Appl. Chem. Eng., Vol. 34, No. 6, December 2023, 640-648
https://doi.org/10.14478/ace.2023.1064

Article

Bioremoval of Cadmium(II), Nickel(II), and Zinc(II) from Synthetic Wastewater by the Purple Nonsulfur Bacteria, Three *Rhodobacter* Species

Jin Yoo, Eun-Ji Oh*, Ji-Su Park, Deok-Won Kim***, Jin-Hyeok Moon****, Deok-Hyun Kim*****, Daniel Obrist****** and Keun-Yook Chung****,†**

Indoor Environment Division, Incheon Research Institute of Public Health and Environment, Incheon 22320, Republic of Korea
**Water and Land Research Group/Division for Natural Environment, Korea Environment Institute, Sejong 30147, Republic of Korea*
***EHS Part, Doosan Corporation Electro-Materials, Gimcheon 39541, Republic of Korea*
****Department of Earth Resources and Environmental Engineering, Hanyang University, Seoul 04763, Republic of Korea*
*****Department of Environmental and Biological Chemistry, Chungbuk National University, Cheongju 28644, Republic of Korea*
******Chemical Accident Investigation Team, National Institute of Chemical Safety, Cheongju 28164, Republic of Korea*
*******Department of Environmental, Earth, and Atmospheric Sciences, University of Massachusetts Lowell, MA 01854, USA*
(Received July 25, 2023; Revised October 31, 2023; Accepted October 31, 2023)

Abstract

The purpose of this study was to determine the inhibitory effect of heavy metals [Cd(II), Ni(II), and Zn(II)] on the growth of *Rhodobacter* species (*Rhodobacter blasticus*, *Rhodobacter sphaeroides*, and *Rhodobacter capsulatus*) and their potential use for Cd(II), Ni(II), and Zn(II) bioremoval from liquid media. The presence of toxic heavy metals prolonged the lag phase in growth and reduced biomass growth for all three *Rhodobacter* species at concentrations of Cd, Ni, and Zn above 10 mg/L. However, all three *Rhodobacter* species also had a relatively high specific growth rate against each toxic heavy metal stress test for concentrations below 20 mg/L and possessed a potential bioaccumulation ability. The removal efficiency by all strains was highest for Cd(II), followed by Ni(II), and lowest for Zn(II), with the removal efficiency of Cd(II) by *Rhodobacter* species being 66% or more. Among the three strains, *R. blasticus* showed a higher removal efficiency of Cd(II) and Ni(II) than *R. capsulatus* and *R. sphaeroides*. Results also suggest that the bio-removal processes of toxic heavy metal ions by *Rhodobacter* species involve both bioaccumulation (intracellular uptake) and biosorption (surface binding).

Keywords: *Bioremoval, Cadmium, Nickel, Zinc, Purple nonsulfur bacteria, Rhodobacter blasticus*

1. Introduction

Toxic heavy metal pollution of the aquatic environment has become a notable problem across the world owing to its non-biodegradability, bioaccumulation, and biomagnification in the food chain[1-3]. Conventionally, heavy metals are defined as elements with metallic features with an atomic number > 20 and atomic weights between 63.5 and 200.6[4-6], and they include a group of metals and metalloids [7,8]. In general, the term “Heavy metal” is considered to be any metallic element with a relatively high density and toxicity or poison at low concentrations. Low concentrations of heavy metals can be harmful to living organisms including humans[9]. Some of these metals, however, are also micronutrients necessary for plant growth (e.g., Co, Cu, Mn, Ni, and Zn), while others have an unknown biological function and are toxic (e.g., Cd, Hg, and Pb)[10].

Along with industrial development, such as metal plating factories, mining operations, fertilizer and pesticides industries, tanneries, battery manufacture, and paper industries, industrial wastewater including heavy metals are directly or indirectly released into the environment. In comparison with organic contaminants, heavy metals are likely to be not biodegradable and bioaccumulated in living cells, and the heavy metal ions can be toxic or carcinogenic. Toxic heavy metals such as cadmium, nickel, zinc, copper, mercury, lead, and chromium cause a significant hazard in industrial wastewater treatment. For example, cadmium exposes human health to severe risks, and chronic exposure to it causes kidney failure, and human exposure to its high levels can result in death[11]. Nickel exceeding critical levels might result in severe lung and kidney problems as well as gastrointestinal distress, pulmonary fibrosis, and skin dermatitis[12]. Moreover, it is known that nickel is a human carcinogen. Zinc is a trace element essential to the maintenance of human health. It is also a key element to the biological functions of the living cell and controls many biochemical processes. However, high levels of zinc can cause critical health problems, such as stomach cramps, skin irritations, vomiting, and anemia[13].

To avoid adverse effects of heavy metals, contaminated wastewater must be treated before being discharged into the environment. Conventional physicochemical techniques for removing heavy metals

† Corresponding Author: Chungbuk National University
Department of Environmental and Biological Chemistry, Cheongju 28644, Republic of Korea
Tel: +82-43-261-3383 e-mail: kychung@cbnu.ac.kr

pISSN: 1225-0112 eISSN: 2288-4505

from wastewater involve lime precipitation, chemical oxidation or reduction, ion exchange, electrochemical treatment, filtration, reverse osmosis, membrane technologies, and evaporative recovery[14]. However, these techniques have significant shortcomings, including low efficiency at low concentrations, high capital investment and operation costs of treatment systems, and production of toxic sludge[15,16]. Therefore, new technologies are required to reduce toxic heavy metal concentrations to environmentally safe levels in a cost-effective and environmentally friendly manner.

Bioremediation, which uses microorganisms to degrade contaminants in the environment, are cost-effective and environmentally friendly way to the treatment of contaminated wastewater with fewer by-products than physicochemical techniques. Bioremediation has recently received wide attention to remediating contaminated environments, including soils, lakes, streams, and groundwater[17-22] and it has been considered an alternative treatment technology[3].

Many investigators also observed that toxic heavy metals harmfully affect microbial growth, morphology, activity, and functioning, resulting in reduced microbial biomass[21,23-25]. On the other hand, microorganisms living in contaminated environments can have the capability to adapt and tolerate harsh environment in the presence of toxic heavy metals. For instance, extracellular polymeric materials or exopolymeric materials excreted by bacteria have been known as functioning as biosorbent agents by accumulating nutrients from the environment and contributing to the biosorption of toxic heavy metals[26-28].

In particular, purple nonsulfur bacteria (in taxonomy, Rhodobacteraceae) have been shown to resist toxic heavy metals[21,24,29-33]. A unique advantage of purple nonsulfur bacteria is that they can use not only solar radiation as an energy source under anaerobic conditions in the presence of light but also can use organic matter as the source of energy and carbon under aerobic and dark conditions[34]. The various metabolic activities such as photosynthesis and respiration by the *Rhodobacter* may permit the species to survive and thrive in diverse habitats[35,36]. Little information is still available about the removal of heavy metals by the PNAS, Rhodobacter. As far as we know, this is the first report about the removal of Cd, Ni, and Zn in industrial wastewater by R. *balsticus* among Rhodobacter species.

This work aimed to investigate: (i) the influence played by toxic heavy metals [Cd(II), Ni(II), and Zn(II)] on the photosynthetic growth of purple nonsulfur bacteria, especially *Rhodobacter* species; (ii) the potential of different *Rhodobacter* species for bioremediation of Cd(II), Ni(II), and Zn(II) in liquid media; and (iii) active and passive removal efficiencies of toxic heavy metals to reveal their biological removal mechanisms possibly through the intracellular uptake (bioaccumulation) and surface binding (biosorption).

2. Materials and methods

2.1. Bacteria and cultivation

Three *Rhodobacter* species used in this study were provided by the Korean Collection for Type Cultures (KCTC). *Rhodobacter blasticus* (KCTC 15056), *Rhodobacter sphaeroides* (KCTC 1434), and *Rhodobacter capsulatus* (KCTC 2583) were used in this study. The cells were inoculated in 100 mL modified ATCC medium No. 1170 at 2% (by volume). The cells were incubated in a shaking incubator having white LED (Light-emitting diode) lamps in properly controlled conditions. The optimum growth conditions were initial pH of 6.5~7; shaking speed of 200 rpm (rev/min); temperature of 30 °C on incubation; and light intensity of 3,000 lux (lm/m^2). To keep a culture for inoculation a live fresh culture, a new modified ATCC medium No. 1170 was inoculated every seven days. Five-day-old cells were inoculated for the growth experiment.

2.2. Liquid media contaminated with toxic heavy metals

To investigate the effects of toxic heavy metals on the growth and removal of toxic heavy metal by *Rhodobacter* species, the bacterial cell cultures grown in the exponential stage were inoculated into the bottles including modified ATCC medium No. 1170 added with Cd(II), Ni(II), and Zn(II). A 1000 mg/L stock solution of Cd(II), Ni(II), and Zn(II) were manufactured by solubilizing 1.2457 g $CdCl_2 \cdot 2½H_2O$, 1.8341 g $NiCl_2 \cdot 6H_2O$, and 1 g $ZnCl_2$, respectively, in 1 L of deionized water. The analytical grade of chemicals was used in the experiment.

2.3. Preparation of cell pellets of *Rhodobacter* species

Harvesting of cells in the exponential growth phase was performed by centrifugation at 8,000 rpm for 15 min and was washed twice with 0.1% of sterilized peptone water to acquire the cell pellets for evaluating the bioremediation of heavy metals.

2.4. Bioremoval of Cd(II), Ni(II), and Zn(II) by *Rhodobacter* species

All experiments were performed using the 200 mL culture flasks with a 150 mL sample of wastewater containing toxic heavy metals. The initial cell concentration was established at about 0.1 g dry wt./L. The culture flasks were grown at 30 °C and shaked at 200 rpm and illuminated at 3000 lux of light intensity by white LED lamps. All the culture flasks were prepared in duplicates.

2.5. Metabolism-independent (metabolic inhibition) study

The cell pellets obtained from each culture in the experiment were collected from a late log phase culture (60 hours) and were suspended in 1 M sodium azide (NaN_3) solution for 45 min to inhibit metabolic activity[33,37-39]. Sodium azide was known to enhance the production of $^1O_2$ in an aqueous solution as the nontoxic antimicrobial agent[38]. Cell suspensions were centrifuged and then washed with the sterilized 0.1% of peptone water to acquire the cell pellets to test the bioremediation of toxic heavy metals.

2.6. Metabolism-dependent study

Wet cell suspensions were manufactured as previously explained except that peptone, yeast extract, Na_2SO_4, and KH_2PO_4 were added to the toxic heavy metal solution at concentrations of 10 mg/L[33,37] and a control set contained no supplement of nutrients added and showed a clear solution.

2.7. Analysis methods

Samples were taken during the treatment for Cd(II), Ni(II), and Zn(II) analysis and photosynthetic bacterial growth measurement. For the analysis of heavy metals, the centrifugation of samples was performed at 3000 rpm for 30 min to prepare the supernatant with no biomass, and the resulting supernatant was then analyzed by following APHA standard methods. The supernatant was sterilized by a filter membrane (0.22 μm) and stored at 4 °C. The heavy metals were analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES 8000, PerkinElmer, America). The photosynthetic bacterial growth was measured at 535 nm[31] using a UV-VIS spectrophotometer (UV Mini 1240 Shimadzu, Japan).

2.8. Calculation of removal efficiency (%)

The removal performance (removal efficiency) by three *Rhodobacter* species was assessed by using the toxic heavy metal removal rate, R (in percent), which was defined by the following equation[40,41]:

$$R = \frac{C_i - C_f}{C_i} \times 100\% \quad (1)$$

Where, C_i and C_f were the concentration of toxic heavy metals in the initial and final stages after treatment in the solution, respectively.

2.9. Data presentation and statistical analysis

All results were obtained from independent triplicates from a single culture. These experiments were simultaneously carried out and results were expressed as mean values with the standard deviations, which were shown by the error bars in the figures.

All data were statistically analyzed by SAS Version 8.2 (SAS Institute, Cary, NC, USA). The significant differences in mean values were statistically analyzed through the use of Fisher's least significant difference test (LSD; P = 0.05).

3. Results

3.1. Effect of Cd(II), Ni(II), and Zn(II) on the photosynthetic bacterial growth

Figure 1 shows the growth curves of three species of *Rhodobacter (R. blasticus*, *R. capsulatus*, and *R. sphaeroides*) in the presence of Cd(II). After the cultures were inoculated, they showed an initial density of about 0.03-0.04 absorbance units (a.u) at 535 nm. *R. blasticus* tended to have long lag periods among three *Rhodobacter* species and entered exponential growth after an average of 75 hours in the presence of Cd(II); the lag period in the presence of Cd(II) was about three times greater than that in plain media without Cd. The maximum absorbance measurements obtained in plain (0 mg/L Cd), 5 mg/L Cd, 10 mg/L Cd, 20 mg/L Cd, and 80 mg/L Cd were 2.21, 1.41, 1.21, 0.27, and 0.30 a.u, respectively. For *R. capsulatus*, exponential growth started concurrently after a lag period of 51 hours on average in the presence of Cd(II), whereas it entered exponential growth after about 24 hours in the absence of Cd(II). Maximum absorbance observed in plain, 5 mg/L Cd, 10 mg/L Cd, 20 mg/L Cd, and 80 mg/L Cd were 2.01, 1.30, 1.17, 0.34, and 0.20 a.u, respectively. For *R. sphaeroides*, exponential growth started after a lag period of 39 hours on average in the presence of Cd(II). Maximum absorbance observed in plain, 5 mg/L Cd, 10 mg/L Cd, 20 mg/L Cd, and 80 mg/L Cd were 2.47, 1.67, 1.47, 0.54, and 0.48 a.u, respectively.

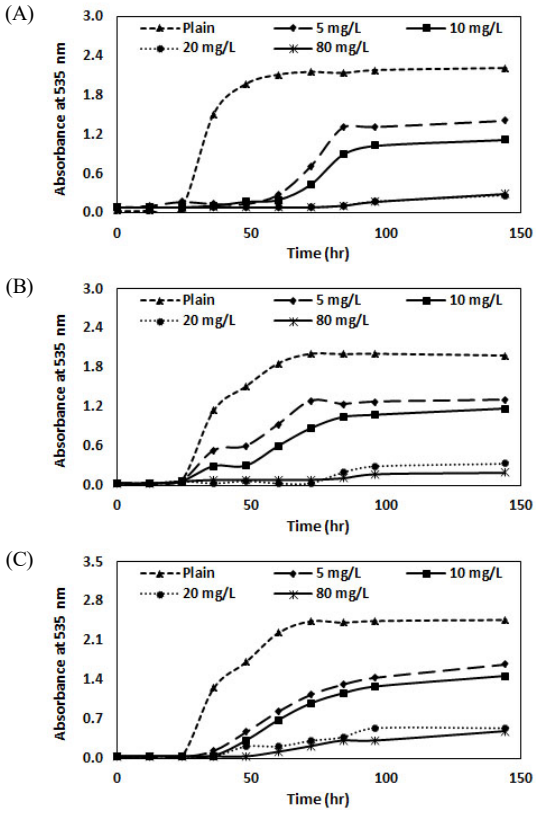

Figure 1. Growth curve of (A) *Rhodobacter blasticus*, (B) *Rhodobacter capsulatus*, and (C) *Rhodobacter sphaeroides* in the presence of Cd(II).

The growth curves for three species of *Rhodobacter* with Ni(II) added were shown in Figure 2. Exposure of the three species to Ni(II) showed similar patterns as observed in the presence of Cd(II) discussed above, with absorbance generally lower with increasing Ni(II) concentrations. The average lag times of the three microorganisms in the presence of Ni(II) were 54 hours, 48 hours, and 39 hours for *R. capsulatus*, *R. blasticus*, and *R. sphaeroides*, respectively.

Figure 3 shows the growth curves of the three *Rhodobacter* species in the presence of Zn(II). Exposure of the three species to Zn(II) again showed similar patterns with absorbance generally lower with increasing Zn(II) concentrations. The average lag time of the three microorganisms in the presence of Zn(II) was 48 hours, 36 hours, and 39 hours for *R. capsulatus*, *R. blasticus*, and *R. sphaeroides*, respectively. In the case of *R. capsulatus*, the absorbance value did not increase by more than 0.1 in 80 mg/L Zn, which means that it did not grow.

3.2. Bioremoval of toxic heavy metals [Cd(II), Ni(II), and Zn(II)] by *Rhodobacter* species

Comparisons between the ability of *Rhodobacter* species for the re-

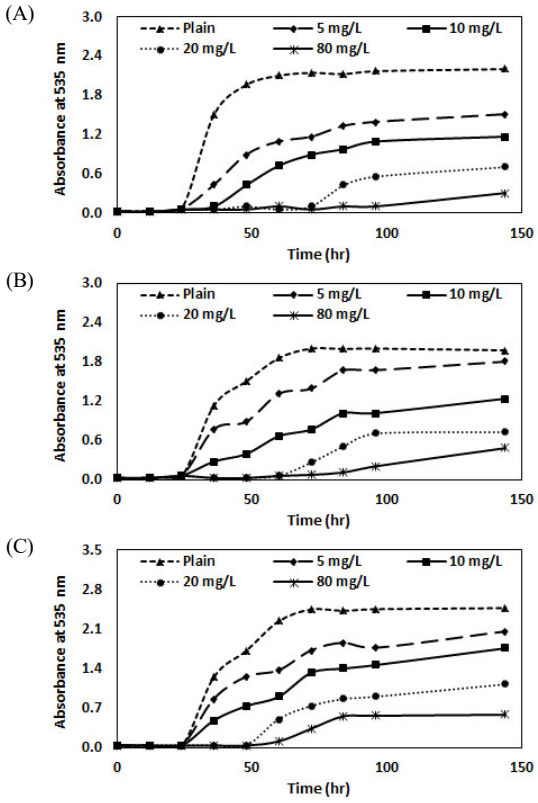

Figure 2. Growth curve of (A) *Rhodobacter blasticus*, (B) *Rhodobacter capsulatus*, and (C) *Rhodobacter sphaeroides* in the presence of Ni(II).

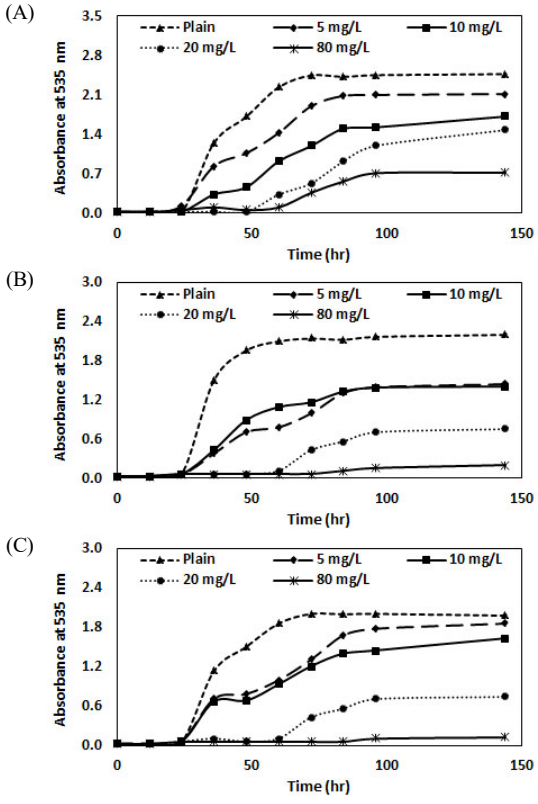

Figure 3. Growth curve of (A) *Rhodobacter blasticus*, (B) *Rhodobacter capsulatus*, and (C) *Rhodobacter sphaeroides* in the presence of Zn(II).

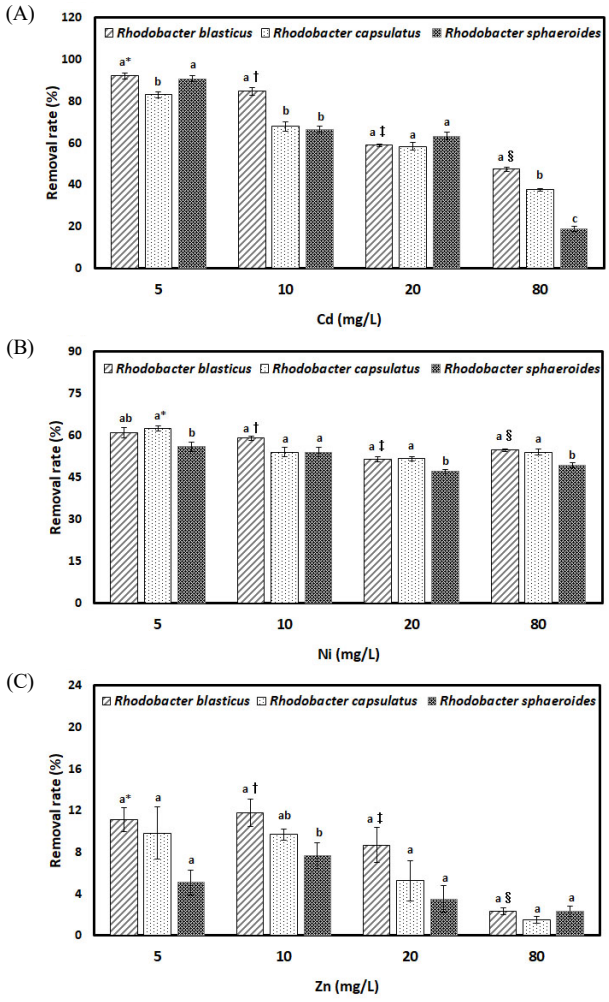

Figure 4. Removal rates of (A) Cd(II), (B) Ni(II), and (C) Zn(II) by *Rhodobacter blasticus*, *Rhodobacter capsulatus*, and *Rhodobacter sphaeroides* in liquid media contaminated under conditions of aerobic light. *, †, ‡, § Lowercase letters with numbers above bars using different letters indicate significant differences ($P < 0.05$).

moval of toxic heavy metals [Cd(II), Ni(II), and Zn(II)] are shown in Figure 4. The removal efficiencies for Cd by R. *blasticus*, R. *capsulatus*, and R. *sphaeroides* under different concentrations of Cd(II) solution (5, 10, 20, and 80 mg/L) were as follows: for 5 mg/L Cd: R. *blasticus*, R. *sphaeroides* > R. *capsulatus*; for 10 mg/L Cd: R. *blasticus* > R. *capsulatus*, R. *sphaeroides*; for 20 mg/L Cd: no significant differences were found for the removal efficiency of Cd by *Rhodobacter* species, and removal rates were 66% or more; and finally in 80 mg/L Cd: R. *blasticus* > R. *capsulatus* > R. *sphaeroides*.

Removal efficiencies for Ni by R. *blasticus*, R. *capsulatus*, and R. *sphaeroides* under different concentrations of Ni(II) solution (5, 10, 20, and 80 mg/L) were about 50%, in the following order: in 5 mg/L Ni, R. *capsulatuss* ≥ R. *blasticus* > R. *sphaeroides*; in 10 mg/L Ni, no significant differences were found; in 20 mg/L Ni: R. *blasticus*, R. *capsulatus* > R. *sphaeroides*; and finally in 80 mg/L Ni: R. *blasticus*, R. *capsulatus* > R. *sphaeroides*.

Overall, R. *blasticus* exhibited a higher ability for removal of Cd(II)

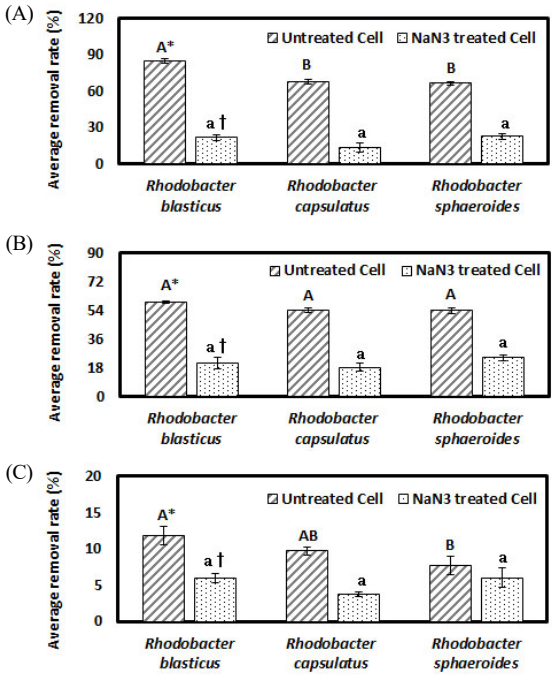

Figure 5. Effect of metabolic inhibition by sodium azide on the removal of (A) Cd(II), (B) Ni(II), and (C) Zn(II) by three *Rhodobacter* species under conditions of aerobic light. * Capital and † lowercase letters above bars using different letters indicate significant differences.

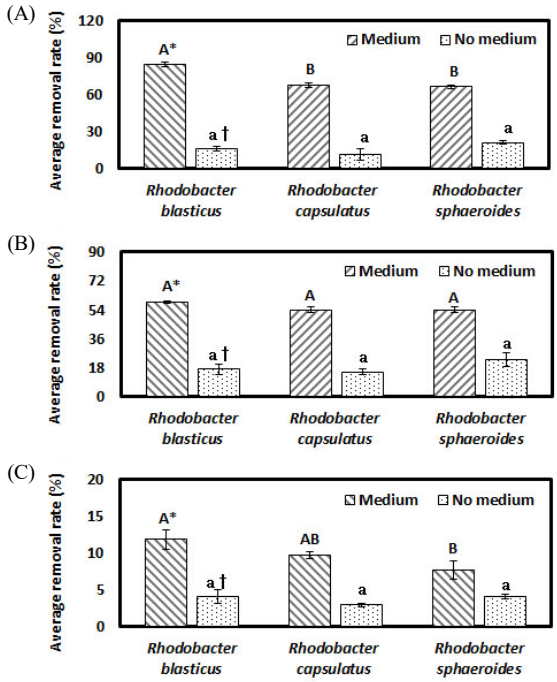

Figure 6. Effect of adding nutrients on the removal of (A) Cd(II), (B) Ni(II), and (C) Zn(II) by three *Rhodobacter* species under conditions of aerobic light. * Capital and † lowercase letters above bars using different letters indicate significant differences.

and Ni(II) compared to Zn(II). The removal efficiencies for Zn by *R. blasticus*, *R. capsulatus*, and *R. sphaeroides* at 5, 10, 20, and 80 mg/L of Zn (II) concentrations were less than 20%. No significant differences were found in the removal efficiency of Zn(II) by *Rhodobacter* species.

3.3. Metabolic study of bioremediation of Cd(II), Ni(II), and Zn(II) by *Rhodobacter* species

3.3.1. Metabolic inhibition study

Sodium azide was known to enhance the production of $^1O_2$ in an aqueous solution as the nontoxic antimicrobial agent[38]. The experimental results obtained from the use of sodium azide as a metabolic inhibitor in Figure 5 indicate that it strongly inhibited the removal of Cd(II) (Figure 5-A), Ni(II) (Figure 5-B), and Zn(II) (Figure 5-C) at concentrations of 10 mg/L by all *Rhodobacter* species. Percentage reductions of Cd removal efficiencies were 63, 55, and 44% in *R. blasticus*, *R. capsulatus*, and *R. sphaeroides* respectively, compared to a set of untreated cells. Percentage reductions of Ni removal efficiencies were 38, 35, and 29% by *R. blasticus*, *R. capsulatus*, and *R. sphaeroides,* respectively. Finally, percentage reductions of Zn removal efficiencies were 6, 6, and 2% by *R. blasticus*, *R. capsulatus*, and *R. sphaeroides,* respectively.

3.3.2. Metabolic dependent study

Results of the removal of toxic heavy metals by *Rhodobacter* species showed that all strains removed toxic heavy metals in the presence of nutrients when compared with those of no added nutrients (Figure 6). When the nutrients were added to the medium, R. *blasticus*, R. *capsulatus*, and R. *sphaeroides* eliminated 85, 68, and 66% of Cd(II), respectively. In the absence of nutrients, the removal efficiencies for Cd (II) by R. *blasticus*, R. *capsulatus*, and R. *sphaeroides* decreased by about 69, 56, and 45%, respectively. The removal efficiencies for Ni (II) by R. *blasticus*, R. *capsulatus*, and R. *sphaeroides* were reduced by 42, 39, and 31%, respectively; the removal efficiencies for Zn removal efficiencies by R. *blasticus*, R. *capsulatus*, and R. *sphaeroides* were decreased by 8, 7, and 4%, respectively.

4. Discussion

Both growth rates and biomass generation rates of microorganisms are profoundly influenced by metal ion concentrations. Usually, growth rates and biomass generation rates are inversely proportional to the toxicity of heavy metals. Our findings corresponded well to this notion; Figures 1~3 showed that growth rates decrease with increasing toxic metal concentration in modified ATCC medium supplemented with Cd(II), Ni(II), and Zn(II). The decline in growth due to toxic heavy metal stress differed in the three *Rhodobacter* species; growth rate declines were highest in *R. blasticus*, followed by *R. capsulatus* and lowest in *R. sphaeroides* in the presence of Cd(Figure 1), Ni(Figure 2), and Zn(Figure 3). Our study's results agree with those of numerous investigations, although the types of microorganisms used were different[42-45]. Acikel and Ersan[44] documented that the growth rates of *R. delemar* declined with increases in Ni concentrations in concentration ranges of 0 mg/L to 50 mg/L. Dursun [[42] reported that two

species of fungi (*Aspergillus niger* and *Rhizopus arrhizus*) were examined for their growth rate in the presence of cadmium ion (Cd^{2+}) and copper ions (Cu^{2+}) in the concentration ranges 0~100 mg/L. While growth inhibition was observed in both fungi species, biomass production in *R. arrhizus* was higher than that in *A. niger*, so Dursun *et al.*[42] concluded that *R. arrhizus* seemed to be a more active and sturdy means for removal of Cd^{2+} and Cu^{2+} from wastewaters than *A. niger*.

Furthermore, increases in concentrations of toxic heavy metals led to prolonged lag phases and reduced microbial biomass in all three *Rhodobacter* species (Figures 1~ 3). *R. blasticus* showed a maximum prolonged lag phase of 63 hours in the presence of Cd(II), 54 hours in the presence of Ni(II), and 48 hours in the presence of Zn(II), compared to 24 hours in plain media. *R. capsulatus* showed a prolonged lag phase of 54 hours during the log phase in the presence of Cd(II), 48 hours in the presence of Ni(II), and 36 hours in the presence of Zn(II) compared to 24 hours in plain media. Finally, *R. sphaeroides* showed a prolonged lag phase of 39 hours in the log phase in the presence of Cd(II), Ni(II), and Zn(II), compared to 24 hours in plain media. Similarly, Bar *et al.*[43] showed an extended lag phase in *K. pneumoniae* of 16 hours in the presence of Co^{2+} compared to 4 hours in the presence of Pb^{2+} and 2 hours in the absence of toxic metals. Wei *et al.*[46] also reported that low concentrations of heavy metals (0.5 mM Pb and 1.0 mM Zn) showed little impact on biomass growth, although at high concentrations (> 1.5 mM Pb and > 2.0 mM Zn) lengthened lag phase and decreased biomass concentrations were observed in bacterial isolates. In summary, it appears that all *Rhodobacter* species investigated in this study showed relatively high specific growth rates and biomass compared to other bacteria and fungi even in the presence of toxic heavy metal stress for concentrations below 20 mg/L (Figures 4, 5, and 6) and therefore possessed an ability for potential bio removal and further bioremediation of heavy metals in the industrial wastewater.

Unlike organic pollutants, toxic heavy metals are not biodegraded into less toxic or non-toxic substances by bacteria because of their chemical characteristics[22]. These metals can not be non-degradable and tend to bioaccumulate in the cells eventually causing health risks. Purple nonsulfur bacteria are a group of microorganisms that are known for removing toxic heavy metals. The present study demonstrated that three *Rhodobacter* species (R. *blasticus*, R. *capsulatus*, and R. *sphaeroides*) could effectively remove Cd(II) and Ni(II) from an aqueous solution (Figure 4). In particular, *R. blasticus* showed a higher removal efficiency of Cd(II) and Ni(II) compared to *R. capsulatus* and *R. sphaeroides.* There have been few studies on the removal of toxic heavy metals by *R. blasticus*. On the other hand, *R. sphaeroides* is the most studied microorganism for hydrogen production and bioremediation among purple nonsulfur bacteria[21,31,47,48]. Like other species of *Rhodobacter*, *R. sphaeroides* is a metabolically diverse organism that is capable of many modes of growth including aerobic respiration, anaerobic anoxygenic photosynthesis, fermentation, and diazotrophic growth[49]. Bai *et al.*,[14] reported that *Rhodobacter sphaeroides* was involved in the uptake and the bioremediation of synthetic solutions of cadmium sulfate. Soluble cadmium (40 mg/L) was almost completely removed from the solution after 42 h (removal rate: 97%). They concluded that abiotic precipitate did not play a great part in the whole process of removal, while bio removal of Cd by the sulfide precipitate was a significant contributor. This result agreed with that reported in the literature[50-52].

The removal efficiency for Ni(II) by *R. blasticus*, *R. capsulatus*, and *R. sphaeroides* at 5, 10, 20, and 80 mg/L of concentrations of Ni solution was approximately 50%. On the other hand, the use of three *Rhodobacter* species to remove Zn(II) was not practical as Zn(II) removal efficiencies were below 20% (Figure 4) for all three species and under different concentrations of Zn solution (5, 10, 20, and 80 mg/L). The removal efficiencies for heavy metals used by all *Rhodobacter* species were in the following order: Cd (II) > Ni (II) > Zn (II). Previously, Panwichian *et al.*,[53] reported that strains of *R. sphaeroides* KMS24 and *Rhodobium marium* NW16 could get rid of toxic heavy metal ions (Cu^{2+}, Zn^{2+}, Cd^{2+}) in the polluted shrimp pond in the aerobic-dark and microaerobic-light conditions. They suggested that the exopolymeric substances produced by the two strains played a great role in the accumulation of those heavy metals, and even they grew much better in the liquid medium including the higher concentration of toxic heavy metals than that of contaminated shrimp pond. *R. capsulatus* also absorbed the metal trivalent aurum[32]. In a separate study, *Rhodovulum* sp. PS88 and *R. spaheroides* S were also shown to eliminate the cadmium in the batch culture system. Under both heterotrophic (aerobic-dark) and photoheterotrophic (anaerobic-light) conditions, the strain PS88 demonstrated a higher constant value and hence the higher uptake of Cd compared to *R. sphaeroide* S[30].

The principal mechanisms used by purple nonsulfur bacteria for the removal of metal ions are considered to include biosorption and bioaccumulation processes[54-56]. The difference between the two is dependent on the energy required. The former does not need the energy whereas the latter requires the energy. In other words, biosorption is the entrapment of metal ions by binding sites on the cellular surface, which is a non-specific, fast, and metabolic-independent uptake, known as “bioabsorption” or “passive uptake”, performed by both living and dead cells[14,57-60]. Bioaccumulation is the result of interaction with the metabolic cycle after the cell enters. This mode of action, slower than subsequent biosorption, also is known as “the active uptake” and is performed only by the actively growing cell cultures[19,61]. It is well known that both these processes are involved in removing the toxic heavy metals from water as not only living cells but also dead cells which could be bound to the toxic heavy metals either actively, or passively or by a combined two processes[21,62,63].

Some purple nonsulfur bacteria may use more than one mechanism to eliminate the metal ions and hence are to be more effective as compared to the ones that use only one mechanism[22]. The type of mechanism to be used by the organism is dependent on the properties of the metal ion that it is exposed to. Our metabolic inhibition (Figure 5) and metabolic-dependent (Figure 6) studies showed that the removal processes of toxic heavy metals by *Rhodobacter* species might involve both bioaccumulation (intracellular uptake) and biosorption (surface binding). In other words, Both bioaccumulation (intracellular uptake in-

side cell and biosorption-energy dependent process-metabolic dependent activity-active removal) (attachment to bacterial cell surface-energy independent process-metabolic independent activity-passive removal) were involved in the removal of three metals.

It is evident from Figure 5 that sodium azide (1 M) significantly reduced the ability of all strains to remove Cd(II), Ni(II), and Zn(II). In other words, when living cells were used, it meant that bioaccumulation was involved in the removal of toxic heavy metals. The results of the present study corresponded well with those found in the earlier experimental study in purple nonsulfur bacteria[33]. It appears that the intracellular accumulation of toxic heavy metals is governed by energy-dependent transport systems. This was also supported by the increment of the toxic heavy metal removal ability after supplementing nutrients to the toxic heavy metal solution(Figure 6). This indicated that metabolic processes promoted the uptake of toxic heavy metals into the cells. Prado Acosta *et al.*[64] indicated that metabolic-dependent processes were also energy-dependent, demanding an active energy-creating system by the cells and presumably via particular transport systems.

The results of this study suggest that R. *blasticus* could be the most potential means of bioremediation to remove cadmium in the industrial wastewater. On the other hand, the tolerance observed towards heavy metals allows proposing the employment of *R. blasticus* in the degradation of organic pollutants in metal-contaminated environments. Furthermore, future work should focus on the modification in the outer membrane proteins of *R. blasticus* with potential bioremediation properties for improving metal binding abilities and the factors involved in improving in situ bioremediation strategies.

4. Conclusions

Our results demonstrated the potential bioremediation capacity of toxic heavy metals from liquid media by different *Rhodobacter* species. Addition of toxic divalent cations such as cadmium (II), nickel (II), and zinc (II) produced a reduced growth rate at the stationary phase, whereas the lag period may become longer. In this study, it was shown that three *Rhodobacter* species (R. *blasticus*, R. *capsulatus*, and R. *sphaeroides*) could effectively eliminate Cd(II) and Ni(II) from an aqueous solution. In particular, *R. blasticus* showed a higher removal efficiency of Cd(II) than *R. capsulatus* and *R. sphaeroides*. It appears that the processes of toxic heavy metals removal by purple nonsulfur bacteria involve intracellular uptake (bioaccumulation) and surface binding (biosorption). Overall, our results suggest that *R. blasticus* may be the most potent means of bioremediation to remove cadmium in the water of the three tested Rodobacter species. It appears that this may be due to the more resistance and resulting greater growth rate of R. blasticus to Cd than the tested other species, resulting in the increased removal of cadmium.

Acknowledgments

This study was conducted with the support of the Research Cooperating Program for the National Research Foundation of Korea (Project No. 2019R1F1A 106325212), NRF, Republic of Korea.

References

1. N. Green, B. Bjerkeng, K. Hylland, A. Ruus, and B. Rygg, Hazardous substances in the European marine environment: Trends in metals and persistent organic pollutants, European Environment Agency (2003).
2. P. B. Tchounwou, C. G. Yedjou, A. K. Patlolla, and D. J. Sutton, Heavy metal toxicity and the environment, *Molecular, Clinical and Environmental Toxicology*, 133-164, Springer, Berlin, Germany (2012).
3. M. Aryal and M. Liakopoulou-Kyriakides, Bioremoval of heavy metals by bacterial biomass, *Environ. Monit. Assess.*, **187**, 4173 (2015).
4. J. H. Duffus, "Heavy metals" - A meaningless term?, *Pure Appl. Chem.*, **74**, 793-807 (2002).
5. K. S. Kumar, H. U. Dahms, E. J. Won, J. S. Lee, and K. H. Shin, Microalgae−A promising tool for heavy metal remediation, *Ecotoxicol. Environ. Saf.*, **113**, 329-352 (2015).
6. Ihsanullah, A. Abbas, A. M. Al-Amer, T. Laoui, M. J. Al-Marri, M. S. Nasser, M. Khraisheh, and M. A. Atieh, Heavy metal removal from aqueous solution by advanced carbon nanotubes: Critical review of adsorption applications, *Sep. Purif. Technol.*, **157**, 141-161 (2016).
7. J. O. Duruibe, M. O. C. Ogwuegbu, and J. N. Egwurugwu, Heavy metal pollution and human biotoxic effects, *Int. J. Phys. Sci.*, **2**, 112-118 (2007).
8. L. R. Herrera-Estrella, A. A. Guevara-García, and J. Lo'pez-Bucio, Heavy metal adaptation, *Encyclopedia of Life Science*, 1-5, Macmillan Publishers, London, United Kingdom (1999).
9. U. Förstner and G. T. Wittmann, *Metal Pollution in the Aquatic Environment*, 2nd ed., 7-11, Springer Science & Business Media, Berlin, Germany (2012).
10. A. Gaur and A. Adholeya, Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils, *Curr. Sci.*, **86**, 528-534 (2004).
11. L. Järup, M. Berglund,, C. G. Elinder, G. Nordberg, and M. Vanter, Health effects of cadmium exposure−A review of the literature and a risk estimate, *Scand. J. Work Environ. Health*, **24**, 1-51 (1998).
12. C. E. Borba, R. Guirardello, E. A. Silva, M. T. Veit, and C. R. G. Tavares, Removal of nickel (II) ions from aqueous solution by biosorption in a fixed bed column: Experimental and theoretical breakthrough curves, *Biochem. Eng. J.*, **30**, 184-191 (2006).
13. N. Oyaro, J. Ogendi, E. N. Murago, and E. Gitonga, The contents of Pb, Cu, Zn and Cd in meat in nairobi, Kenya, *J. Food Agric. Environ.*, **5**, 119-121 (2007).
14. S. S. Ahluwalia and D. Goyal, Microbial and plant derived biomass for removal of heavy metals from wastewater, *Bioresour. Technol.*, **98**, 2243-2257 (2007).
15. A. M. Y. Chong, Y. S. Wong, and N. F. Y. Tam, Performance of different microalgal species in removing nickel and zinc from industrial wastewater, *Chemosphere*, **41**, 251-257 (2007).
16. A. Khosmanesh, F. Lawson, and I. G. Prince, Cadmium uptake by unicellular green microalgae, *Chem. Eng. J. Biochem. Eng. J.*, **62**, 81-88 (1996).

17. M. Farhadian, C. Vachelard, D. Duchez, and C. Larroche, In situ bioremediation of monoaromatic pollutants in groundwater: A review, *Bioresour. Technol.*, **99**, 5296-5308 (2008).
18. V. Radhika, S. Subramanian, and K. A. Natarajan, Bioremediation of zinc using Desulfotomaculum nigrificans: Bioprecipitation and characterization studies, *Water Res.*, **40**, 3628-3636 (2006).
19. A. Malik, Metal bioremediation through growing cells, *Environ. Int.*, **30**, 261-278 (2004).
20. G. M. Gadd, Bioremedial potential of microbial mechanisms of metal mobilization and immobilization, *Curr. Opin. Biotechnol.*, **11**, 271-279 (2000).
21. H. J. Bai, Z. M. Zhang, G. E. Yang, and B. Z. Li, Bioremediation of cadmium by growing *Rhodobacter sphaeroides*: Kinetic characteristic and mechanism studies, *Bioresour. Technol.*, **99**, 7716-7722 (2008).
22. A. Idi, M. H. M. Nor, M. F. A. Wahab, and Z. Ibrahim, Photosynthetic bacteria: An eco-friendly and cheap tool for bioremediation, *Rev. Environ. Sci. Biotechnol.*, **14**, 271 (2015).
23. P. K. Sarkar and A. K. Banerjee, The effect of nickel on growth, morphology and photopigments of *Rhodospirillum photometricum*, *Folia Microbiol. (Praha)*, **32**, 48-54 (1987).
24. B. B. Nepple, I. Flynn, and R. Bachofen, Morphological changes in phototrophic bacteria induced by metalloid oxyanions, *Microbiol. Res.*, **154**, 191-198 (1999).
25. S. Mohamed Fahmy Gad El-Rab, A. Abdel-Fattah Shoreit, and Y. Fukumori, Effects of cadmium stress on growth, morphology, and protein expression in *Rhodobacter capsulatus* B10, *Biosci. Biotechnol. Biochem.*, **70**, 2394-2402 (2006).
26. A. Iyer, K. Mody, and B. Jha, Biosorption of heavy metals by a marine bacterium, *Mar. Pollut. Bull.*, **50**, 340-343 (2005).
27. A. Pal and A. K. Paul, Microbial extracellular polymeric substances: central elements in heavy metal bioremediation, *Indian J. Microbiol.*, **48**, 49 (2008).
28. M. Pandi, V. Shashirekha, and M. Swamy, Bioabsorption of chromium from retan chrome liquor by cyanobacteria, *Microbiol. Res.*, **164**, 420-428 (2009).
29. A. Smiejan, K. J. Wilkinson, and C. Rossier, Cd bioaccumulation by a freshwater bacterium, *Rhodospirillum rubrum*, *Environ. Sci. Technol.*, **37**, 701-706 (2003).
30. M. Watanabe, K. Kawahara, K. Sasaki, and N. Noparatnaraporn, Biosorption of cadmium ions using a photosynthetic bacterium, *Rhodobacter sphaeroides* S and a marine photosynthetic bacterium, *Rhodovulum* sp., and their biosorption kinetics, *J. Biosci. Bioeng.*, **95**, 374-378 (2003).
31. L. Giotta, A. Agostiano, F. Italiano, F. Milano, and M. Trotta, Heavy metal ion influence on the photosynthetic growth of *Rhodobacter sphaeroides*, *Chemosphere*, **62**, 1490-1499 (2006).
32. Y. Feng, Y. Yu, Y. Wang, and X. Lin, Biosorption and bioreduction of trivalent aurum by photosynthetic bacteria *Rhodobacter capsulatus*, *Curr. Microbiol.*, **55**, 402-408 (2007).
33. S. Panwichian, D. Kantachote, B. Wittayaweerasak, and M. Mallavarapu, Isolation of purple nonsulfur bacteria for the removal of heavy metals and sodium from contaminated shrimp ponds, *Electron. J. Biotechnol.*, **13**, 3-4 (2010).
34. J. F. Imhoff and H. G. Trüper, Purple nonsulfur bacteria, *Bergey's Manual of Systematic Bacteriology*, 3rd ed., 1658-1661, Williams & Wilkins, Philadelphia, United States (1989).
35. K. R. Girija, C. Sasikala, C. V. Ramana, C. Spröer, S. Takaichi, V. Thiel, and J. F. Imhoff, *Rhodobacter johrii* sp. nov., an endospore-producing cryptic species isolated from semi-arid tropical soils, *Int. J. Syst. Evol. Microbiol.*, **60**, 2099-2107 (2010).
36. L. I. Crouch and M. R. Jones, Cross-species investigation of the functions of the *Rhodobacter* PufX polypeptide and the composition of the RC−LH1 core complex, *Biochim. Biophys. Acta Bioenerg.*, **1817**, 336-352 (2012).
37. R. Gourdon, E. Rus, and S. Bhende, Sofer SS Mechanism of cadmium uptake by activated sludge, *Appl. Microbiol. Biotechnol.*, **34**, 274-278 (1990).
38. L. Huang, Y. Xuan, Y. Koide, T. Zhiyentayev, M. Tanaka, and M. R. Hamblin, Type I and Type II mechanisms of antimicrobial photodynamic therapy: An in vitro study on gram-negative and gram-positive bacteria, *Lasers Surg. Med.*, **44**, 490-499 (2012).
39. E. Barbot, I. Seyssiecq, N. Roche, and B. Marrot, Inhibition of activated sludge respiration by sodium azide addition: Effect on rheology and oxygen transfer, *Chem. Eng. J.*, 230-235 (2010).
40. X. Yang and X. Cui, Adsorption characteristics of Pb(II) on alkali-treated tea residue, *Water Resour. Ind.*, **3**, 1-10 (2013).
41. R. Mopoung and N. Kengkhetkit, Lead and cadmium removal efficiency from aqueous solution by NaOH treated pineapple waste, *Int. J. Appl. Chem.*, **12**, 23-35 (2016).
42. A. Y. Dursun, G. Uslu, O. Tepe, Y. Cuci, and H. I. Ekiz, A comparative investigation on the bioaccumulation of heavy metal ions by growing Rhizopus arrhizus and Aspergillus niger, *Biochem. Eng. J.*, **15**, 87-92 (2003).
43. C. Bar, R. Patil, J. Doshi, M. J. Kulkarni, and W. N. Gade, Characterization of the proteins of a bacterial strain isolated from contaminated site involved in heavy metal resistance—A proteomic approach, *J. Biotechnol.*, **128**, 444-451 (2007).
44. Ü. Açıkel and M. Erşan, Acid phosphatase production by *Rhizopus delemar*: A role played in the Ni (II) bioaccumulation process, *J. Hazard. Mater.*, **184**, 632-639 (2010).
45. A. Mishra and A. Malik, Recent advances in microbial metal bioaccumulation, *Crit. Rev. Environ. Sci. Technol.*, **43**, 1162-1222 (2013).
46. G. Wei, L Fan, W. Zhu, Y. Fu, J. Yu, and M. Tang, Isolation and characterization of the heavy metal resistant bacteria CCNWRS33-2 isolated from root nodule of Lespedeza cuneata in gold mine tailings in China, *J. Hazard. Mater.*, **162**, 50-56 (2009).
47. K. Takeno, K. Sasaki, M. Watanabe, T. Kaneyasu, and N. Nishio, Removal of phosphorus from oyster farm mud sediment using a photosynthetic bacterium, *Rhodobacter sphaeroides* IL106, *J. Biosci. Bioeng.*, **88**, 410-415 (1999).
48. F. Italiano, A. Buccolieri, L. Giotta, A. Agostiano, L. Valli, F. Milano, and M. Trotta, Response of the carotenoids mutant *Rhodobacter sphaeroides* growing cells to cobalt and nickel exposure, *Int. Biodeterior. Biodegradation*, **63**, 948-957 (2009).
49. N. W. Woodbury, J. P. Allen, R. E. Blankenship, M. T. Madigan, and C. E. Bauer, The pathway, kinetics and thermodynamics of electron transfer in wild type and mutant reaction centers of purple nonsulfur bacteria, *Anoxygenic Photosynthetic Bacteria*, 527-557, Springer, Berlin, Germany (1995).
50. C. L. Wang, P. C. Michels, S. C. Dawson, S. Kitisakkul, J. A. Baross, J. D. Keasling, and D. S. Clark, Cadmium removal by a new strain of *Pseudomonas aeruginosa* in aerobic culture, *Appl. Environ. Microbiol.*, **63**, 4075-4078 (1997).
51. C. L. Wang, P. D. Maratukulam, A. M. Lum, D. S. Clark, and J.

D. Keasling, Metabolic engineering of an aerobic sulfate reduction pathway and its application to precipitation of cadmium on the cell surface, *Appl. Environ. Microbiol.*, **66**, 4497-4502 (2000).
52. C. L. Wang, A. M. Lum, S. C. Ozuna, D. S. Clark, and J. D. Keasling, Aerobic sulfide production and cadmium precipitation by *Escherichia coli* expressing the Treponema denticola cysteine desulfhydrase gene, *Appl. Microbiol. Biotechnol.*, **56**, 425-430 (2001).
53. S. Panwichian, D. Kantachote, B. Wittayaweerasak, and M. Mallavarapu, Removal of heavy metals by exopolymeric substances produced by resistant purple nonsulfur bacteria isolated from contaminated shrimp ponds, *Electron. J. Biotechnol.*, **14**, 2 (2011).
54. L. E., Macaskie and A. C. R. Dean, Microbial metabolism, desolubilization, and deposition of heavy metals: Metal uptake by immobilized cells and application to the detoxification of liquid wastes, *Advances in Biotechnological Processes*, 159-201, lan R. Liss, Inc., New York, United States (1989).
55. F. Veglio, F. Beolchini, and A. Gasbarro, Biosorption of toxic metals: an equilibrium study using free cells of *Arthrobacte*r sp, *Process Biochem.*, **32**, 99-105 (1997).
56. E. I. Yilmaz, Metal tolerance and biosorption capacity of *Bacillus circulans* strain EB1, *Res. Microbiol.*, **154**, 409-415 (2003).
57. J. E. Sloof, A. Viragh, and B. Veer, Kinetics of cadmium uptake by green algae, *Water Air Soil Pollut.*, **83**, 105-122 (1995).
58. J. S. Chang, R. Law, C. C. Chang, Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21, *Water Res.*, **31**, 1651-1658 (1997).
59. I. Bakkaloglu, T. J. Butter, L. M. Evison, F. S. Holland, and I. C. Hancock, Screening of various types biomass for removal and recovery of heavy metals (Zn, Cu, Ni) by biosorption, sedimentation, and desorption, *Water Sci. Technol.*, **38**, 269-277 (1998).
60. M. Torres, J. Goldberg, and T. E. Jensen, Heavy metal uptake by polyphosphate bodies in living and killed cells of *Plectonema boryanum* (cyanophycae), *Microbios*, **96**, 141-147 (1998).
61. R. Munoz and B. Guieysse, Algal-bacterial processes for the treatment of hazardous contaminants: A review, *Water Res.*, **40**, 2799-2815 (2006).
62. F. A. Al-Momani, A. M. Massadeh, and Y. A. Hadad, Uptake of zinc and copper by halophilic bacteria isolated from the Dead Sea shore, Jordan, *Biol. Trace Elem. Res.*, **115**, 291-300 (2007).
63. M. Gavrilescu, Removal of heavy metals from the environment by biosorption, *Eng. Life Sci.*, **4**, 219-232 (2004).
64. M. Prado Acosta, E. Valdman, S. G. Leite, F. Battaglini, and S. M. Ruzal, Biosorption of copper by *Paenibacillus polymyxa* cells and their exopolysaccharide, *World J. Microbiol. Biotechnol.*, **21**, 1157 (2005).

Authors

Jin Yoo; M.Sc., Researcher, Indoor Environment Division, Incheon Research Institute of Public Health and Environment, Incheon 22320, Republic of Korea; yoojin529@korea.kr

Eun-Ji Oh; M.Sc., Researcher, Water and Land Research Group/ Division for Natural Environment, Korea Environment Institute, Sejong 30147, Republic of Korea; qwqw990@naver.com

Ji-Su Park; M.Sc., Researcher, EHS Part, Doosan Corporation Electro-Materials, Gimcheon 39541, Republic of Korea; wltn3812@naver.com

Deok-Won Kim; M.Sc., Ph.D. Student, Department of Earth Resources and Environmental Engineering, Hanyang University, Seoul 04763, Republic of Korea; kghkgkr1@naver.com

Jin-Hyeok Moon; In Undergraduate, Undergraduate Student, Department of Environmental and Biological Chemistry, Chungbuk National University, Cheongju 28644, Republic of Korea; moonjh2323@naver.com

Deok-Hyun Kim; M.Sc., Researcher, Chemical Accident Investigation Team, National Institute of Chemical Safety, Cheongju 28164, Republic of Korea; foseja1324@korea.kr

Daniel Obrist; Ph.D., Professor, Department of Environmental, Earth, and Atmospheric Sciences, University of Massachusetts Lowell, MA 01854, USA; daniel_obrist@uml.edu

Keun-Yook Chung; Ph.D., Professor, Department of Environmental and Biological Chemistry, Chungbuk National University, Cheongju 28644, Republic of Korea; kychung@cbnu.ac.kr