

Development of a Microbe-Zeolite Carrier for the Effective Elimination of Heavy Metals from Seawater

In Hwa Kim^{1†}, Jin-Ha Choi^{1†}, Jeong Ock Joo², Young-Kee Kim³, Jeong-Woo Choi^{1,2}, and Byung-Keun Oh^{1,2*}

¹Department of Chemical and Biomolecular Engineering, ²Interdisciplinary Program of Integrated Biotechnology, Sogang University, Seoul 121-742, Republic of Korea

³Department of Chemical Engineering, Hankyong National University, Ansong 456-749, Republic of Korea

Received: April 24, 2015
Revised: May 28, 2015
Accepted: June 1, 2015

First published online
June 2, 2015

*Corresponding author
Phone: +82-2-705-8478;
Fax: +82-2-717-7008;
E-mail: bkoh@sogang.ac.kr

†These authors contributed
equally to this work.

pISSN 1017-7825, eISSN 1738-8872

Copyright© 2015 by
The Korean Society for Microbiology
and Biotechnology

The purpose of this study was to investigate the potential of zeolite-supported sulfate-reducing bacteria (SRB) in enhancing the removal of Cu²⁺, Ni²⁺, and Cr⁶⁺ in contaminated seawater. Our results show that SRB-immobilized zeolite carriers can enhance the removal of heavy metals. In addition, heavy metals were generally better removed at conditions of 37°C. Cu²⁺, Ni²⁺, and Cr⁶⁺ were effectively removed by 98.2%, 90.1%, and 99.8% at 100 parts per million concentration of the heavy metals, respectively. These results indicate that SRB-zeolite carriers hold great potential for use in the removal of cationic heavy metal species from marine environment.

Keywords: Zeolites, sulfate-reducing bacteria, carrier, bioremediation, heavy metal, *Desulfovibrio desulfuricans*

Several toxic heavy metals can accumulate in organisms, thus causing numerous diseases and disorders; these metals have been discharged into the environment as industrial waste, causing serious soil and water pollution [7, 13]. They are frequently used and discharged by many industries such as the leather tanning, mining operations, electroplating, metal finishing, paint and pigments, alloy, and storage batteries industries [18]. Various techniques can be employed to treat metal-contaminated water, such as electrochemical precipitation, ion exchange, ultrafiltration, and reverse osmosis [6, 12, 16, 17]. However, there are many major drawbacks involved in the pre- and post-treatment of wastewater, including high cost, low removal efficiency, and residual sludge [9].

The capability of zeolites to exchange cations is one of their most useful properties and determines their ability to remove heavy metals from industrial and processing waters and wastewaters. In structure, zeolites are composed of three-dimensional frameworks of SiO₄ and AlO₄ tetrahedra. The isomorphous replacement of Si⁴⁺ by Al³⁺, which is small enough to occupy the position in the center of the

tetrahedron of four oxygen atoms, produces a negative charge in the lattice. The net negative charge is balanced by the exchangeable cation (sodium, potassium, or calcium) and these cations can be replaced by heavy metal cations in solutions such as lead, cadmium, zinc, and manganese [1, 2]. Based on this property of zeolite, it can be suitable for the removal of heavy metal ions effectively from industrial effluent waters. For instance, cesium and strontium radioisotopes was removed and purified from wastewater by using natural zeolite [6].

Bioremediation, the use of microorganisms or microbial processes to degrade and detoxify environmental contaminants, is an attractive alternative to purify heavy-metal-contaminated water and sediments [11]. Bioremediation allows the selective elimination of heavy metals, can be economically performed at room temperature, and has a relatively high rate and good reproducibility for the removal of heavy metals [5]. For these reasons, there are various studies investigating the application of bioremediation using microbes. However, most research has been carried out in freshwater with aerobic bacteria, which is difficult to

translate to heavy metal removal in seawater because of the different conditions. In seawater, the oxygen concentration is relatively low and the salt concentration is higher than that in freshwater. Therefore, to apply this microorganism to the marine environment, they must be able to resist high salt concentrations as well as to adapt to low oxygen and conditions of non-existing sunlight.

In the present study, we fabricated a novel carrier for the effective removal of the heavy metals Cu^{2+} , Ni^{2+} , and Cr^{6+} in artificial seawater using *Desulfovibrio desulfuricans*, which belongs to the sulfate-reducing bacteria (SRB), attached to zeolite. *D. desulfuricans* is suitable for use in seawater with a high salt concentration and low oxygen environment. Moreover, SRB can effectively remove heavy metals by virtue of their physical adsorption and ionic attraction through metabolic processes with sulfates reduction [8, 14]. With these advantages, it is possible to maximize their removal capability using zeolite as a microbe carrier. The SRB-zeolite carrier is formed with a combining complex, which attaches SRB to the surface of the zeolite. It can function to efficiently eliminate heavy metals as a result of the combined removal effects of SRB and zeolite. Furthermore, it exhibits a superior removal capability and reusability than SRB alone because of zeolite's intensive immobilization on the zeolite surface and relative ease of collection. In this work, batch experiments were performed on two carriers, zeolite and the SRB-zeolite complex, by varying the metal ion concentration and temperature to study the effects on the removal of heavy metals.

Desulfovibrio desulfuricans (KCTC 5768) was used in this study. The cell culture medium was composed of K_2HPO_4 0.5 g/l, NH_4Cl 1 g/l, Na_2SO_4 1 g/l, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 2 g/l, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.1 g/l, and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g/l produced by Junsei, Japan; Resazurin 1 mg/l and sodium thioglycolate 0.1 g/l produced by Alfa Aesar, England; Yeast extract 1 g/l manufactured by Becton Dickinson, USA; and DL-sodium lactate 2 g/l and ascorbic acid 0.1 g/l made by Daejung, Korea. Artificial seawater medium was composed of NaCl 24 g/l, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 12 g/l, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 1 g/l, KCl 0.75 g/l, NaNO_3 0.04 g/l, K_2HPO_4 0.001 g/l, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 2 mg, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 0.5 mg/l, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 0.2 mg/l, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 2 μg /l, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 2 μg /l, $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ 1 mg/l, thiamine hydrochloride 300 μg /l, Na_2EDTA 12 mg/l, animobenzoate 20 μg /l, calcium pantothenate 10 μg /l, and cyanocobalamin 4 μg /l produced by Junsei; Tris-[hydroxymethyl]-aminomethane 1 g/l manufactured by USB, USA; and yeast extract 10 g/l [10]. The medium was purged with nitrogen gas to maintain an anaerobic environment. It was separated into a serum bottle and blocked off from air using

a rubber stopper and aluminum seal. The microorganism was inoculated at 2% (v/v) and incubated at 37°C for 48 h at pH 7.8. All media were sterilized for 15 min at 121°C. All experiments were performed in an anaerobic chamber.

We used zeolite as a carrier. Zeolite was filtered on a sieve to achieve a uniform size of 180–500 μm . It was subsequently washed with distilled water until free of impurities and dried for 24 h in an oven. Next, it was continuously stirred for 24 h in 0.1 M CaCl_2 solution for surface modification. Finally, it was washed twice with distilled water and dried in an oven for 24 h. *Desulfovibrio desulfuricans* adhered to the sterilizing carrier after culturing the microorganism for 48 h. The incubator temperature was 37°C and the microbe was stirred at 180 rpm for 7 days.

The resultant microbe carrier was washed twice with artificial seawater before heavy metal solution was added. We used scanning electron microscopy (SEM) to ensure that the microorganisms were well attached to the surface of the zeolite. First, 2% paraformaldehyde, 2% glutaraldehyde, and 0.05 M sodium cacodylate buffer were used in a modified Karnovsky's fixative process, followed by treatment with a 2% osmium tetroxide and 0.01 M sodium cacodylate buffer. Then the microbe carrier was measured by field emission-SEM. We used copper nitrate trihydrate ($\text{CuN}_2\text{O}_6 \cdot 4\text{H}_2\text{O}$), nickel sulfate hexahydrate ($\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$), and chromium oxide (CrO_3) produced by Sigma Aldrich, USA. The heavy metal was dissolved in distilled water and sterilized for 15 min at 121°C. The carrier was cultured in artificial seawater medium for 7 days at three different temperatures (5°C, 15°C, and 25°C) and three different concentrations (50, 100, and 200 ppm) of heavy metal solution. Then inductively coupled plasma (ICP) analysis was conducted.

D. desulfuricans, an SRB microbe, and zeolite are both effective materials for removal of heavy metal ions in wastewater. It is reasonably expected therefore that an SRB-attached zeolite carrier would exhibit high-performance removal capability. Fig. 1 shows the diverse processes used to treat heavy metal using an SRB-zeolite complex. It can be broadly divided into two mechanisms; metabolism-dependent and metabolism-independent processes. Metabolism-independent processes are based on a physical adsorption mechanism, which works *via* charge attraction. Generally, the surface of the microbe has a negative charge that can easily adsorb heavy metal cations. Zeolite also has a net negative charge due to the isomorphous replacement of Si^{4+} by Al^{3+} . Moreover, the crystalline pore structure of zeolite produces a widened surface area on which heavy metal cations can be adsorbed. Sulfate reduction and its precipitated

complex results from the second metabolism-independent process of the SRB microbe, which includes active transportation by the electron transport system. It has advantages for the sustainable elimination of heavy metal through the continuous sulfate reduction reaction and cell growth, derived from the metabolic mechanism.

In order to induce the metabolic effect of the SRB, as well as physical adsorption, it is necessary to attach additional SRB to the surface of zeolite. Because SRB have a strong negative charge, the negatively charged zeolite is temporarily made a positive direction by adding CaCl_2 in the reaction solution. Slightly negatively charged zeolites compared with natural zeolite become a superior supporting material for microbe attachment. As shown in Fig. 2, many SRB

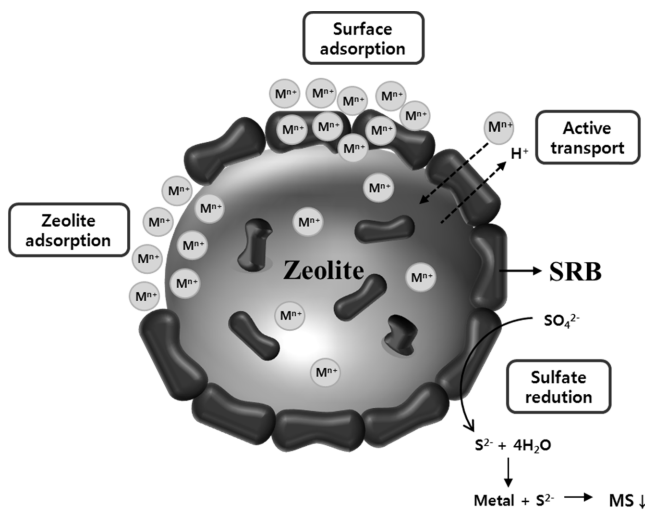


Fig. 1. Mechanism of heavy metal removal by the SRB-zeolite carrier complex.

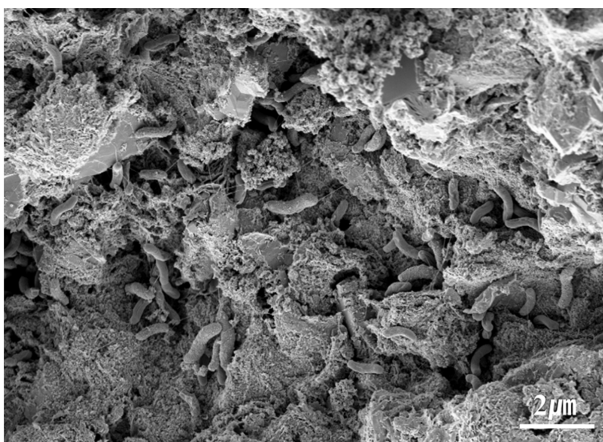


Fig. 2. SEM images of SRB-attached zeolite surface with CaCl_2 -treated solution.

microbes attach to the zeolite surface. It has a slightly negatively charged surface due to the Ca^{2+} attached to the surface of the zeolite, which helps the zeolite to adsorb more SRB and endows the zeolite with a greater removal ability.

To determine the heavy-metal-removing ability of the SRB-zeolite complex, the concentrations of heavy metals were measured by ICP analysis with natural zeolite and the SRB-zeolite carrier (Fig. 3A). Before the assay, appropriate amounts of heavy metal (100 ppm) were treated in the artificial seawater solution and calibrated to 100% quantity. In the case of the natural zeolite carrier, a relatively low

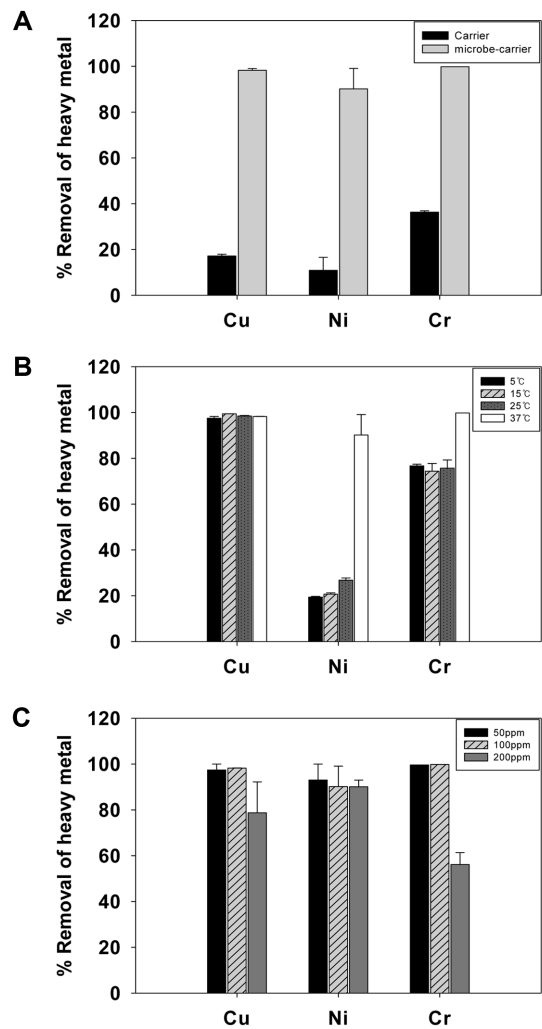


Fig. 3. Effective removal of heavy metals by using an SRB-zeolite complex.

(A) Removal ratio of heavy metal by natural zeolite and SRB-zeolite carrier in artificial seawater. (B) Heavy metal removal by the SRB-zeolite carrier at different temperatures. (C) Heavy metal removal by the SRB-zeolite carrier at various heavy metal concentrations.

removal of heavy metal was observed, from 10% to 40%. In contrast, the SRB-zeolite carrier demonstrated remarkably effective removal capacity, showing approximately 100% reduction for all three heavy metals. The SRB-zeolite carrier exhibited high removal efficiency, at least 2.5-fold greater than the natural zeolite carrier. In particular, the SRB-zeolite carrier removed 90.1% Ni²⁺, which was 8-times greater than what was obtained with the natural zeolite. These results therefore demonstrate that the SRB-zeolite carrier is an effective material for the reduction of heavy metals. SRB play an important role in the removal of heavy metal owing to their physical adsorption and metabolic processes.

The temperature of seawater varies depending on the area and vertical position, from -1°C to 30°C. Hence, it is necessary to study the effect of temperature on the removal of heavy metals by SRB. Fig. 3B shows the removal ratio of Cu²⁺, Ni²⁺, and Cr⁶⁺ at 5°C, 15°C, 25°C, and 37°C. The temperature of 37°C maximized the removal of heavy metals, except Cu²⁺, which was approximately 100% removed in all temperatures. Additionally, there were no significant differences between 5°C, 15°C, and 25°C. These results determine that 37°C is the optimal temperature for the effective elimination of heavy metals by an SRB-zeolite carrier. This might be related to the optimal temperature of the microbe, which is 37°C, typical of most microorganisms. Microbes function and grow actively in their optimal conditions, especially temperature, which is also directly related to the ability to remove heavy metal. Okabe *et al.* [15] also indicated that growth rate is related to temperature and dramatically decreases outside the optimal temperature range. Therefore, it is reasonable to consider 37°C the optimal temperature for an SRB-zeolite carrier to remove heavy metal.

Heavy-metal-contaminated sites in marine environments exist at a range of pollution levels, and each heavy metal has different environmental regulation levels. To assess the dependence of heavy metal removal on concentration, we carried out the experiment at three concentrations: 50 ppm, 100 ppm, and 200 ppm (Fig. 3C). At 50 ppm, Cu²⁺, Ni²⁺, and Cr⁶⁺ were eliminated by 97.4%, 93.0%, and 99.6%, respectively. At 100 ppm, these heavy metals were removed by 98.2%, 90.1%, and 99.8%, respectively. At 200 ppm, Cu²⁺, Ni²⁺, and Cr⁶⁺ were removed by 78.7%, 90.1%, and 56.1%, respectively. These results indicate that the developed carrier complex operates generally well in various concentrations of heavy metals. However, the removal ratio of Cr⁶⁺ dropped drastically at a concentration of 200 ppm, which might reflect the limited removal capability of the SRB-zeolite

carrier. Decreasing removal was also exhibited for Cu²⁺, which had a decrement of approximately 20%. Cabrera *et al.* [3] showed similar results, in that a high concentration of heavy metal is not well removed by bacteria.

In conclusion, we developed a novel biocarrier consisting of zeolite as a support carrier and *D. desulfuricans*, an SRB, for the effective removal of the heavy metals Cu²⁺, Ni²⁺, and Cr⁶⁺. The SRB-zeolite carrier could be applied as a novel, highly efficient capping technique for bioremediation in contaminated marine sediments. Thus, it is amongst the new technologies that derive their scientific justification from the emerging concepts of green chemistry and green engineering. This fast growing and highly promising remediation technique is increasingly being studied and applied practically for removing pollutants.

Acknowledgments

This research was a part of the project titled "Development of Sustainable Remediation Technology for Marine Contaminated Sediments" funded by the Ministry of Land, Transport and Maritime Affairs, Korea, and by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIP) (2015R1A2A2A01007843), and by the Sogang University Research Grant of 2014 (201410057.01).

References

1. Barer RM. 1978. *Zeolites and Clay Minerals as Sorbent and Molecular Sieves*. Academic Press, London-New York.
2. Breck DW. 1964. Crystalline molecular sieves. *J. Chem. Educ.* **12**: 678.
3. Cabrera G, Perez R, Gomez JM, Abalos A, Cantero D. 2006. Toxic effects of dissolved heavy metals on *Desulfovibrio vulgaris* and *Desulfovibrio* sp. strains. *J. Hazard. Mater.* **135**: 40-46.
4. Dabrowski A, Hubicki Z, Podkościelny P, Robens E. 2004. Selective removal of the heavy metal ions from waters and industrial wastewaters by ion-exchange method. *Chemosphere* **56**: 91-106.
5. Erdem E, Karapinar N, Donat R. 2004. The removal of heavy metal cations by natural zeolites. *J. Colloid Interf. Sci.* **280**: 309-314.
6. Hafez MB, Nazmy AF, Salem F, Eldesoki M. 1978. Fixation mechanism between zeolite and some radioactive elements. *J. Radioanal. Nucl. Chem.* **47**: 115.
7. Inglezakis VJ, Loizidou MD, Grigoropoulou HP. 2003. Ion exchange of Pb²⁺, Cu²⁺, Fe³⁺, and Cr³⁺ on natural clinoptilolite: selectivity determination and influence of acidity on metal uptake. *J. Colloid Interf. Sci.* **261**: 49-54.

8. Jong T, Parry DL. 2004. Adsorption of Pb(II), Cu(II), Cd(II), Zn(II), Ni(II), Fe(II), and As(V) on bacterially produced metal sulfides. *J. Colloid Interf. Sci.* **275**: 61-71.
9. Kadirvelu K, Thamaraiselvi K, Namasivayam C. 2001. Removal of heavy metals from industrial wastewaters by adsorption onto activated carbon prepared from an agricultural solid waste. *Bioresour. Technol.* **76**: 63-65.
10. Kim SJ, Park KW, Hur BK. 2000. Characteristics of linoleic acid production by marine fungi in sea water media. *Biotechnol. Bioeng.* **15**: 195-200.
11. Kumar A, Bisht BS, Joshi VD, Dhewa T. 2011. Review on bioremediation of polluted environment: a management tool. *Int. J. Environ. Sci.* **1**: 1079-1093.
12. Kurniawan TA, Chan GY, Lo WH, Babel S. 2006. Physico-chemical treatment techniques for wastewater laden with heavy metals. *Chem. Eng. J.* **118**: 83-98.
13. Lin SH, Juang RS, Hazard J. 2002. Heavy metal removal from water by sorption using surfactant-modified montmorillonite. *J. Hazard. Mater.* **92**: 315-326.
14. Muyzer G, Stams AJM. 2008. The ecology and biotechnology of sulphate-reducing bacteria. *Nat. Rev. Microbiol.* **6**: 441-454.
15. Okabe S, Nielsen PH, Charcklis WG. 1992. Factors affecting microbial sulfate reduction by *Desulfovibrio desulfuricans* in continuous culture: limiting nutrients and sulfide concentration. *Biotechnol. Bioeng.* **40**: 725-734.
16. Qdais HA, Moussa H. 2004. Removal of heavy metals from wastewater by membrane processes: a comparative study. *Desalination* **164**: 105-110.
17. Trivunac K, Stevanovic S. 2006. Removal of heavy metal ions from water by complexation-assisted ultrafiltration. *Chemosphere* **64**: 486-491.
18. Wu Y, Zhang S, Guo X, Huang H. 2008. Adsorption of chromium(III) on lignin. *Bioresour. Technol.* **99**: 7709-7715.