Catena 148 (2017) 46–51

Contents lists available at ScienceDirect

Catena

journal homepage: www.elsevier.com/locate/catena

# Influence of indigenous bacteria stimulation on arsenic immobilization in field study

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ARTICLE INFO

*Article history:*
Received 5 January 2015
Received in revised form 6 July 2016
Accepted 13 August 2016
Available online 22 August 2016

*Keywords:*
Soil contamination
Arsenic
Microbial immobilization
Sulfate reducing bacteria

ABSTRACT

Microbial immobilization of arsenic by stimulation of sulfate reducing bacteria in contaminated paddy soil in the vicinity of an abandoned Au-Ag mine was investigated. Soil samples were mainly contaminated with arsenic due to mining activities. Batch experiments in which glucose and sulfate were amended for bacterial stimulation indicated that indigenous sulfate reducing bacteria could reduce iron and sulfate and subsequently produce metal sulfides. The concentrations of nickel and arsenic decreased in biotic experiment, and nickel-bearing iron sulfide was identified by X-ray diffraction analysis. The total concentration of arsenic in the soil was observed to be similar before and after supply of glucose and sulfate at field experiments; however, readily extractable arsenic decreased by 60%, 60% and 75% at depth of 0.2, 0.5, and 0.8 m, respectively, after indigenous bacteria stimulation. The results indicated that indigenous bacteria stimulation might improve the As immobilization in contaminated soil.



## 1. Introduction

Mining and mineral processing have produced a huge amount of wastes. Numerous researchers reported various problems related with mining processes, e.g. production of acid mine drainage (AMD), release of heavy metals and arsenic, and soil pollution (Akcil and Koldas, 2006; Bhumbla and Keefer, 1994; Cheng et al., 2009; Jung, 2001). Especially, heavy metal contamination of soil caused by mine wastes induces serious problems, such as soil texture destruction, shortage of nutrient, groundwater pollution, and decrease in biological diversity (Liu et al., 2008). Soil washing, physicochemical solidification/stabilization (S/S), phytoremediation, and electrokinetics have been proposed for heavy metal contaminated soil remediation. However, the proposed remediation methods are often commercially unfeasible because of the cost and operating difficulties.

Stabilization processes are widely used to remediate the soil contaminated with heavy metals. The main purpose of stabilization is immobilization of heavy metals using various agents, such as cement, quick lime, fly ash, phosphate, and organic matters (Basta and McGowen, 2004; Brown et al., 2004; Dermatas et al., 2004; Moon and Dermatas, 2007; Moon et al., 2008; Vandecasteele et al., 2002). Recently, many researchers are highly interested in microbial immobilization as well as chemical stabilization, since the microbial immobilization has been proposed as an eco-friendly, economical, and efficient alternative. Main mechanisms of microbial immobilization are based on the immobilization of heavy metals in soil via microbial activity e.g. sorption, precipitation, oxidation, and reduction.

Microbial activity can mediate the behavior of heavy metals and metalloids, especially arsenic (As). For example, microbial iron (Fe) reduction, which reduces $Fe^{3+}$ to $Fe^{2+}$, can enhance the mobilization of As by reductive dissolution of Fe (oxy)-hydroxides. Meanwhile, sulfate ($SO_4^{2-}$) reduction can decrease the As mobilization via generation of sulfide minerals such as realgar (AsS) and orpiment ($As_2S_3$). The effects of Fe and $SO_4^{2-}$ reduction on As behavior have been reported by many researchers (Campbell et al., 2006; Kirk et al., 2010; O'Day et al., 2004; Reza et al., 2013).

Sulfate reducing bacteria (SRB) is a distinctive microbe for heavy metal immobilization by sulfide generation. The SRB are obligate anaerobes characterized by their ability to perform dissimilatory sulfate reduction, with simultaneous oxidation of organic substrates (Postgate, 1984). Microbial sulfate reduction and heavy metal immobilization processes can be described as the following Eqs.;

$$\text{Organic matter } (C, H, O) + SO_4^{2-} \xrightarrow{SRB} HS^- + HCO_3^- \quad (1)$$

$$Me^{2+} + HS^- \rightarrow MeS(\downarrow) + H^+ \quad (2)$$

where, $Me^{2+}$ is the free metal ion, and MeS is insoluble metal sulfide. Sulfate reduction by SRB metabolism has been applied for immobilization of heavy metals in contaminated water (Chang et al., 2000; Elliott

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http://dx.doi.org/10.1016/j.catena.2016.08.022
0341-8162/

et al., 1998). The microbial sulfide precipitation process was mainly used for AMD remediation (Benedetto et al., 2005; Jong and Parry, 2003; Tsukamoto et al., 2004); however, applications of microbial immobilization process are unusual approaches for soil remediation.

The objective of the present study was to examine the potential for As immobilization in soil by stimulating the indigenous SRB under reducing condition. The batch experiments were carried out anaerobically with glucose amendment for stimulation of indigenous bacteria. Field experiments were conducted to examine the microbial immobilization of As as well. In order to assess immobilization efficiency, partial and total extraction of As from the soil were conducted before and after the supply of carbon source and sulfate.

## 2. Materials and methods

### 2.1. Soil sampling from an abandoned Au-Ag mine

Soil samples for the study were collected from the paddy field in the vicinity of the Sanyang Au-Ag mine, located in Boseong, Korea. The mine produced mainly gold (Au) until 1935. During 60 years of operation, 675 $m^3$ of mining waste was dumped without proper environmental management and led to As contamination to nearby environment (KMoE, 2001). Soil samples were taken from four points at the paddy field according to KMoE (2010). Soil samples were air dried at room temperature, and sieved using 10 and 80 mesh sieve. Soil pH, cation exchange capacity (CEC), loss on ignition (LOI), and size distribution were measured using under 10 mesh soil samples. In order to determine the extractable content of heavy metals (e.g. Cu, Pb, Cd, and Cr) and As, 0.1 N and 1 N of HCl were applied, respectively. The total concentrations of heavy metals and As were determined via aqua regia digestion, using under 80 mesh soil samples.

### 2.2. Batch experiments for the immobilization of As

The microbial immobilization of As in soil was carried out using 600 mL of serum bottles with butyl rubber cap for 80 days. Four soil samples were mixed to a composite sample for the batch experiments. Sieved soil (<2 mm) of 18 g was added to serum bottles and 540 mL of sterilized deionized water (DIW) was added. Glucose (5 mM) was supplied into the serum bottles as a carbon source. The injected glucose was sterilized using 0.2 μm syringe filter. Nitrogen ($N_2$) flushing was conducted during 1 h, at the beginning of the experiment, to remove oxygen in the bottles. The serum bottles were sterilized using autoclave at 121 °C for 15 min after $N_2$ flushing. The initial pH was adjusted at 6.5 using HCl (0.1 M) and NaOH (1 M).

Indigenous microbe inoculums were extracted from the studied soil under wet condition. For the inoculum extraction from the soil, 5 g of wet soil were mixed with sterilized 5 mM HEPES solution (50 mL). The mixture was agitated at 150 rpm for 1 h, and supernatant (10 mL) was transfer to microbial experimental sets. Sterilized DIW of the same amount with bacterial inoculum was added to abiotic, control sets. The biotic and abiotic experimental serum bottles were stored at 25 °C and, periodically, solution samples were taken with syringe for measurement of pH, As, Fe, and $SO_4^{2-}$. All experiments were carried out duplicated.

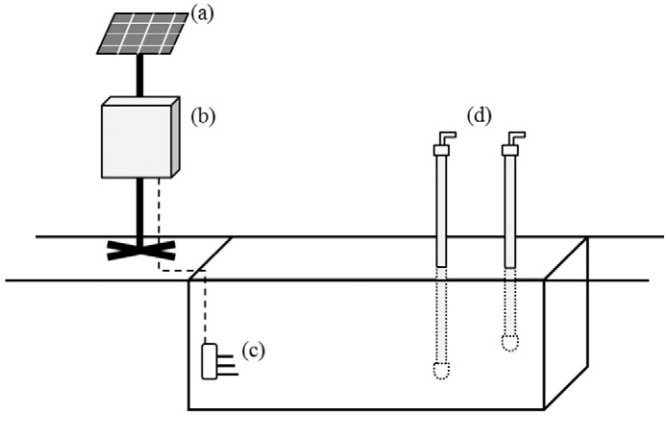


**Fig. 1.** Schematic diagram of field experimental plots. (a) solar cell, (b) data loader, (c) in-situ electrode, and (d) lysimeter.

**Table 1**
Physical and chemical properties of the studied soil.

| Properties | The studied sample[1)] | Reference values for Korean soil[2)] |
|---|---|---|
| pH | 5.9 ± 0.2 | 5.5–6.0 |
| Organic matter (%) | 3.0 ± 0.4 | 2–5 |
| CEC (mEq 100 $g^{-1}$) | 10.3 ± 0.3 | 0.4–37.8 |
| Sand (%) | 75.9 ± 5.1 | 38.3 |
| Silt (%) | 23.9 ± 5.0 | 41.8 |
| Clay (%) | 0.2 ± 0.1 | 20.0 |
| Soil texture | Loamy sand | |

1) Mean ± standard deviation of 4 samples.
2) ASI (1985) and RDA (1988).

### 2.3. Field experiments

Immobilization of As in contaminated soil was investigated by stimulation of indigenous SRB at field site. In order to implement the field study, three experimental plots were prepared in the paddy field near the abandoned Au-Ag mine. The field plot size was 2 m × 2 m × 0.5 m (Fig. 1). Waterproof cloths were located on the side of experimental field plots. Lysimeters were installed at depths of 0.2 m, 0.5 m, and 0.8 m, to collect pore water from field plots. After 100 days, 100 L of 20 mM glucose was added to plots A and B; in addition, the same amount of groundwater was supplied to plot C as a control. Sulfate (1000 mg $L^{-1}$) was added only to plot B, to enhance the microbial sulfate reduction process. Periodically, pore water samples were taken using lysimeter with depth of 0.2 m, 0.5 m, and 0.8 m, to determine pH and concentration of As and heavy metals. The three depth soil samples were collected from two points using hand auger after 200 days.

### 2.4. Analytical methods

The pH was measured using a glass electrode connected to an Orion Model 720 A pH meter. Soluble iron, $Fe^{2+}$, concentration was determined by a modified ferrozine method (Lovley and Phillips, 1987; Stookey, 1970). Filtered aliquots were mixed with 0.1% ferrozine solution in a 0.5% ammonium acetate buffer of pH 7.0 (adjusted with NaOH) and mixing ratio of aliquots and ferrozine was 1:50. The solution was agitated, and analyzed using UV–vis spectrophotometer (UV-MINI-1240) at 562 nm. The concentration of $Fe^{3+}$ was calculated from the total dissolved Fe subtracted by the measured $Fe^{2+}$ concentration. The

**Table 2**
As and heavy metal content in the soil after total and partial extraction.

| Elements (mg $kg^{-1}$) | Total extraction[a,1)] | Partial extraction[a,2)] | Tolerable level (Kloke, 1979) |
|---|---|---|---|
| As | 71.2 ± 16.0 | 21.2 ± 3.2 | 20 |
| Cd | 4.8 ± 0.0 | 2.1 ± 0.6 | 3 |
| Cr | 66.1 ± 0.1 | 0.6 ± 0.3 | 100 |
| Cu | 30.1 ± 2.3 | 6.4 ± 1.2 | 100 |
| Fe (%) | 3.4 ± 0.2 | | |
| Ni | 26.6 ± 1.4 | | 50 |
| Pb | 47.6 ± 5.8 | 12.5 ± 3.6 | 100 |
| Zn | 104.3 ± 13.1 | | 300 |

1) Aqua regia digestion.
2) 0.1 N and 1 N HCl extraction.
[a] Mean ± standard deviation of 4 samples.