J. Soil Groundw. Environ. Vol. 19(2), p. 16~24, 2014 http://dx.doi.org/10.7857/JSGE.2014.19.2.016

≪ Research Paper ≫

Effects of Extracellular Electron Shuttles on Microbial Iron Reduction and Heavy Metals Release from Contaminated Soils

Yun Ho Hwang[1] · Moo Joon Shim[2] · Du Hyun Oh[2] · Jung-Seok Yang[2] · Man Jae Kwon[1,2]*

[1]*Green School, Korea University*

[2]*Korea Institute of Science and Technology*

ABSTRACT

To test the potential effects of extracellular electron shuttles (EES) on the rate and extent of heavy metal release from contaminated soils during microbial iron reduction, we created anaerobic batch systems with anthraquinone-2,6-disulfonate (AQDS) as a surrogate of EES, and with contaminated soils as mixed iron (hydr)oxides and microbial sources. Two types of soils were tested: Zn-contaminated soil A and As/Pb-contaminated soil B. In soil A, the rate of iron reduction was fastest in the presence of AQDS and > 3500 mg/L of total Fe(II) was produced within 2 d. This suggests that indigenous microorganisms can utilize AQDS as EES to stimulate iron reduction. In the incubations with soil B, the rate and extent of iron reduction did not increase in the presence of AQDS likely because of the low pH (< 5.5). In addition, less than 2000 mg/L of total Fe(II) was produced in soil B within 52 d suggesting that iron reduction by subsurface microorganisms in soil B was not as effective as that in soil A. Relatively high amount of As (~500 mg/L) was released to the aqueous phase during microbial iron reduction in soil B. The release of As might be due to the reduction of As-associated iron (hydr)oxides and/or direct enzymatic reduction of As(V) to As(III) by As-reducing microorganisms. However, given that Pb in liquid phase was < 0.3 mg/L for the entire experiment, the microbial reduction As(V) to As(III) by As-reducing microorganisms has most likely occurred in this system. This study suggests that heavy metal release from contaminated soils can be strongly controlled by subsurface microorganisms, soil pH, presence of EES, and/or nature of heavy metals.

Key words : Extracellular Electron Shuttles, Anthraquinone-2,6-disulfonate, Iron reducing bacteria, Heavy metals release

1. Introduction

Iron is one of the most abundant elements in the earth's crust at approximately 5.0% by weight (McDonough and Sun, 1995). Iron is a redox sensitive element and exists mainly as two transition states, Fe(III) and Fe(II), according to redox states in surrounding area. In particular, Fe(III) is an important electron acceptor for microbial respiration (e.g., dissimilatory iron reducing bacteria (DIRB)) and DIRB can reduce Fe(III) to Fe(II) for their growth. In addition, iron plays an important role in contaminant reduction as reduced form of iron (i.e., Fe(II) phases) can reduce various inorganic and organic contaminants including heavy metals, explosives, and radionuclides (Fredrickson et al., 2000; Kwon and Finneran, 2010).

A variety of microorganisms in subsurface environments have shown to reduce Fe(III) to Fe(II). In particular, DIRB (e.g., *Geobacter* spp., *Shewanella* spp., *Anaeromyxobacter* spp.) can grow via reducing a variety of iron phases (e.g., soluble Fe(III), Fe(III) (hydr)oxides, mixed Fe(III)-Fe(II) iron oxides) coupled with the oxidation of inorganic (i.e., H_2) or organic compounds (e.g., acetate, lactate)(Caccavo et al., 1992; Roden and Zachara, 1996; Zachara et al., 2002).

Extracellular electron shuttles (EES) have been proposed as an electron transfer mediator between microorganisms and solid-phase minerals to stimulate Fe(III) reduction by eliminating the need for physical contact between microorganisms and iron (hydr)oxides (Lovley et al., 1996). EES are catalytic, therefore even small amount of EES can stimulate iron reduction. In addition, the ubiquity of Fe(III)-

*Corresponding author : mkwon@kist.re.kr

Received : 2014. 3. 12 Reviewed : 2014. 4. 30 Accepted : 2014. 4. 30

Discussion until : 2014. 6. 30

and EES-reducing microorganisms increases the likelihood that EES-mediated iron reduction can occur in many subsurface environments (Coates et al., 1998). In fact, many studies have shown that EES increased iron reduction under various conditions. For example, Lovley et al. (1998) reported that humic substances as EES stimulated the reduction of structural Fe(III) in clay, poorly crystalline Fe(III) hydroxides, and crystalline Fe(III) (hydr)oxides (i.e., goethite and hematite)(Lovley et al., 1998). A recent study also showed that the presence of anthraquinone-2,6-disulfonate (AQDS) enhanced the microbial reduction of iron oxides more than 15 times with xylose as a carbon source (Gerlach et al., 2011).

Strong correlations between iron (hydr)oxides and heavy metals have been reported in many pristine and contaminated environments (Cummings et al., 1999; Gounou et al., 2010; Lee et al., 2009). Heavy metals can be adsorbed onto iron (hydr)oxides which, in general, have large surface area. In addition, during the formation or precipitation of iron (hydr)oxides from groundwater and other aqueous environments, heavy metals can be incorporated in the structure of newly formed minerals. Therefore, heavy metals can be naturally eliminated from groundwater or surface water by adsorption or co-precipitation on these iron (hydr)oxides (Yun et al., 2001). However, these heavy metals can be released again by the physical, chemical, and biological processes occurring in contaminated environments. In particular, heavy metal release during iron reduction by subsurface microorganisms have drawn much wide attention (Gounou et al., 2010; Mitsunobu et al., 2012; Treeby et al., 1989) because of the abundance and ubiquity of iron (hydr)oxides and microbial iron reduction in subsurface environments. The release of heavy metals during microbial iron reduction can negatively impact on surrounding ecosystems. However, it is also possible that heavy metal release during microbial iron reduction can be applied for heavy metal leaching process for the remediation of contaminated soils (Ayyasamy et al., 2009).

Although many studies have reported the enhancement of iron reduction in the presence of EES, heavy metal release during EES-mediated iron reduction by indigenous subsurface microorganisms is largely unknown (Fig. 1). In this study, two types of soils contaminated by heavy metals were

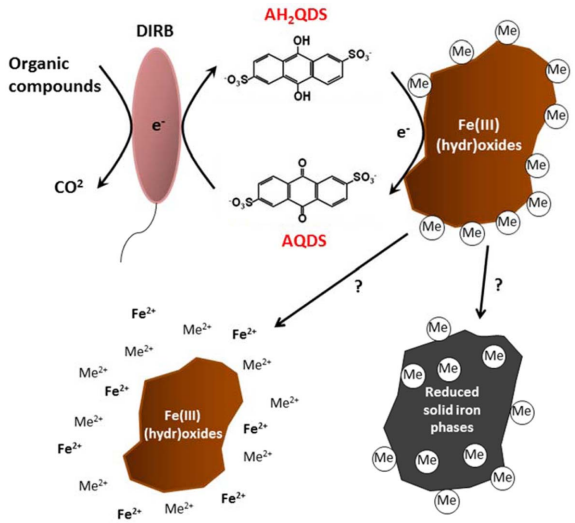

Fig. 1. Schematics of electron shuttle-mediated iron reduction and its potential impacts on heavy metal release or immobilization. DIRB: dissimilatory iron reducing bacteria, AQDS: anthraquinone-2,6-disulfonate, AH_2QDS: anthrahydroquinone-2,6-disulfonate, Me: metal.

tested for iron reduction and heavy metal release during microbial iron reduction in the presence or absence of AQDS. We used AQDS as a surrogate of EES and organic carbon compounds (i.e., acetate, lactate, glucose) as the electron donor to stimulate microbial iron reduction.

The objectives of this study are to 1) determine whether EES will stimulate biological iron reduction in the presence or absence of electron donor, 2) investigate whether EES will enhance heavy metal release under various experimental conditions, and 3) examine the various environmental factors controlling heavy metal release from contaminated soils.

2. Material and methods

2.1. Chemicals

AQDS was obtained from Sigma Aldrich (USA). Sodium hydroxide, nitric acid, and hydrochloric acid were obtained from Sigma Aldrich (USA) or Junsei Chemical Co. (Japan). All chemicals used were of reagent grade quality or higher, and all aqueous solutions were prepared with 18-Mohm-cm water. De-ionized water was prepared using a Millipore water purification system (Barnstead, USA).

2.2. Soils

Soil A was obtained from waste pile left after soil

washing process while soil B was collected from near weathered mine tailings in Gangneung, S. Korea. To minimize microbial growth and contamination, soils were kept in a refrigerator (< 4°C) and in airtight container before batch test.

The soils were homogenized in a N_2-filled glove bag prior to processing for individual experiments. Each soil was mixed well after eliminating visible gravels, roots using sterilized spatula.

2.3. Soil incubations

Approximately 50 g of soil and 100 mL of freshwater (FW)-medium were dispensed into 160 mL serum bottles in an $N_2 : H_2$ (= 95 : 5)-filled glove bag that were then sealed with a thick butyl-rubber stopper. After removal from the glove bag, the headspace of each bottle was flushed with 80 : 20 (vol/vol) $N_2 : CO_2$ that had been passed over hot copper filings to remove traces of oxygen. Acetate, lactate, or glucose was added as a sole electron donor at a final concentration of 10 mM. AQDS was added as EES at 0.1 mM. All amendments were made from sterile, anaerobic stock solutions. All subsequent amendments or transfers were made using sterile needles and syringes that had been flushed with anaerobic gas. Experimental conditions including the composition of FW-medium are shown in Table 1.

All experiments were controlled in anaerobic conditions. All the bottles were incubated in the dark at room temperature without agitation. In order to generate abiotic controls, the sediment bottles were autoclaved for 1 h per day for 3 consecutive days (Finneran and Lovley, 2001). All experiments except controls were performed in duplicate.

Samples (~3.6 mL) were collected periodically for total Fe(II) and heavy metal analysis via anoxic syringe and needle. The reduction of iron (hydr)oxides was monitored by measuring the Fe(II) content of 0.5 M HCl extracts of the suspensions (one volume of suspension added to an equal volume of anoxic 1 M HCl) using the ferrozine assay as described in the next section. Samples for heavy metal analysis were filtered through centrifugation prior to analyses. No more than seven samples were taken from any incubation; therefore, the final volume of each incubation was approximately ~75 mL at the end of the experiments (75% volume remaining).

Table 1. Experimental conditions and medium compositions

Experimental Conditions	
Temp (°C)	~ 25
Medium volume (mL)	100
Anoxic	$N_2 : CO_2 = 80 : 20$
Aquifer material (g)	50
AQDS (mM)	0.1
Electron donor (mM)	
Acetate	10
Lactate	10
Glucose	10
Bicarbonate buffer (mM)	30
Medium Composition	**/L**
Millli QH_2O (mL)	800
$NaHCO_3$ (g)	2.5
NH_4Cl (g)	0.25
NaH_2PO_4-H_2O (g)	0.6
KCl (g)	0.1
Vitamin mix (mL)	10
Mineral mix (mL)	10
1 mM Na_2SeO_4 (mL)	1

The similar batch systems were created to determine the concentrations of FW-medium extractable heavy metals. The soils with FW-medium were incubated by shaking at 120 rpm for 3 days at room temperature. Samples (2 mL) were collected at day 3 via anoxic syringe and needle, and samples were filtered through centrifugation prior to analyses.

2.4. Analytical techniques.

Total Fe(II)(i.e., 0.5 N HCl-extractable Fe(II)) was quantified by the Ferrozine assay (Stookey, 1970). Briefly, 1 mL of HEPES (50 mM)-buffered ferrozine reagent was added to 0.05 mL of sample, and the Fe(II) concentration was measured at 562 nm with a spectrophotometer (DR2800, HACH, USA). Total concentrations of heavy metals were determined using aqua regia digestion with nitric acid and hydrochloric acid (1 : 3). The concentrations of As, Cd, Cu, Ni, Pb, Zn in the filtrated samples were analyzed by an inductively coupled plasma-optical emission spectrophotometer (ICP-OES, 730-ES, Varian Inc., USA). Sulfate concentrations in aqueous phases were determined by a colorimetric method (DR2800, HACH, USA)(Bertolacini and Barney, 1957). Initial sulfate concentration was <0.8 mg/L. The pH was

Table 2. The concentrations of heavy metals, water contents, dissolved organic carbons in Soil A and B. [1)]Total concentrations of heavy metals by aqua regia digestion; [2)]Concentrations of heavy metals by freshwater(FW)-medium extraction; [3)]ND: not detected; [4)]DOC: dissolved organic carbon by FW-medium extraction

		Soil A		Soil B	
		Total[1)]	FW-ext.[2)]	Total	FW-ext.
Metals (mg/kg)	As	15.3	0.1	26,862	0.9
	Cd	1.8	ND[3)]	2.4	ND
	Cu	29.7	ND	145.0	0.1
	Ni	6.8	ND	10.7	0.1
	Pb	153.3	ND	3,203	0.2
	Zn	401.8	0.8	190.1	4.3
Water content (%)		20.1		4.6	
Organic Carbon (%)		6.8		0.5	
Organic Matter (%)		9.4		0.6	
DOC[4)] (mg/L)		51.8		66.8	

measured by using pH meter (Mettler Toledo, USA) electrode. Loss on ignition (LOI) was determined according to the previous study (Howard and Howard, 1990). Dissolved organic carbon (DOC) in aqueous phases during FW-medium extraction was analyzed using a high temperature combustion process (Shimadzu TOC-VCPH, Japan).

3. Results and Discussion

3.1. Characterization of heavy metal contaminated soils

The initial concentrations of total and FW-medium extractable heavy metals are shown in Table 2. Total concentration of Zn in soil A was slightly higher than 400 mg/kg (worrisome level of Zn in Korean soil contamination standards is 400 mg/kg). However, the concentrations of all heavy metals extracted by the FW-medium were low. In case of soil B, total concentrations of As and Pb were extremely high while FW-medium extractable heavy metals were low, except Zn (4.3 mg/L). Water contents of soil A and B were approximately 20% and 5%, respectively. LOI of soil A and B were approximately 10% and 2%, respectively. The contents of DOC in solution phases of soil A and B were approximately 52 and 67 mg/L, respectively. The results of LOI and DOC indicated that the contents of organic matter was higher in soil A than in soil B, but organic matter in soil B existed as the easily soluble form.

3.2. Electron shuttle-mediated iron reduction in the presence of specific electron donor

To investigate the effects of electron donor and shuttle on microbial iron reduction, the rate and extent of iron reduction in soil A and B were monitored in the presence or absence of AQDS and specific electron donor. The pH in soil A with acetate or lactate was close to 7 and was maintained neutral for the entire experiment, while the pH with glucose pH decreased to below 6 in 8 days (data not shown). The pH decreases in glucose-amended incubations might be due to the fermentation of glucose to organic acid (e.g., acetate, lactate and propionate) and CO_2 by subsurface microbial communities under anaerobic conditions (Gounou et al., 2010). In fact, substantial headspace pressure in glucose-amended incubations was occurred during sampling events over time. The pH in glucose-amended incubations in soil A increased again from 6 to 7 within 42 days. Meanwhile pH in electron shuttles- and acetate-amended conditions was slightly decreased from 7 to 6.5. The pH in sterilized controls remained at 7 within 42 days. The pH in no addition control of soil B was 3.5 at the beginning of the experiment and then increased to 4.5. All other incubations of soil B showed the increase in pH from 4 to 5 over 52 days.

The concentrations of total Fe(II) increased within 2 days in both soil A and B (Fig. 2). Although microbial iron reduction in real soils generally took a few weeks to months, iron reduction in both soil A and B was occurred within 2 days suggesting that iron reducing microbial

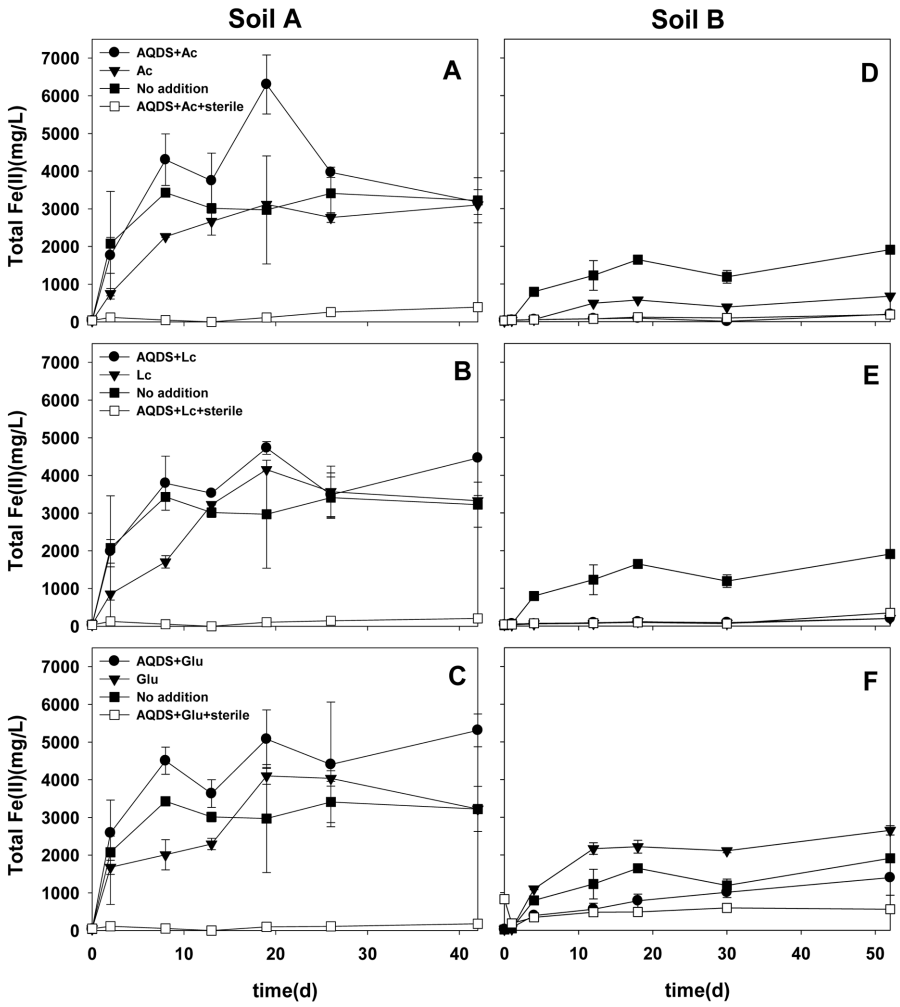

Fig. 2. Variation of total Fe(II) concentrations during microbial iron reduction in the presence or absence of AQDS as an electron shuttling compound and acetate, lactate, or glucose as an electron donor in contaminated soils. Soil A with acetate (A), lactate (B), or glucose (C). Soil B with acetate (D), lactate (E), or glucose (F). AQDS: anthraquinone -2,6-disulfonate, Ac: acetate, Lc: lactate, Glu: glucose.

community in these soils might be well adapted in the current experimental conditions including the FW-medium.

Microbial iron reduction by adding electron donors such as acetate, lactate and glucose was expected. Interestingly, the electron donor did not stimulate iron reduction in soil A as expected (Fig. 2A-C). This suggests that additional electron donors were not necessary for microbial growth in soil A. Assuming that most DOC (52 and 67 mg/L in soil A and B, respectively) were present as acetate, approximately 2 mM of acetate could be pre-existed in soil solutions in both soils, which is enough for and utilized by microbial growth as a carbon and energy source (Kwon et al., 2008).

Microbial iron reduction was enhanced in the presence of AQDS in soil A, while iron reduction was not stimulated in soil B (Fig. 2). It is well known that the reducing capacity of reduced AQDS decreases when pH is decreased from 9.2 to 6.2 (Kwon and Finneran, 2008). Thus, no increase in iron reduction in the presence of AQDS might be due to the lower pH (i.e., pH < 5) in the incubations of soil B. Unexpectedly, the microbial iron reduction in soil B was even decreased in the presence of AQDS. Although toxic effects of AQDS on acetoclastic methanogenic activity have been reported at the concentrations of 5-25 mM (Cervantes et al., 2000), it is not clear why the rate and extent of microbial iron reduction was decreased in the presence of low AQDS (0.1 mM) in the current study.

In soil B, iron reduction in no addition controls was initiated within 4 days. Amendment of acetate or lactate in

soil B decreased the rate and extent of iron reduction, but glucose stimulated iron reduction. Approximately 2000 mg/L of iron was reduced suggesting amending glucose might play an important role in higher extent of total Fe(II) in soil B. In fact, total Fe(II) did not increase in sterilized control, but remained stable at around 550 mg/L for the entire experiment. This suggested that the increase in total Fe(II) in glucose-amended incubations of soil B might be due to the dissolution of Fe(II) from soil B by glucose rather than the stimulation of glucose-fermenting microorganisms capable of iron reduction.

Less than 2000 mg/L of total Fe(II) was produced in soil B within 52 days, but total Fe(II) in soil A accumulated up to (> 6000 mg/L) indicating that iron reduction by subsurface microorganisms in soil B was not as effective as that in soil A. Lower extent of microbial iron reduction might be due to lower pH and higher concentrations of heavy metals in soil B. It is also possible that microbial iron reduction in soil B might be limited in the presence of nitrates (Cooper et al., 2003) because of preferential respiratory electron flow to nitrate over Fe(III). However, as shown in figure 2D, the iron reduction occurred in 4 days and total Fe(II) concentrations were relatively stable for the rest of the experiments suggesting that the inhibition of iron reduction by the presence of nitrate was not significant. Also, electron donors in the soil incubation were not limited and thus microbial competition could be diminished.

3.3. Heavy metal release during electron shuttle-mediated iron reduction

Effects of EES-mediated iron reduction on heavy metal release from contaminated soils were investigated and compared between soil A and B (Fig. 3). Again, AQDS as EES stimulated microbial iron reduction only in soil A because of lower pH in soil B. In the incubations of soil A, the concentrations of As, Zn, and Pb released in aqueous phases were insignificant within 26 days. The concentrations of these dissolved metals were lower than those in solution during FW-medium extraction (Table 2). These results suggest that microbial iron reduction and EES-mediated iron reduction may not release heavy metals from contaminated soils. Previous studies reported that microbial iron reduction of heavy metal contaminated soil and sediments can release heavy metals into porewater and groundwater under anaerobic conditions (Cummings et al., 1999; Gounou et al., 2010; Mcheik et al., 2013). However, the reductive transformation of iron (hydr)oxides can result in the formation of secondary iron minerals such as magnetite, siderite, vivianite, and mackinawite depending on surrounding geochemical conditions (Bae and Lee, 2013; Zachara et al., 2002). Therefore, it is possible that heavy metals might be adsorbed again on the surfaces of these newly formed secondary minerals or incorporated in their structures.

In soil B, the concentrations of dissolved Pb and Zn were close to those by FW-medium extraction and decreased over time (Fig. 3G and 2H). Dissolved Zn in the sterilized control of soil B was stable at around 3 mg/L, except at day 30. Lower pH might result in higher concentrations of dissolved Zn in the sterilized control of soil B. It is well known that solubilities of heavy metals are relatively high under acidic conditions (Chuan et al., 1996). However, reduced amounts of dissolved Zn in AQDS or no addition incubations suggest that Zn might be removed by adsorption or incorporation on and/or in a newly formed iron minerals.

Unlike Zn and Pb, the concentrations of dissolved As in soil B increased up to 500 mg/L in the presence or absence of of AQDS, while those in the sterilized control were less than 1.3 mg/L at the end of the experiments (Fig. 3F). The results suggest that As was released in the solution during microbial iron reduction. Two possibilities can result in the increase in dissolved As in soil B. First, direct enzymatic reactions of As(V)-reducing bacteria might reduce As(V) to As(III) which is more soluble than As(V), and therefore As might be released in the aqueous phases. It has been reported that over 95% of As in soils collected from the same area of soil B was in the form of scorodite ($Fe_3As_5O_4 \cdot 2H_2O$), as confirmed by X-ray Absorption Fine Structure (XAFS) analysis (Kwon et al., 2013). Second, As might be released during microbial iron reduction. Total Fe(II) and dissolved As during incubations regardless of the presence of AQDS were highly correlated ($r^2 = 0.69$-0.87). Therefore, it is possible that As sorbed on or incorporated in iron (hydr)oxides in soil B might be released during reductive dissolution of iron (hydr)oxides by dissimilatory

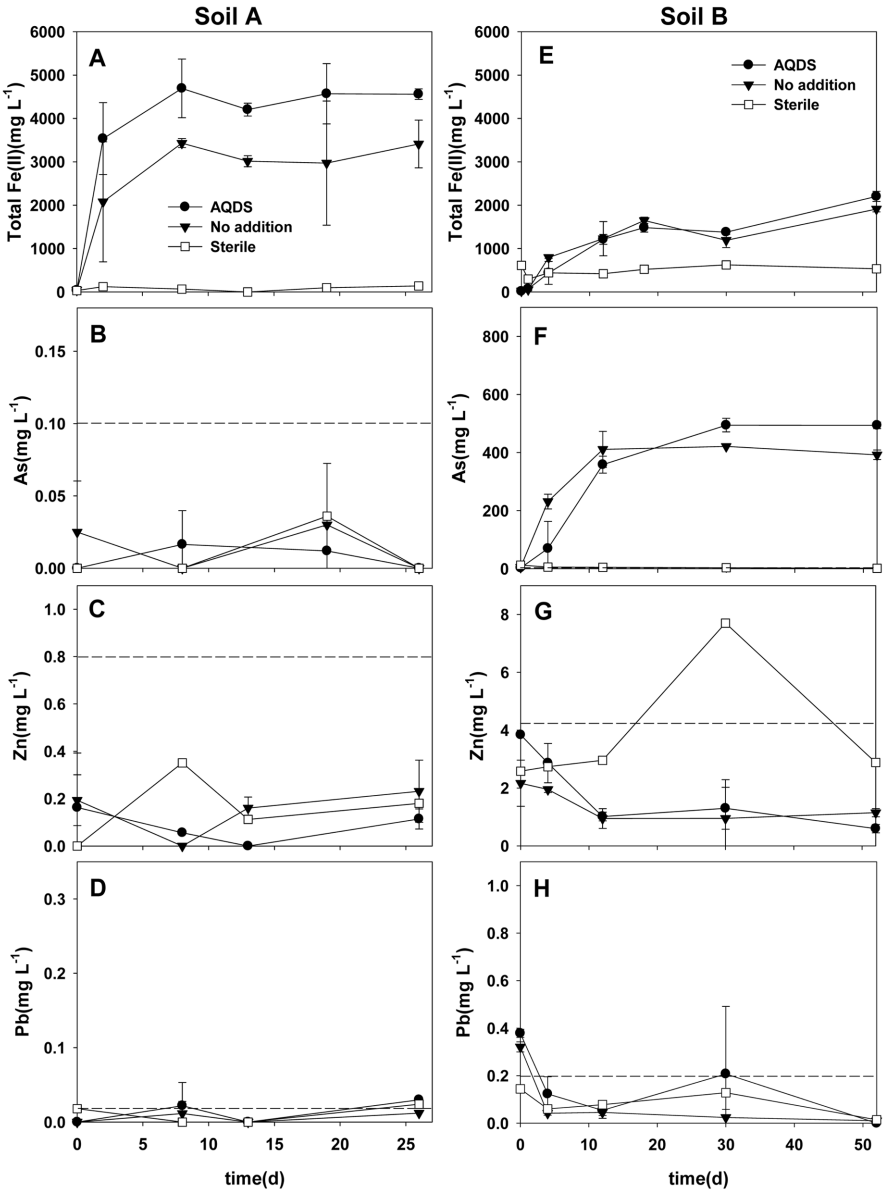

Fig. 3. Variation of total Fe(II) and dissolved heavy metal concentrations during microbial iron reduction in the presence or absence of AQDS in contaminated soils. Total Fe(II) (A), As (B), Zn (C), or Pb (D) in soil A. Total Fe(II) (E), As (F), Zn (G), or Pb (H) in soil B. The dashed lines indicate the concentrations of dissolved heavy metals by freshwater(FW)-medium extraction. AQDS: anthraquinone-2,6-disulfonate.

iron reducing microbial communities. However, given that Pb and Zn in aqueous phase under the same conditions were < 0.3 mg/L and < 4 mg/L, respectively, for the entire experiment, the microbial reduction As(V) to As(III) by As-reducing microorganisms was most likely occurred in this system. In addition, As(III) produced by the microbial As reduction might be complexed with DOC (Liu and Cai, 2010), which could subsequently accelerate As release rate from soil B.

4. Conclusions

The results demonstrated that AQDS as EES stimulated microbial iron reduction in soil A with pH > 6.5, but did not stimulate iron reduction in soil B with pH < 5.5. This suggests that AQDS-mediated iron reduction is highly pH dependent. In addition, adding acetate, lactate, or glucose as the electron donor did not stimulate the microbial iron reduction likely due to the preexistence of organic carbons

in both soils. This implies that organic carbons in these soils were present as easily usable form (mainly easily extractable organic carbon) for iron reducing microorganisms. The results also showed that microbial iron reduction and/or EES-mediated iron reduction did not result in the release of heavy metals from the contaminated soils (except As in soil B). This is possibly because of adsorption or incorporation of dissolved heavy metals on and in the newly formed secondary iron minerals. However, As in soil B was released in the aqueous phases during microbial iron reduction. Direct enzymatic reduction of As(V) to As(III) might be responsible for the increase in As under this condition.

The findings in this study suggest that heavy metal release from contaminated soils can be strongly controlled by subsurface microorganisms, soil pH, presence of EES, and/or nature of heavy metals. This study also implies that immobilization or mobilization of heavy metals from contaminated soil and sediment during in-situ bioremediation should be carefully examined for successful outcomes.

Acknowledgements

This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government (MEST) (2012, University-Institute cooperation program) and the Korea Ministry of Environment under "The GAIA Project-2012000550004 and 2013000540005".

References

Ayyasamy, P.M., Chun, S., and Lee, S., 2009, Desorption and dissolution of heavy metals from contaminated soil using *Shewanella* sp. (HN-41) amended with various carbon sources and synthetic soil organic matters, *J. Hazard. Mater.*, **161**, 1095-1102.

Bae, S. and Lee, W., 2013, Biotransformation of lepidocrocite in the presence of quinones and flavins, *Geochim. Cosmochim. Acta*, **114**, 144-155.

Bertolacini, R. and Barney, J., 1957, Colorimetric determination of sulfate with barium chloranilate, *Anal. Chem.*, **29**, 281-283.

Caccavo, F., Blakemore, R.P., and Lovley, D.R., 1992, A hydrogen-oxidizing, Fe (III)-reducing microorganism from the Great Bay Estuary, New Hampshire, *Appl. Environ. Microbiol.*, **58**, 3211-3216.

Cervantes, F.J., van der Velde, S., Lettinga, G., and Field, J.A., 2000, Competition between methanogenesis and quinone respiration for ecologically important substrates in anaerobic consortia, *FEMS Microbiol. Ecol.*, **34**, 161-171.

Chuan, M., Shu, G., and Liu, J., 1996, Solubility of heavy metals in a contaminated soil: Effects of redox potential and pH, *Water Air Soil Poll.*, **90**, 543-556.

Coates, J.D., Ellis, D.J., Blunt-Harris, E.L., Gaw, C.V., Roden, E.E., and Lovley, D.R., 1998, Recovery of humic-reducing bacteria from a diversity of environments, *Appl. Environ. Microbiol.*, **64**, 1504-1509.

Cooper D.C., Flynn, W. P., Arndt, S., and Aaron, J.C., 2003, Chemical and Biological Interactions during Nitrate and Goethite Reduction by *Shewanella putrefaciens* 200, *Appl. Environ. Microbial.*, **69**, 6 3517-3525.

Cummings, D.E., Caccavo, F., Fendorf, S., and Rosenzweig, R.F., 1999, Arsenic mobilization by the dissimilatory Fe (III)-reducing bacterium *Shewanella alga* BrY, *Environ. Sci. Technol.*, **33**, 723-729.

Finneran, K.T. and Lovley, D.R., 2001, Anaerobic degradation of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA), *Environ. Sci. Technol.*, **35**, 1785-1790.

Fredrickson, J.K., Zachara, J.M., Kennedy, D.W., Duff, M.C., Gorby, Y.A., Li, S.-m.W., and Krupka, K.M., 2000, Reduction of U(VI) in goethite (á-FeOOH) suspensions by a dissimilatory metal-reducing bacterium, *Geochim. Cosmochim. Acta*, **64**, 3085-3098.

Gerlach, R., Field, E.K., Viamajala, S., Peyton, B.M., Apel, W.A., and Cunningham, A.B., 2011, Influence of carbon sources and electron shuttles on ferric iron reduction by *Cellulomonas* sp. strain ES6, *Biodegrad.*, **22**, 983-995.

Gounou, C., Bousserrhine, N., Varrault, G., and Mouchel, J.-M., 2010, Influence of the iron-reducing bacteria on the release of heavy metals in anaerobic river sediment, *Water Air Soil Poll.*, **212**, 123-139.

Howard, P. and Howard, D., 1990, Use of organic carbon and loss-on-ignition to estimate soil organic matter in different soil types and horizons, *Biol. Fert. Soils*, **9**, 306-310.

Kwon, M. and Finneran, K., 2008, Biotransformation products and mineralization potential for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in abiotic versus biological degradation pathways with anthraquinone-2,6-disulfonate (AQDS) and *Geobacter metallireducens*, *Biodegrad.*, **19**, 705-715.

Kwon, M. and Finneran, K., 2010, Electron shuttle-stimulated RDX mineralization and biological production of 4-nitro-2,4-diazabutanal (NDAB) in RDX-contaminated aquifer material,

Biodegrad., **21**, 923-937.

Kwon, M., Ham, B., Hwang, Y., Choi, J., Boyanov, M., Kemner, K., O'Loughlin, E., and Yang, J.-S., 2013, Geochemical characteristics and microbial community composition of toxic metal-rich sediments contaminated from mine tailings, *Mineral. Mag.*, **77**, 1533.

Kwon, M.J., Sanford, R.A., Park, J., Kirk, M.F., and Bethke, C.M., 2008, Microbiological response to well pumping, *Ground Wate*., **46**, 286-294.

Lee, J.-U., Lee, S.-W., Chon, H.-T., Kim, K.-W., and Lee, J.-S., 2009, Enhancement of arsenic mobility by indigenous bacteria from mine tailings as response to organic supply, *Environ. Int.*, **35**, 496-501.

Liu, G. and Cai, Y., 2010, Complexation of arsenite with dissolved organic matter: Conditional distribution coefficients and apparent stability constants, *Chemosphere*, **81**, 890-896.

Lovley, D.R., Coates, J.D., Blunt-Harris, E.L., Phillips, E.J.P., and Woodward, J.C., 1996, Humic substances as electron acceptors for microbial respiration, *Nature*, **382**, 445-448.

Lovley, D.R., Fraga, J.L., Blunt-Harris, E.L., Hayes, L.A., Phillips, E.J.P., and Coates, J.D., 1998, Humic substances as a mediator for microbially catalyzed metal reduction, *Acta Hydrochim. Hydrobiol.*, **26**, 152-157.

McDonough, W.F. and Sun, S.-S., 1995, The composition of the earth, *Chem. Geol.*, **120**, 223-253.

Mcheik, A., Fakih, M., Bousserrhine, N., Toufaily, J., Garnier-Zarli, E., and Hamieh, T., 2013, Biomobilization of heavy metals from the sediments affect the bacterial population of Al-Ghadir river (Lebanon), *Agriculture, Forestry and Fisheries*, **2**, 116-125.

Mitsunobu, S., Shiraishi, F., Makita, H., Orcutt, B.N., Kikuchi, S., Jorgensen, B.B., and Takahashi, Y., 2012, Bacteriogenic Fe (III) (oxyhydr)oxides characterized by synchrotron microprobe coupled with spatially resolved phylogenetic analysis, *Environ. Sci. Technol.*, **46**, 3304-3311.

Roden, E.E. and Zachara, J.M., 1996, Microbial reduction of crystalline iron (III) oxides: Influence of oxide surface area and potential for cell growth, *Environ. Sci.Technol.*, **30**, 1618-1628.

Stookey, L.L., 1970, Ferrozine-a new spectrophotometric reagent for iron, *Anal. Chem.*, **42**, 779-781.

Treeby, M., Marschner, H., and Römheld, V., 1989, Mobilization of iron and other micronutrient cations from a calcareous soil by plant-borne, microbial, and synthetic metal chelators, *Plant Soil*, **114**, 217-226.

Yun, S.-T., Jung, H.-B., and So, C.-S., 2001, Transport, fate and speciation of heavy metals (Pb, Zn, Cu, Cd) in mine drainage: Geochemical modeling and anodic stripping voltammetric analysis, *Environ. Technol.*, **22**, 749-770.

Zachara, J.M., Kukkadapu, R.K., Fredrickson, J.K., Gorby, Y.A., and Smith, S.C., 2002, Biomineralization of poorly crystalline Fe(III) oxides by dissimilatory metal reducing bacteria (DMRB), *Geomicrobiol. J.*, **19**, 179-207.