



Desorption and dissolution of heavy metals from contaminated soil using *Shewanella* sp. (HN-41) amended with various carbon sources and synthetic soil organic matters

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ABSTRACT

Heavy metals in soil are considered a major environmental problem facing many countries around the world. Contamination of heavy metals occurs in soil due to both anthropogenic and natural causes. During the last two decades, extensive attention has been paid to the management and control of soil contamination. Decontamination of heavy metals in the soil has been a challenge for a long time. Microbial solubilization is one of promising process for remediation of heavy metals from contaminated sites. In this study, we attempted to treat soil contaminated with heavy metals using a facultative anaerobic bacterium *Shewanella* sp. (HN-41). The effect of carbon sources on the dissolution and conversion of heavy metals was first investigated using a defined medium containing 1 g of highly contaminated soil to select the most effective carbon source. Among three carbon sources, namely glucose, acetic acid and lactic acid, glucose at 10 mM was found to be the most effective. Therefore, glucose was used as a representative carbon source for the second part of the biological treatment in the defined medium, amended with humic acid (HA) and anthraquinone-2,6-disulfonate (ADQS), respectively. Among the heavy metals, iron and manganese exhibited the highest dissolution efficiency in the medium supplemented with glucose at 10 mM. The rates of dissolution and removal of heavy metals were little bit higher in the medium amended with humic acid and ADQS. Per these results outlined above, a combined system of humic acid and ADQS incorporated with glucose was found to be effective for the removal of heavy metals from soil.

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1. Introduction

Heavy metals are a serious threat to soil quality due to their persistence after entering the soil [1]. It has been demonstrated repeatedly that heavy metals adversely affect biological functions in soil [2,3]. Commonly found heavy metals include iron, lead, mercury, arsenic, chromium, cadmium, nickel, zinc and copper [4]. Unlike most organic pollutants, heavy metals cannot be chemically or biologically degraded. Due to these constraints, remediation of sorbed metals in soil and metal crusts in earth is considered essential worldwide.

Several techniques are available for decontaminating soils containing heavy metals; most of them fall into two major categories: immobilization [5] and extraction [6]. Immobilization involves the fixation of heavy metals by solidification or stabilization, thereby preventing their migration into the ground water. Extraction procedures employ a combination of physical, chemical and biological

methods for the actual removal of heavy metals from soil; soil washing is a promising extraction technique that involves the transfer of heavy metals into a wash solution either by desorption or solubilization [7]. Different extractants, such as acids, bases, chelating agents, oxidizing agents and surfactants have been used for this purpose [8], but although these techniques are efficient in removing heavy metals from contaminated soil, they are very expensive and complicated for earth systems so that they have a limited potential application. The use of biological systems is of particular interest because of their environmentally friendly and biodegradable nature [9].

The effects of carbon sources on the remediation of heavy metals from water and soil system for biological processes have captivated the attention of many researchers. Webb et al. [10] used four different carbon sources, lactate, acetate, propionate and glycerol for the reduction of heavy metals and the production of sulphide from natural and constructed wetland. They observed that the bacterial growth and the rate of iron removal were higher after 67 h using acetate and propionate. Stoll and Duncan [11] measured the effectiveness of glucose for the bioaccumulation of metals (Cu^{2+} , Cr^{6+} , Cd^{2+} , Ni^{2+} and Zn^{2+}) from wastewater systems using *Sac-*

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charomyces cerevisiae. In another study by Lovley et al. [12], three different carbon sources, acetate, lactate and succinate were investigated for their potential use in the conversion of ferric iron from sediment samples. Recently, Sirianuntapiboon and Ungkaprasatcha [13] performed removal of lead and nickel from synthetic solutions amended with glucose as carbon sources using a bio-sludge process.

Humic substances are present in soil, water and sediment in both soluble and insoluble forms. These macromolecular substances are derived from plant, algal and microbial material [14]. The complexation of metal ions by humic acid is extremely important in affecting the retention and mobility of metal contaminants in soil and water. Arnold et al. [15] studied the association of triorganotin compounds with dissolved humic acid and found that assimilation is governed by the complexation of the corresponding triorganotin cation by negatively charged ligands (i.e., carboxylate and phenolate groups) of the humic acids. Tan et al. [9] reported that the total acidity values can be attributed to the sum of the carboxyl- and phenolic-OH content and that they indicate the cation exchange and complexing capacities of humic matter. A high total acidity value is indicative of a high cation exchange capacity (CEC) and complexing power.

Due to its enormous chelating capacity, humic acid is capable of detoxifying lakes that are affected by metal pollution [9]. Misra et al. [16] and Pandey et al. [17] reported the role of humic acid in the sequestration of metals by formation of soluble complexes. Stone et al. [18] achieved the removal of hematite from sediment using anthraquinone-2,6-disulfonate (ADQS) with ferrozine and zinc as complexants. Several authors used individual carbon substrates and synthetic soil organic matter for the removal of heavy metals from water systems. However, there is no combined system yet tested using carbon sources and soil organic matter on the desorption and dissolution of heavy metals from soil. Therefore, in this study a combined system is attempted, using different carbon sources such as glucose, acetic acid and lactic acid incorporated with humic acid and ADQS for the efficient transformation of heavy metals from soil.

2. Materials and methods

2.1. Sample collection and analysis

Metal contaminated soil was collected in pre-cleaned, acid washed plastic containers for the analysis of heavy metals; the samples came from a mountain slope in the Bucheon region located in South Korea. Soil was carefully transported to the laboratory in an ice-box and stored at 4 °C in the laboratory. Soil pH was measured by pH-electrode with ORION 520A digital meter. Carbon and sulphur were analyzed using an elemental analyzer (Multi EA 2000, Analytik Jena) and heavy metals were analyzed using ICP-AES (Spectro, Genesis) according to norms APHA [19]. The quality assurance of trace metal analysis in the soil was checked by routinely analysing certified international reference material (SRM2709 for soils) and including reagent blanks in digestion. The precision and bias of chemical analyses were less than 10%.

2.2. Organisms and adaptation

In this study, *Shewanella* sp. was selected because of its commercial availability, metal degrading abilities and physical versatility. *Shewanella* sp. (HN-41) was obtained from the Department of Earth and Environmental Sciences, Chonnam National University, South Korea. Cells were grown in 500 ml of triptic soy broth (30 g/l) and incubated on a rotary shaker at 30 °C and 120 rpm for 15 h. Cells

were then centrifuged at 10,000 rpm for 20 min and washed three times in PIPES [piperazine-1,4-bis(2-ethanesulfonic acid)] solution at 20 mM concentration.

Prior to use, multi-tolerance was developed for this organism using triptic soy agar with various heavy metals included (Al^{2+} , As^{5+} , Mn^{4+} , Fe^{3+} , Cr^{6+} , Cu^{2+} , Cd^{2+} , Ni^{2+} , Pb^{3+} and Zn^{2+}). Triptic soy agar (per liter containing 17 g of casein, 5 g of NaCl, 3 g of soybean meal, 2.5 g of K_2HPO_4 , 2.5 g of glucose and 15 g of agar) was prepared and sterilized for 15 min at 15 lb pressure. About 100 mg/l of aluminium, manganese, iron, chromium, copper, cadmium, nickel, lead and zinc (filter sterilized, 0.2 μ m) was added and the medium was aseptically transferred into sterile Petri plates. The plates were kept overnight for surface drying. The *Shewanella* sp. (HN-41) was tested by spot inoculation on agar surface against various heavy metals. The plates were incubated at 30 °C for 72 h. After 72 h, the growth of the isolates was observed and the resistant isolates were stored on the same medium at 4 °C for further studies.

2.3. Preparation of inoculum

A loopful of culture was inoculated in presterilized 100 ml triptic soy broth. The flask was kept in a shaker at 120 rpm for 12 h at 30 °C. The culture broth was centrifuged at 10,000 rpm for 20 min. Cell suspension was prepared using sterile distilled water and adjusted to 0.5 OD (10^7 CFU/ml) using a UV-vis Spectrophotometer (model: HACH DR/4000U) at 600 nm. One milliliter (10^7 CFU/ml) of the above suspension was used as inoculum for the dissolution and removal of heavy metals from soil.

2.4. Effect of various carbon sources on metal removal in defined medium

Synthetic defined medium (DM) was used for this entire study and consisted of the following materials (per liter containing): 2.5 g of $NaHCO_3$, 0.08 g of $CaCl_2 \cdot 2H_2O$, 1 g of NH_4Cl , 0.2 g of $MgCl_2 \cdot 6H_2O$, 10 g of NaCl, 7.2 g of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 0.5 g of yeast extract [20]. About 200 ml of the defined medium was prepared in a 500-ml screw cap bottle and supplemented with 10 mM each of glucose, acetic acid and lactic acid, respectively, then sterilized for 15 min at 15 lb pressure. Further 1% of filter sterilized (Whatman No. 1 filter at 0.2 μ m pore size) trace elements (per liter containing 1.5 g of $C_6H_9NO_6$, 0.2 g $FeCl_2 \cdot 4H_2O$, 0.1 g of $MgCl_2 \cdot 6H_2O$, 0.02 g of $Na_2WO_4 \cdot 2H_2O$, 0.1 g of $MnCl_2 \cdot 4H_2O$, 0.1 g of $CoCl_2 \cdot 6H_2O$, 1 g of $CaCl_2 \cdot 2H_2O$, 0.05 g of $ZnCl_2$, 0.002 g of $CuCl_2 \cdot 2H_2O$, 0.005 g of H_3BO_3 , 0.01 g of $Na_2MoO_4 \cdot 2H_2O$, 1 g of NaCl, 0.017 g of Na_2SeO_3 and 0.024 g of $NiCl_2 \cdot 6H_2O$) and 0.1% of vitamin solution [per liter containing 0.02 g of $C_{10}H_{16}N_2O_3S$, 0.02 g of $C_{19}H_{19}N_7O_6$, 0.1 g of B_6 (pyridoxine) HCl, 0.05 g of B_1 (thiamine) HCl, 0.05 g of B_2 (riboflavin), 0.05 g of nicotinic acid, 0.05 g of nicotin acid, 0.05 g of pantothenic acid, 0.001 g of B_{12} (cyanocobalamin), 0.05 g of *para*-amino benzoic acid (PABA) and 0.05 g of lipolic acid] were added. To this 1 g of metal contaminated soil and 2 ml of bacterial inoculum (10^7 CFU/ml of the cells) were aseptically added and closed with a screw cap cork. Non-sterile soil was used in this study according to the natural soil environment. The control case was maintained with the same medium with soil but without the carbon sources and bacterial inoculum. Strict anaerobic conditions were maintained by flushing N_2 gas (99.99%) and keeping the test materials in a shaker (120 rpm) at 30 °C for 10 days. Every 24 h samples were drawn aseptically using a 5-ml micro-syringe and analyzed for bacterial growth by pour plate technique, for the amount of dissolved metals (Al, Cr, Mn, Fe, Cu, Ni, Zn, Cd and Pb) using ICP-AES (Spectro, Genesis) and for ferrous iron using the ferrozine assay method [21]. At the same time, the redox potential (Eh)

and pH of the samples were measured by an ORION 520A digital meter. At the end of 10th day, soils were separated by centrifugation (4000 rpm) and dried at 60 °C overnight, then analyzed for various heavy metals (Al, Cr, Mn, Fe, Cu, Ni, Zn, Cd and Pb) remaining in the soil.

2.5. Effect of humic acid and ADQS on the removal of heavy metals in the synthetic medium

Defined medium (DM) supplemented with 10 mM glucose was prepared and sterilized. Glucose was found to be an ideal carbon source in metal removal when compared to acetic acid and lactic acid; hence, glucose was used as a carbon source for this study. To this medium, 1 mg/l each of filter sterilized humic acid (HA) and anthraquinone-2,6-disulphonate (ADQS) were added individually as an electron shuttle. Further, 1 g of soil and 10^7 CFU/ml of the bacterial cells were added to the medium and kept in a shaker (120 rpm) at 30 °C for 10 days. The defined medium, with and without glucose, was maintained as a control, to compare efficiency of dissolution and removal of heavy metals by bacterial species. Periodically samples were drawn and analyzed for bacterial growth, Eh, pH and dissolved trace metals. At the end of 10th day, the heavy metals (Al, Cr, Mn, Fe, Cu, Ni, Zn, Cd and Pb) remaining in the soil were also estimated.

2.6. Statistical analysis

All experiments were conducted in triplicates and the rate of dissolution and the percentage of metal removal from soil were calculated with error bars. Both mean and standard deviation were performed where appropriate, using the statistical package within Microsoft® Excel (Version 2003).

3. Results

3.1. Characteristics of soil

The concentration of heavy metals and significant characteristics of soil were analyzed and the results are given in Table 1. Soil was found to be neutral and rich in heavy metals. Among the heavy metals in soil, the concentration iron was maximum (73,996 mg/kg) followed by aluminium (65,490 mg/kg). The presence of total organic matter (18,304 mg/kg) and elemental sulphur (685 mg/kg) in the soil act as an energy source for *Shewanella* sp. (HN-41) during the experiment. In order to improve the efficiency on the dissolution and removal of heavy metals, selected carbon source and synthetic soil organic matter were applied.

Table 1
Characteristics of soil used in dissolution study

S. No.	Heavy metals	Concentrations
1	pH	7.5 ± 0.3
2	TOC	18304 ± 98.3
3	Sulphur	685 ± 103.6
4	Aluminium	65490 ± 194
5	Chromium	176.4 ± 2.8
6	Manganese	722.8 ± 41.04
7	Iron	73995.6 ± 599.6
8	Copper	78.4 ± 2
9	Nickel	219.6 ± 20.2
10	Zinc	278.4 ± 5.29
11	Cadmium	8.8 ± 0.4
12	Lead	242.8 ± 20.5

Values are the mean (±S.E.) of triplicates. Values (except pH) are expressed in mg/kg.

3.2. Effect of carbon sources on the bacterial growth and dissolution of heavy metals from soil

The growth of *Shewanella* sp. (HN-41) and the dissolution of iron in the synthetic medium containing soil associated with the biological treatment system are shown in Fig. 1 for different carbon sources. The effects of various carbon sources (glucose, acetic acid and lactic acid) on the growth of bacterial species were found to be maximum (48×10^7 CFU/ml) in the medium supplemented with glucose, followed by acetic acid (46×10^7 CFU/ml) at 10th day. For all carbon sources the rate of dissolution increased rapidly up to 8 days and then maintained stable thereafter.

Rapid dissolution of iron was noticed in the medium supplemented with glucose at a concentration of 10 mM. The dissolution of ferrous iron increased up to 2087 mg/kg in the medium amended with glucose (Fig. 1b). In the cases of acetic acid and lactic acid, the dissolution showed increases of only 90 and 106 mg/kg, respec-

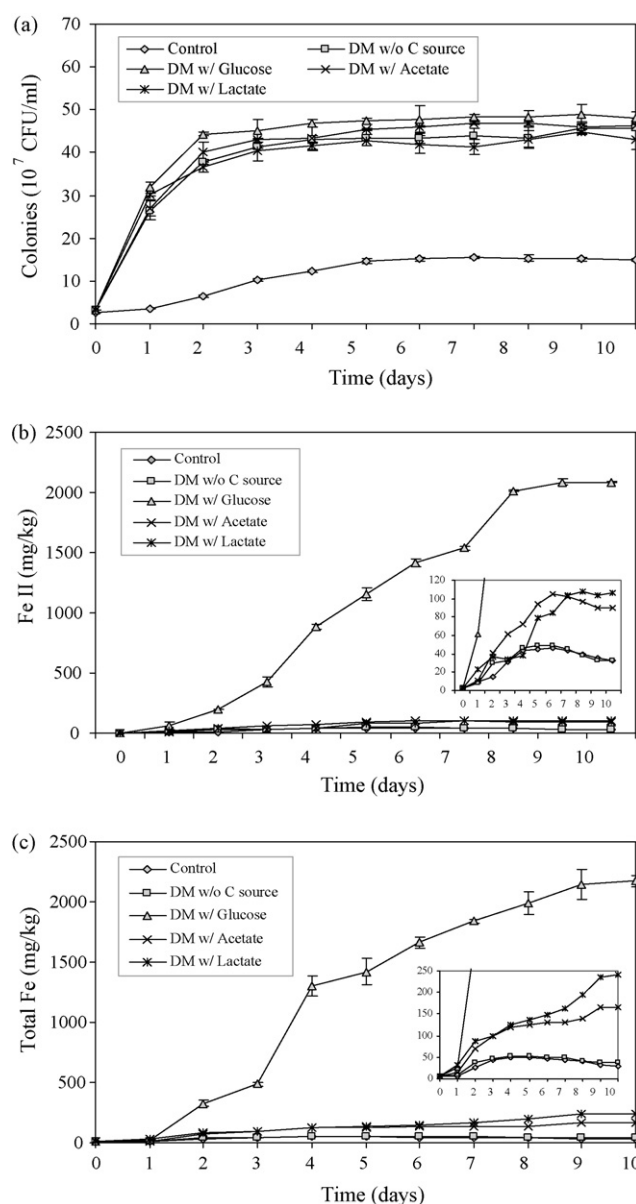


Fig. 1. Effect of various carbon sources on bacterial growth and dissolution of iron from soil: (a) bacterial growth; (b) dissolution of Fe(II); (c) dissolution of total Fe. DM, defined medium; w/, with; w/o, without.

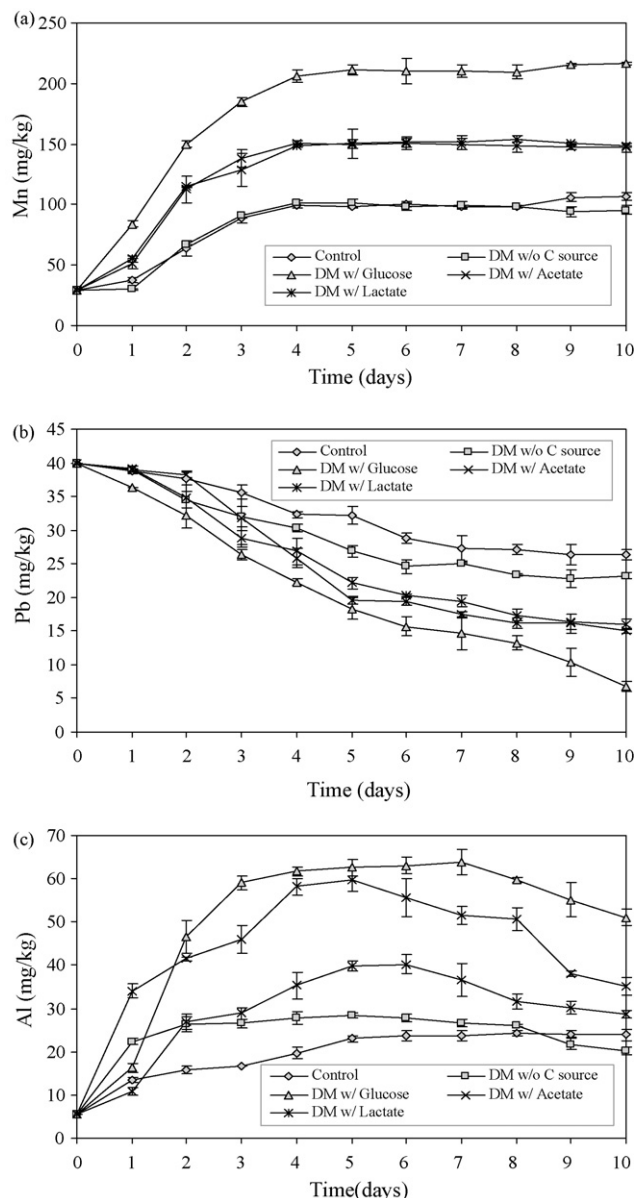


Fig. 2. Effect of various carbon sources on the dissolution of manganese, lead and aluminium from soil: (a) dissolution of Mn; (b) dissolution of Pb; (c) dissolution of Al. DM, defined medium; w/, with; w/o, without.

tively. Similar efficiency was noticed on the dissolution of total iron (Fig. 1c). The concentration of Fe was found to be higher in the synthetic medium compared with other heavy metals such as manganese, lead and aluminium.

Dissolutions of manganese, lead and aluminium in the synthetic medium containing soil are shown in Fig. 2. The oxidation, reduction and mineralization properties of manganese are similar to those of iron. In this element, the maximum dissolution of 211.5 mg/kg was recorded in the medium supplemented with glucose at 5 days followed by 150.2 mg/kg and 149.8 mg/kg using lactic acid and acetic acid, respectively (Fig. 2a). However, the rates of dissolution in the medium without carbon sources and in the control were found to be more or less similar. In the case of lead, the initial concentration was much higher (40 mg/kg) than iron, manganese and aluminium. It was decreased drastically from 40 to 6.8 mg/kg in the medium supplemented with glucose (Fig. 2b).

The dissolution of aluminium was found to be maximum (62.6 mg/kg) in the medium with glucose as a sole carbon source during 5th day incubation, compared with dissolution in the medium supplemented with acetic acid and lactic acid. For all carbon sources the rate of dissolution of aluminium increased rapidly up to 5 days and then decreased gradually thereafter (Fig. 2c). In other elements, such as chromium, copper, cadmium and nickel the dissolutions were found to be very negligible in the medium amended with various carbon sources (data not shown). Among the three candidate carbon sources, glucose at 10 mM showed the highest rate of metal dissolution; thus, glucose was selected as a representative carbon source for the dissolution of heavy metals in the second attempt in the synthetic medium supplemented with humic acid and ADQS.

3.3. Effect of humic acid and ADQS on the bacterial growth and dissolution of heavy metals from soil

The growth of *Shewanella* sp. (HN-41) and the dissolution of iron in the synthetic medium supplemented with humic acid and ADQS are shown in Fig. 3. The maximum growth of 50×10^7 CFU/ml was recorded on the 6th day in the synthetic medium supplemented with both humic acid and ADQS at a concentration of 1 mg/l as an additional nutrient substrate. The synthetic medium without humic acid and ADQS, the growth was found to be much less. The bacterium (HN-41) metabolized the maximum level of iron from the soil and increased the rate of dissolution in the medium amended with 1 mg/l of ADQS after incubation for 10 days.

In this study the maximum concentration of ferrous iron was noted in the synthetic medium supplemented with ADQS (2657 mg/kg), followed by humic acid (2242 mg/kg). In the medium without humic acid and ADQS, the dissolution of ferrous iron was much less. Similar efficiency was recorded for the level of total dissolved iron, in the medium supplemented with humic acid and ADQS.

The maximum dissolution of manganese (220 mg/kg) was noticed on 10th day, in the medium with ADQS as an additional nutrient supporter, whereas in the medium with humic acid the dissolution was less (214.4 mg/kg) (Fig. 4). The initial concentration of lead was found to be 40 mg/kg and *Shewanella* sp. (HN-41) gradually reduced it from 40 to 11.4 mg/kg in the medium amended with ADQS. The dissolution of other elements, such as chromium, copper, cadmium and nickel was found to be much less in the synthetic medium treated with humic acid and ADQS.

3.4. Effect of carbon sources, humic acid and ADQS on the removal of heavy metals from soil

The concentration of metals remaining in the soils was estimated at the end of the 10th day. Among the heavy metals in the soil, removal of manganese was highest (58.22%) in the medium amended with glucose as a sole carbon source, followed by cadmium (50%) and lead (23%). The removal of other heavy metals, such as aluminium, chromium, iron, copper, nickel and zinc were 17, 11, 14, 8, 20 and 4%, respectively. Among the three carbon sources, glucose showed the highest metal removal rate (Fig. 5a).

In the study performed with humic acid and ADQS, manganese showed the maximum (59%) removal in the medium amended with ADQS as an additional nutrient source, followed by lead (23%) and nickel (21%) (Fig. 5b). There was negligible metal removal noted in the medium without organic matters. However, there was no significant variation in the experiment conducted with humic acid and ADQS as additional growth promoters, compared with other carbon sources.

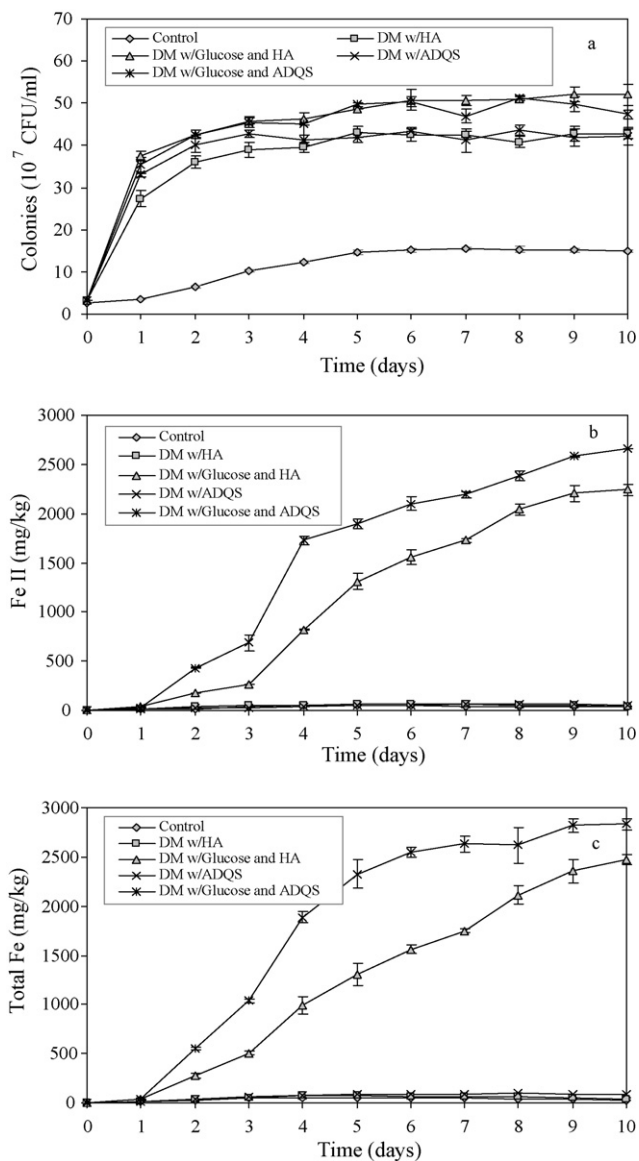


Fig. 3. Effect of humic acid and ADQS on bacterial growth and dissolution of iron from soil: (a) bacterial growth; (b) dissolution of Fe(II); (c) dissolution of total Fe. DM, defined medium; w/, with; w/o, without.

3.5. Effect of carbon sources and synthetic soil organic matter on Eh and pH of the medium

The influence of various carbon sources (glucose, acetic acid and lactic acid) on the Eh of the medium are shown in Fig. 6. The initial value of redox potential (Eh) was 168 mV, but after the 10th day it was reduced by *Shewanella* sp. (HN-41) from 168 to -26.2 mV in the synthetic medium supplemented with 10 mM glucose. Similarly the range of redox potential was reduced to 17 and 0.4 mV, respectively, in the medium with lactic acid and acetic acid. Among the three carbon sources, the Eh was reduced the most when using glucose at a concentration of 10 mM. The experiment studied with humic acid and ADQS produced somewhat less reduction of redox potential than the previous study; Eh was reduced from 168 to -42.2 and -25.4 mV, respectively, in the medium with humic acid and with ADQS.

The pH was decreased slightly from 7 to 6.84 in the synthetic medium supplemented with glucose, whereas in the medium with

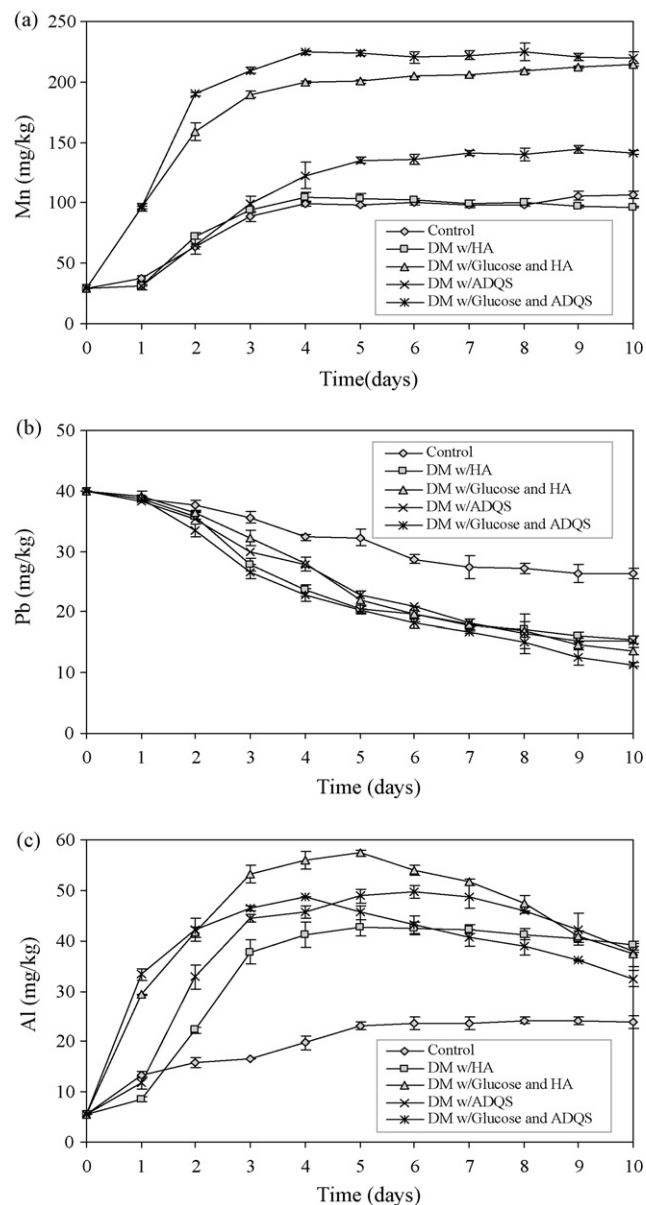


Fig. 4. Effect of humic acid and ADQS on the dissolution of manganese, lead and aluminium from soil: (a) dissolution of Mn; (b) dissolution of Pb; (c) dissolution of Al. DM, defined medium; w/, with; w/o, without.

acetic acid and lactic acid the pH was elevated to 7.3 (Fig. 6). The change of pH was significant in the medium without supplementation and bacterial inoculum, where it drastically increased from 7 to 7.65. Similar observation was noted in the synthetic medium supplemented with humic acid and ADQS. The result clearly indicates that the rate of dissolution and metal removal were drastically increased when the medium was slight acidic and had low redox potential.

4. Discussion

4.1. Dissolution of heavy metals in the synthetic medium with carbon sources, humic acid and ADQS

The *Shewanella* sp. (HN 41) solubilized a maximum amount of iron in the synthetic medium supplemented with glucose (10 mM). The concentration of ferrous iron was increased up to 2087 mg/kg.

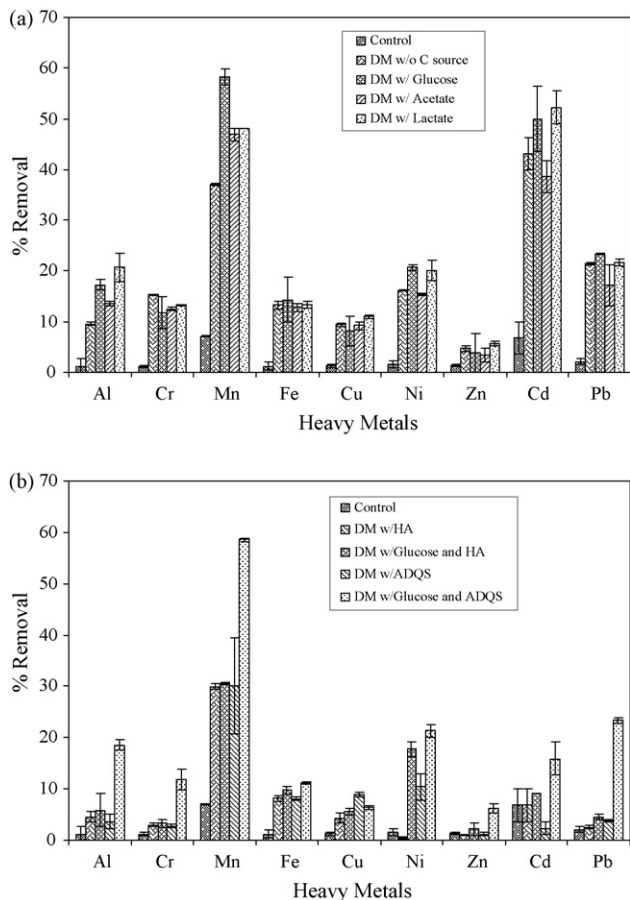


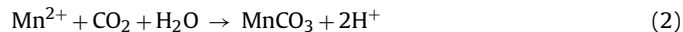
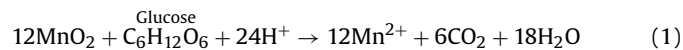
Fig. 5. Effect of various carbon sources, humic acid and ADQS on the transformation of heavy metals from soil: (a) DM amended with carbon sources; (b) DM amended with humic acid and ADQS. DM, defined medium; w/, with; w/o, without.

This might be due to the utilization of DM oxygen as an electron acceptor by bacteria and to the dissimilatory enzymatic action from the input concentration of 73,996 mg/kg of iron in soil. Arnold et al. [15] reported that *Shewanella putrefaciens* under anaerobic conditions is capable of respiratory-chain linked, high rate dissimilatory iron reduction, via both a constitutive and an inducible ferric iron reducing system. Other evidence indicates that ferric iron reduction may involve extracellular cytochromes which shuttle electrons to insoluble ferric iron [22]. Turick et al. [23] reported that *Shewanella* alga produced an extra cellular melanin that contained quinoid compounds that could act as the sole terminal electron acceptor for growth. Bacterially reduced melanin can also reduce ferric iron in the absence of cells, suggesting that the melanin can act as an electron shuttle between the cells and ferric iron oxides.

The efficiency of glucose in dissolving irons in the medium can be explained by the fact that our bacterial culture is a heterotroph and is capable of utilizing glucose as a carbon source. Most of the *Shewanella* sp. utilizes glucose as a nutrient substrate [24]. Our result clearly indicates that *Shewanella* sp. (HN 41) is capable of gaining energy from glucose as an electron donor and ferric iron as an electron acceptor. Under oxygen-limiting conditions, ferric iron can be reduced to ferrous iron. The dissimilatory ferric iron reduction under anoxic state runs usually according to the following reaction stated by Coates et al. [25]:



Like iron, the dissolved manganese was found to be at its maximum in the medium supplemented with glucose. Lee et al. [26] have used glucose as an organic substrate, to provide reducing power for the elimination of manganese, cobalt and nickel from manganese nodules. They also reported that insoluble Mn^{4+} in the nodules could be reduced to soluble Mn^{2+} by dissimilatory manganese reducing bacteria that use a carbon source and Mn^{4+} as their electron donor and acceptor, respectively. When glucose is supplemented under anaerobic conditions, the overall reactions involved in manganese reduction by Mn-reducing bacteria can be expressed by [27]:



Our results are in good agreement with the previous observation [26] where the glucose was used as an electron donor for the leaching of manganese from the nodules. Similarly the maximum dissolution of lead and aluminium were recorded in the medium supplemented with glucose. For all carbon sources, the dissolution of aluminium increased rapidly up to 5 days and then decreased gradually. This was mainly due to the active bacterial growth during the exponential stage metabolized metals as micro-elements, since metals were the only nutrient in the synthetic medium when a carbon source was not supplemented. However, the dissolution of other elements, such as chromium, copper, cadmium and nickel were found to be negligible in the synthetic medium with carbon sources. This may be due to the fact that iron and manganese may predominantly stimulate the bacterial growth as an additional micronutrient and thereby influence the dissolution of chromium, cadmium and nickel.

In the subsequent study using additional humic acid and ADQS, *Shewanella* sp. (HN-41) metabolized maximum levels of soil iron with glucose at 10 mM and ADQS at 1 mg/l. The concentration of ferrous iron was at maximum in the synthetic medium supplemented with ADQS. However, the dissolution of ferrous iron was relatively low in the medium with humic acid. According to the investigations reported by Lovley et al. [28] and Gonzalez-Gil et al. [29], ADQS acted as an electron shuttle between the bacterial species and the ferric iron in the soil and thereby greatly accelerated the rate of ferric iron reduction.

Bond and Lovley [30] studied the reduction of ferric iron oxide by methanogens in the presence and absence of ADQS. They found that addition of such organic matter produces greater efficiency in the dissolution of ferric iron when compared to the medium without supplementation. Lee et al. [31] used *Shewanella* sp. to hasten the dissolution of iron and chromium from Fe–Cr oxides with ADQS as electron transporter. Their result indicates that the rate of dissolution was notably enhanced in the medium amended with ADQS. In our study, the maximum dissolution of manganese and lead was noticed in the medium using ADQS as an additional nutrient supporter, whereas in the medium with humic acid the rate of dissolution was much less.

Removal of manganese was higher in the medium amended with glucose as a sole carbon source, followed by cadmium and lead. Similar efficiency was observed in the medium incorporating glucose and ADQS as an addition nutrient source. The rate of iron reduction was lower in the soil samples of higher iron contents (73,996 mg/kg). Iron oxides are supplying good sites for heavy metal adsorption in soils. When the soil is reduced, the iron oxides are reductively dissolved into ferrous iron and then the adsorbed metals are released as the iron oxides are dissolved. The dissolved ferrous iron in the medium formulated into complexation with bacterial biomass, organic

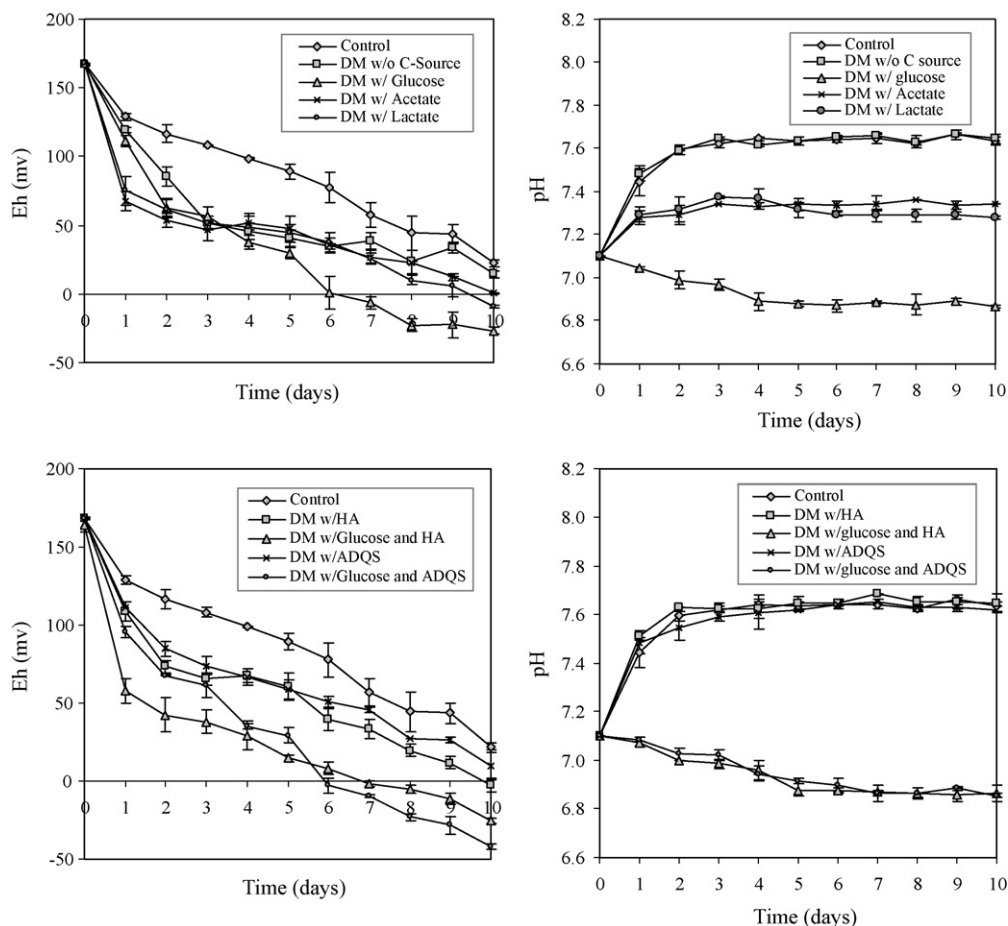


Fig. 6. Effect of various carbon sources, humic acid and ADQS on the range of Eh and pH during transformation of heavy metals from soil. DM, defined medium; w/, with; w/o, without.

matters and co precipitate with other heavy metals such as manganese, lead, aluminium, chromium, copper, cadmium, nickel and zinc.

4.2. Redox potential (Eh) and pH changes in the synthetic medium

The initial redox potential (Eh) was oxidizing, recording 168 mV. When media with bacterial inoculum were bubbled with nitrogen gas, redox potential decreased automatically from 167 to -26.2 mV in 10 days. Among the three carbon sources (glucose, acetic acid and lactic acid), Eh decreased intensely in the synthetic medium containing glucose. This might be due to more bacterial growth and the absence of dissolved oxygen in the medium. In the subsequent study, performed with humic acid and ADQS, the redox potential was found to be very low.

Glucose did not cause a major change in the pH level, whereas it was elevated up to 7.3 in the medium amended with acetic acid and lactic acid. The change of pH was also significant in the medium without carbon sources and bacterial inoculum, showing an increase from 7 to 7.65. Similar results were observed in the synthetic medium supplemented with humic acid and ADQS. Both redox potential and pH were found to greatly influence the solubility of heavy metals in soil. The rate of dissolution was drastically increased when the medium was slight acidic and had low redox potential. Chuan et al. [32] also investigated the effects of redox potential and pH value on metal solubilities in soil. They found that metal solubilities were higher under slightly acidic conditions (pH 5) and lower redox potential (-140 mV).

5. Conclusions

The rate of dissolution and reduction of heavy metals from soil by *Shewanella* sp. (HN-41) was influenced by various carbon sources, humic acid and ADQS. The removal of heavy metals was higher in the synthetic medium supplemented with 10 mM of glucose as a sole carbon source, compared with those in the media supplemented with acetic acid and lactic acid under anaerobic conditions at room temperature (30°C). Another set of experiments, conducted with humic acid and ADQS as an electron transporter, did not show no remarkable difference. Glucose at 10 mM could be suggested as the best carbon source to support the bacterial growth and also removal of heavy metals in soil.

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