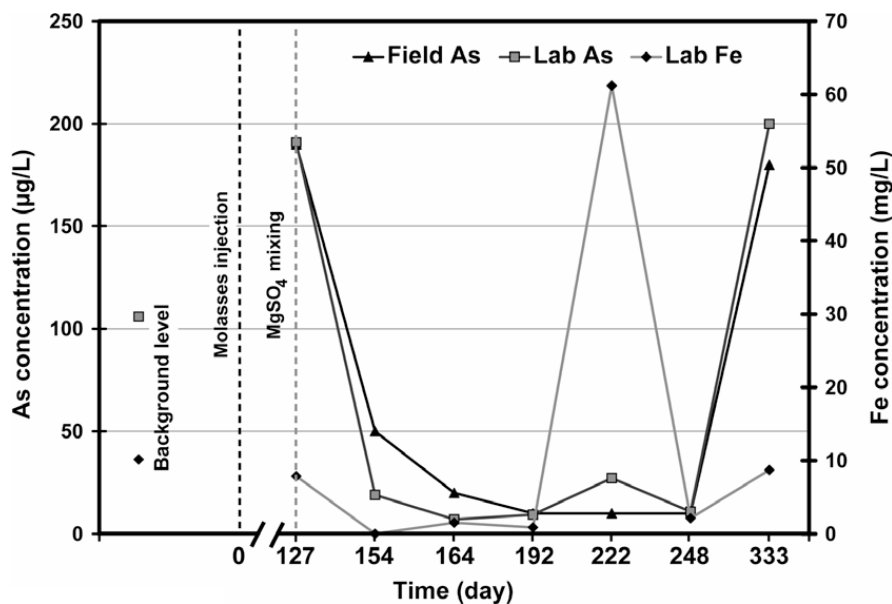
| Sulfur (S) and Arsenic (As) fraction | S    | As   | Molecular weight | $\Delta G_f^\circ$ (FeSAs) | S(x) | As(y) | $2.303RT$ ( $\log x + \log y$ ) | $\Delta G_f^\circ$ ( $\text{FeS}_x\text{As}_y$ ) |                | $\Delta G^\circ$ for the reaction | $\log K$ |
|--------------------------------------|------|------|------------------|----------------------------|------|-------|---------------------------------|--|----------------|-----------------------------------|----------|
| $\text{S}_{1.99}\text{As}_{0.01}$    | 1.99 | 0.01 | 120.396          | –141.6                     | 2.00 | 0.00  | –25.29                          | –166.89  | $\text{FeS}_2$ | –1147.58                          | 199.78   |
| $\text{S}_{1.98}\text{As}_{0.02}$    | 1.98 | 0.02 | 120.824          | –141.6                     | 1.99 | 0.01  | –5.62                           | –147.22  | Fe–S–As        | –1145.63                          | 199.44   |
| $\text{S}_{1.97}\text{As}_{0.03}$    | 1.97 | 0.03 | 121.253          | –141.6                     | 1.98 | 0.02  | –4.63                           | –146.23  | Solid          | –1143.27                          | 199.02   |
| $\text{S}_{1.96}\text{As}_{0.04}$    | 1.96 | 0.04 | 121.681          | –141.6                     | 1.97 | 0.03  | –4.06                           | –145.66  | Solution       | –1140.74                          | 198.58   |
| $\text{S}_{1.95}\text{As}_{0.05}$    | 1.95 | 0.05 | 122.110          | –141.6                     | 1.96 | 0.04  | –3.65                           | –145.25  |                | –1138.12                          | 198.13   |
| $\text{S}_{1.94}\text{As}_{0.06}$    | 1.94 | 0.06 | 122.539          | –141.6                     | 1.95 | 0.05  | –3.34                           | –144.94  |                | –1132.72                          | 197.19   |
| $\text{S}_{1.93}\text{As}_{0.07}$    | 1.93 | 0.07 | 122.967          | –141.6                     | 1.94 | 0.06  | –3.08                           | –144.68  |                | –1129.97                          | 196.71   |
| $\text{S}_{1.92}\text{As}_{0.08}$    | 1.92 | 0.08 | 123.396          | –141.6                     | 1.93 | 0.07  | –2.87                           | –144.47  |                | –1127.19                          | 196.23   |
| $\text{S}_{1.91}\text{As}_{0.09}$    | 1.91 | 0.09 | 123.825          | –141.6                     | 1.92 | 0.08  | –2.69                           | –144.29  |                | –1124.4                           | 195.74   |
| $\text{S}_{1.90}\text{As}_{0.10}$    | 1.90 | 0.10 | 124.253          | –141.6                     | 1.91 | 0.09  | –2.53                           | –144.13  |                |                                   |          |
|                                      |      |      |                  | –141.6                     | 1.90 | 0.10  | –2.38                           | –143.98  |                |                                   |          |
|                                      |      |      |                  | –141.6                     | 1.00 | 1.00  | 0.00                            | –141.60  | FeSAs          |                                   |          |

$\Delta G^\circ$  values of two end-member pure phases  $\text{FeS}_2$  and FeSAs are also shown. The values of equilibrium constant  $\log K$  were calculated for the reaction:  $\text{FeS}_x\text{As}_y + x\text{H}_2\text{O} + y\text{O}_2(\text{aq}) \rightarrow \text{Fe}^{2+} + x\text{SO}_4^{2-} + y\text{As}(\text{OH})_4^- + z\text{H}^+$ , X, Y and Z are the stoichiometric coefficients of  $\text{H}_2\text{O}$ ,  $\text{O}_2(\text{aq})$  and  $\text{H}^+$  in the reaction; x and y are the molar ratio of S and As in the Fe–S–As solid solution.

the experiment where Fe reduction of aquifer HFO was presumably occurring. Thus perhaps Fe-reduction began again at the end of the experiment. In contrast, at 175 days, Cd and Zn remain very low and this could be due to adsorption of those contaminants on the newly formed (Zn-) sulfide phases as proposed in the Alabama study (Lee and Saunders, 2003; Saunders et al., 2005b). Similarly, Fe-sulfides may continue to adsorb As at the end of the experiment.

The Oklahoma experiment suggested that SRB metabolism might lead to As removal, this hypothesis was again tested in Bangladesh, where drinking water (tube) wells

were sampled in the Manikganj district of Bangladesh (Fig. 3). Some tube wells in the Manikganj area contain elevated As concentrations up to 50–200  $\mu\text{g/L}$  (Fig. 3). Sediments in the Manikganj area are characterized by Quaternary alluvial deposits, composed mostly of sand, silt and clay. Two major fining upward alluvial sequences were identified in two 200 m deep drilled holes in Manikganj (Shamsudduha, 2007). Continuous core sediments recovered from these two wells revealed three major stratigraphic units: (i) a yellowish-brown to yellowish-gray lower unit ( $\sim 100$  m) consisting of fine to medium sand with occasional coarse sand, (ii) the middle unit of mostly



**Fig. 4.** Plot of results of the single-well bioremediation test at Manikganj, Bangladesh. Groundwater is initially moderately reducing and interpreted to be controlled by FeRB. Injection of molasses initially causes dissolved As to increase, yet dissolved Fe remains relatively constant. At the 127 day mark, a source of  $\text{SO}_4$  (Epsom's salt was added), and about 4 weeks later, it appeared that biogenic  $\text{SO}_4$  reduction was occurring.

gray (~80 m) medium to fine sand; and (iii) the gray to light yellowish-gray top (~10 m) fine sand, silt and clay (Shamsudduha et al., in press). Groundwater in the Manikganj area is typically Fe-rich and has the general Ca–Mg– $\text{HCO}_3$  composition, neutral pH, and low  $\text{SO}_4$  content (Shamsudduha, 2007) that occur widely in Bangladesh (Nickson et al., 2000; Smedley and Kinniburgh, 2002). A tube well was selected (Fig. 3) that yielded the highest As concentration in the area (~100 µg/L from depth of 37 m) for the field single-well bioremediation experiment. Approximately ~11 kg of molasses was first dissolved into 200 L of groundwater from the site, and the solution poured into the well by gravity injection. Treated groundwater was sampled periodically over the next six months for geochemical analyses. Injection of molasses raised the dissolved As concentration (Fig. 4) during the early stage because it created Fe-reducing conditions and mobilized As from HFO. The same results of As increases were observed during the experiment at the Okalahoma site (Fig. 2) and similar field tests in Bangladesh (Harvey et al., 2002). At the 127 day mark, a source of  $\text{SO}_4$  (4 kg of Epsom's salt,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ) was added, and about 4 weeks later biogenic  $\text{SO}_4$  reduction was occurring, as indicated by very low Eh values. Interestingly,  $\text{SO}_4$  reduction also occurred at about the same interval after organic C was added in the Oklahoma and Alabama experiments (Lee and Saunders, 2003; Saunders et al., 2005b). Once biogenic  $\text{SO}_4$  reduction began, both Fe and As decreased dramatically (days 154–192). From days 248–333, As increased to levels similar to day 127, prior to injection of  $\text{MgSO}_4$ ; such levels are higher than at the beginning of the experiment. Iron returned to background levels (~10 mg/L) at 333 days. It is interesting to note that As returned to the concentration at the time of injection of the  $\text{SO}_4$  source, as opposed to the lower background level. This suggests that at the end of the experiment in Bangladesh,

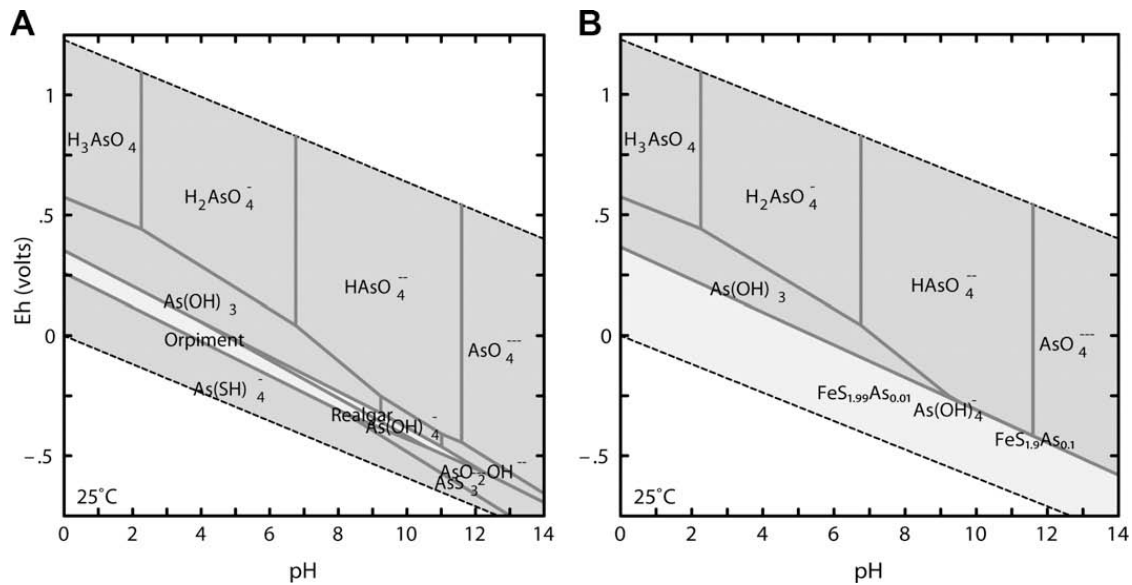
Fe-reducing conditions had returned again and that perhaps there was enough organic C left after depletion of injected  $\text{SO}_4$  to fuel continued Fe-reduction and As release from aquifer sediments. It should be noted that the dissolved As level at the end of the experiment is higher than that at the start. It is likely that the induced Fe-reduction process allows more As to be released from HFO. Moreover,  $\text{H}_2\text{S}$  produced by SRB could enhance As solubility when dissolved Fe is exhausted (Lee et al., 2005).

In comparing results of USA and Bangladesh, it was noted that there was more dissolved Fe at the Oklahoma site prior to  $\text{SO}_4$  reduction, and As removal also seemed more effective and perhaps longer lived there than in Bangladesh. Thus the effects of substituting  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  for Epsom's salt as a source of both  $\text{SO}_4$  and Fe are currently being evaluated in a second (ongoing) experiment in Bangladesh. It is hypothesized that if Fe-sulfide phases can be precipitated in the vicinity of the well, they may be capable of removing As after biogenic  $\text{SO}_4$  reduction has ceased.

#### 4. Geochemical modeling: results and discussion

##### 4.1. Arsenic speciation

Phase diagrams for As speciation in the presence of S were calculated using the ACT2 sub-program of GWB with added thermodynamic data for thioarsenite species and arsenian pyrite. Fig. 5A shows that under oxidizing conditions  $\text{H}_2\text{AsO}_4^-$  and  $\text{H}_3\text{AsO}_4$  are dominant at low pH (<7) while  $\text{HAsO}_4^{2-}$  and  $\text{AsO}_4^{3-}$  become dominant at higher pH. Under reducing conditions  $\text{H}_3\text{AsO}_3$  predominates over a wide range of pH values. Under even more reducing conditions, the solid As sulfides orpiment ( $\text{As}_2\text{S}_3$ ) and realgar (AsS) or thioarsenite aqueous complexes become the dominant phases. Whereas in the system containing a small amount of Fe ( $\text{Fe}^{2+}$  activity =  $10^{-8}$ ), arsenian pyrite



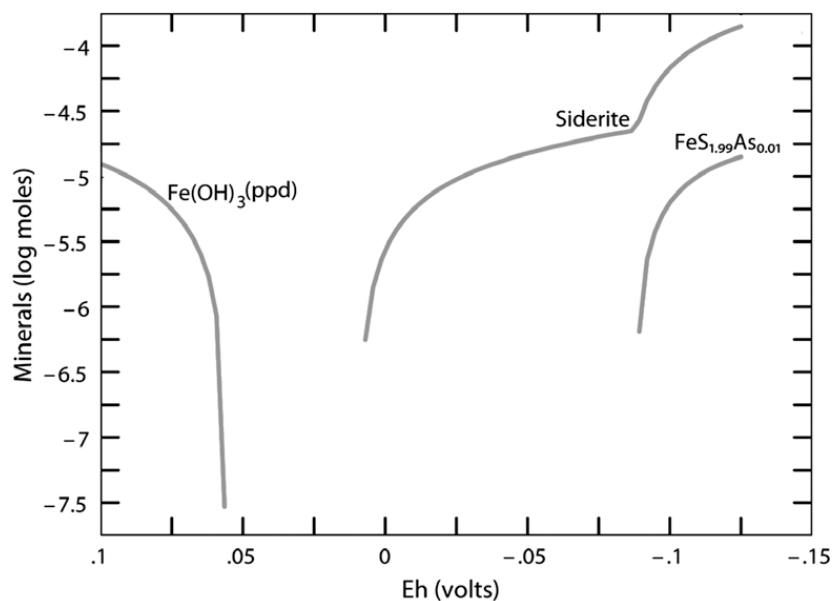
**Fig. 5.** (A) Eh–pH diagrams for As drawn at 25 °C and fixed As and  $\text{SO}_4^{2-}$  activities of  $10^{-2}$ . Dashed lines show stability limits of water at 1 bar pressure. (B) Eh–pH diagrams for As drawn at 25 °C with fixed As and  $\text{SO}_4^{2-}$  activities of  $10^{-2}$  and  $\text{Fe}^{2+}$  activity of  $10^{-8}$ . Arsenian pyrite ( $\text{FeS}_{1.99}\text{As}_{0.01}$ – $\text{FeS}_{1.90}\text{As}_{0.10}$ ) solid solution is included in the thermodynamic database.

( $\text{FeS}_{1.99}\text{As}_{0.01}$ ) replaces the pure As sulfide (orpiment) and thioarsenite aqueous complex (Fig. 5B). This result suggests that arsenian pyrite is thermodynamically more stable than pure As sulfides under  $\text{SO}_4$ -reducing conditions. Moreover, pure orpiment rarely forms in natural waters because its precipitation is kinetically inhibited at near-neutral pH conditions (Webster, 1990).

#### 4.2. Arsenic precipitation under sulfate reducing conditions

How bacterial  $\text{SO}_4$  reduction induces the precipitation of metal sulfides and As from a groundwater in Bangladesh was investigated. To begin an As-enriched groundwater

from Bangladesh was equilibrated as the initial condition in the simulation. The calculation used the water chemical data collected from the BGS-DPHE well site 297\_00331 (BGS-DPHE). This groundwater has elevated As concentrations of 2540  $\mu\text{g/L}$  and is under near-neutral pH condition. To model the effect of SRB bioremediation, fluid reactants containing 300  $\mu\text{mol}$  of  $\text{Fe}^{2+}$  and  $\text{SO}_4^{2-}$  were added into the initial system and the values of Eh changed from +150 mV to –150 mV over the reaction path. The predicted mineral reactions (Fig. 6) show that  $\text{Fe}(\text{OH})_3$  in the initial system first becomes thermodynamically unstable during bacterial reduction and reduced Fe species also combine with  $\text{HCO}_3^-$  released from organic sources to form the



**Fig. 6.** Predicted mineral reactions of As-rich Bangladesh groundwater as Eh decreases during bacterial  $\text{SO}_4$  reduction.

carbonate mineral siderite ( $\text{FeCO}_3$ ). Authigenic (biogenic) siderite has been found in Mississippi and Alabama coastal plain sediments (Saunders and Swann, 1992; Lee et al., 2007) and alluvial sediments in India and Bangladesh (Pal et al., 2002; Sengupta et al., 2004). Under highly reducing conditions, reduced aqueous  $\text{Fe}^{2+}$  reacts with  $\text{H}_2\text{S}$  to form arsenian pyrite (instead of pure pyrite), which can remove As from groundwater by co-precipitation. The formation of arsenian pyrite as predicted by the geochemical model agrees well with the occurrence of As-bearing biogenic pyrite (containing 1–6 wt.% As) in As-enriched groundwaters of Bangladesh and USA under reducing conditions.

### 5. As-bearing pyrite and its role in arsenic removal: discussion

Lee and Saunders (2003) and Saunders et al. (2005b) have shown that injection of labile organic C into groundwater first stimulates biogenic Fe reduction, and then as geochemical conditions become more reducing, bacterial  $\text{SO}_4$  reduction commences. This progression of biogeochemical conditions is interpreted as being a result of depletion of ferric hydroxide phases that serve as an electron acceptor for anaerobic bacteria. Chapelle and Lovley (1992) and Lovley and Chapelle (1995) have shown that there is more energy to be derived from Fe reduction than  $\text{SO}_4$  reduction, and that FeRB and SRB compete with each other in anaerobic groundwater for electron donors (e.g., organic C). Saunders et al. (2005a) have shown that both viable FeRB and SRB are present in naturally As-contaminated groundwater at Korte's (1991) Kansas City, MO (USA) site.

In natural waters, pyrite is the common authigenic mineral that forms under  $\text{SO}_4$ -reducing conditions, provided that ample sources of Fe and  $\text{SO}_4$  are available (Morse et al., 1987). There is a kinetic inhibition for direct precipitation of pyrite under  $\text{SO}_4$ -reducing conditions, and typically amorphous Fe-S phases form initially and then invert to pyrite (Morse et al., 1987). In anoxic marine sediments of the Gulf of Mexico, Huerta-Diaz and Morse (1992) found that Fe-sulfides (amorphous FeS and pyrite) incorporate trace elements such as As, Co, Ni, Mo into pyrite and acid volatile sulfides. Similarly, Saunders et al. (1997) documented that As, and to a lesser extent Co and Ni, were incorporated into authigenic pyrite in a Holocene alluvial aquifer in central Alabama under  $\text{SO}_4$ -reducing conditions. In the Alabama setting, coarse-grained (up to 1.5 cm in diameter) As-bearing pyrite grains encrust and replace wood fragments and bulk S-isotope data indicated that SRB lead to pyrite formation. Pyrite  $\delta^{34}\text{S}$  values are isotopically light as expected as a consequence of bacterial  $\text{SO}_4$  reduction. Pyrite is also zoned with respect to As content, and electron microprobe analyses show that individual zones contain up to 1 wt.% As. More recently, detailed ion microprobe analyses confirmed that the isotopically lightest S in the core of the pyrite grain corresponds with the highest As content (Saunders et al., 2005a).

Many recent studies have conclusively shown that As-bearing pyrite is the principal authigenic sulfide phase in

As-enriched groundwaters in West Bengal, India (Mukherjee et al., 2001; Acharyya and Shah, 2007) and Bangladesh (Nickson et al., 2000; Lowers et al., 2007) and in a Holocene alluvial aquifer in USA (Saunders et al., 1997; Southam and Saunders, 2005). Arsenic-sulfides such as orpiment and realgar have been reported at one site where As contamination occurs (O'Day et al., 2004; O'Day, 2006) using XANES and EXAFS techniques. However, no X-ray diffraction data confirming their presence has been published, although the As-S phases could be too fine-grained for detection by X-ray diffraction. From the field observations and those of others cited above, along with the geochemical modeling presented here incorporating thermodynamic data for As-bearing pyrite, it appears that As-bearing pyrite is the principal solid phase incorporating As under  $\text{SO}_4$ -reducing conditions in natural waters. Oxidation of As-bearing sulfides can cause As enrichment (Welch et al., 2000; Smedley and Kinniburgh, 2002). In Holocene alluvial aquifers, however, As-bearing pyrite is a sink rather than a source of As (Saunders et al., 2005a; Lee et al., 2005). Nickson et al. (2000) and other researchers have shown that there is an inverse relationship between As and  $\text{SO}_4$  in Bangladesh; which reflects the fact that a lack of dissolved  $\text{SO}_4$  in groundwater limits the metabolism of SRB and As removal (Chapelle and Lovley, 1992).

Assuming Fe-sulfide phases form as a consequence of biogenic  $\text{SO}_4$  reduction and that pyrite is the thermodynamically favored phase under most situations (Morse et al., 1987), how is As incorporated into these phases to make As-bearing pyrite? Farquhar et al. (2002), Bostick and Fendorf (2003) and Wolthers et al. (2005) have all shown that As is strongly sorbed to the surfaces of Fe-sulfide phases, under reducing conditions where such sulfides are stable. As the Fe-sulfide phases grow, it is probable that formerly sorbed As is incorporated into the crystal structure of pyrite and other Fe-sulfides, and taken together, these processes can be called "coprecipitation". Thus it appears from the present data that pyrite forms with ~1 wt.% As under reducing conditions where there is sufficient dissolved Fe, As, S and an organic C source to drive biogenic  $\text{SO}_4$  reduction. In laboratory experiments with SRB in a Fe-As-S system, Rittle et al. (1995) actually synthesized arsenopyrite ( $\text{FeAsS}$ ). Because of the abundance of Fe in the crust, it is likely that As-Fe-sulfides will be the predominant As phases forming under reducing conditions.

The bioremediation experiments (and recent laboratory investigations by Keimowitz et al., 2007) suggest that As is removed under  $\text{SO}_4$ -reducing conditions when the SRB are actively metabolizing. Thus the question remains: what is causing the As to be removed? Perhaps an As-Fe-S phase like that observed by Rittle et al. (1995) could explain that result, but more likely, it is suggested that sorption of As on Fe-S phases formed during SRB metabolism is the most likely explanation. Over time As could be incorporated into the pyrite crystal lattice as the amorphous FeS precursors age and ripen. In the Oklahoma experiment, after  $\text{SO}_4$  reduction ceased and dissolved  $\text{SO}_4$  and Fe increased late in the experiment (Fig. 2), As did not show a parallel increase in solution. It is hypothesized that Fe-sulfide phases formed during biogenic  $\text{SO}_4$  reduction have some residual capability of removing As by sorption even though bio-

genic  $\text{SO}_4$  reduction has ceased. In Bangladesh, the experiments were designed to first assess the results of adding labile organic C and  $\text{SO}_4$ , and then to investigate the effect of additional dissolved Fe. In the initial Bangladesh experiment (Fig. 4) biogenic  $\text{SO}_4$  reduction does remove As as was observed in the Oklahoma experiment, but As levels returned to background levels over time. Thus, a second experiment was designed to increase the amount of Fe-sulfide formed, and is currently ongoing. It is hoped that in situ bioremediation can be optimized to become an appropriate, inexpensive, technology for removing As from drinking water in Bangladesh and Indian tube wells.

## 6. Practical considerations for field application

The technique described here has the potential for becoming a low-cost, appropriate technology to remedy As enrichment of groundwater abstracted by tube wells in Southeast Asia. The necessary constituents (molasses and  $\text{FeSO}_4$ ) are produced locally in the region, although an “ideal” concentration or amount of ingredients to optimize the technique cannot be recommended at this point. It generally requires approximately two months for the biogenic Fe-sulfide phases to form in the aquifer adjacent to the treated tube well, and thus the wells cannot be used during that time. The monsoon season typically lasts for ~2 months, so rain water is available for use during that time, or perhaps neighboring wells can be utilized during the time the well is undergoing treatment. The biodegradation of the sugars in molasses does produce noxious smelling water prior to complete degradation. If any of these compounds remain after the treatment process, developing of the wells by pumping will remove them prior to water use. In Bangladesh, wells are typically >100 m apart, and hand pumping of one tube well does not impact neighboring wells, nor would the bioremediation efforts. Although results presented here indicate that As can be removed by SRB metabolism, the technique is designed to continue to remove As by sorption on Fe-sulfide surfaces after bacterial metabolism ceases. Long-term monitoring of treated wells should be done (at least initially) to evaluate if there is a finite time interval where the process is effective. The precipitated Fe-sulfides should be stable as long as they remain below the water table in the moderately reducing aquifers where As enrichment is a problem.

## 7. Conclusions

From the field bioremediation experiments and similar observations by others (Harvey et al., 2002; Keimowitz et al., 2005) it is clear that addition of labile organic C to shallow aquifers containing As-bearing HFO can cause significant release of As to groundwater. This As mobilization is caused by the metabolism of the common anaerobic FeRB such as *Geobacter*, which use organic C as an electron donor causing the reductive dissolution of HFO phases and the release of sorbed and coprecipitated As. This water-sediment-bacteria interaction causes widespread natural As enrichment in Holocene alluvial aquifers throughout the world, including the worst affected areas in Bangladesh

and India. Groundwater in Bangladesh and India is typically low in dissolved  $\text{SO}_4$ , which limits the metabolism of SRB. Kirk et al. (2004) observed that As was low in groundwaters that exist under  $\text{SO}_4$ -reducing conditions, so that if biogenic  $\text{SO}_4$  reduction can be engineered by amending groundwater with appropriate electron donors and acceptors (e.g., Fe), then perhaps this can be used to remediate groundwater containing As.

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