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Evaluation of nitrogen, phosphorus and heavy metals removal from wastewater by *Rhodobacter Blasticus*

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Abstract. The potential for the removal of nitrogen, phosphorus, and heavy metals such as copper and nickel by *Rhodobacter blasticus* known as purple non-sulfur bacteria was evaluated in this study. In addition, the resistance of *R. blasticus* to two heavy metals and the effects of these metals on its cell morphology were investigated. The heavy metal adsorption characteristics by *R. blasticus* were confirmed using Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDX) and Fourier Transform Infrared Spectroscopy (FT-IR). The nitrogen and phosphorus removal efficiencies of *R. blasticus* decreased as the concentration of nitrogen and phosphorus increased from 50 to 500 mg/L. The resistance, based on the minimum inhibitory concentration of *R. blasticus* growing under aerobic-light conditions, was found to be in the order of $Ni^{2+} > Cu^{2+}$. SEM analysis revealed that copper ions altered the morphology of *R. blasticus*, while nickel treatment did not differ from the control group. Futhermore, this study confirmed that the removal efficiencies for Cu^{2+} and Ni^{2+} by *R. blasticus* were 53±0.02% and 55±0.02%, respectively. The surface of bacterial cells where heavy metals were absorbed was also confirmed by SEM-EDX. According to the FT-IR analysis results, the increased transmittance (%) after heavy metal adsorption indicates that these heavy metals were absorbed by the functional groups on the cell surface of *R. blasticus*.

Keywords: adsorption; bioremediation; heavy metals; nutrients; *Rhodobacter blasticus*

1. Introduction

The demand for freshwater is increasing with population growth, so wastewater treatment is very important from the perspective of environmental preservation and reuse of purified water (Cambie *et al*. 2016, Connor 2015).

Various methods exist for removing heavy metals and nutrients from wastewater (Duraisamy *et al*. 2015, Min *et al*. 2021, Sinharoy *et al*. 2024, Uslu *et al*. 2014). The detection concentration ranges of TN, TP, Ni, and Cu in domestic wastewater are 0.44~660, 0.244~575, 0~20.103, and 0~6.902, respectively (Ahn *et al*. 2016). However, biological treatment technologies that decompose and immobilize environmental pollutants have become more widely adopted due to their low cost and safety. Photosynthetic bacteria have a variety of metabolic functions and are commonly distributed in environments such as lakes, seas, rivers, and soil (Chitapornpan *et al*. 2013, Prachanurak *et al*. 2014, Wang *et al*. 2016, Zhou *et al*. 2014). The bacterial can use a variety of organic compounds as energy and carbon sources, so they have been used to treat various types of wastewaters (Chitapornpan *et al*. 2013, Idi *et al*. 2015a, b, Prachanurak *et al*. 2014, Wang *et al*. 2016, Zhou *et al*. 2014). Photosynthetic bacteria are classified into several groups, inclduing cyanobacteria, prochloropytes, purple sulfur bacteria (PSB), green sulfur bacteria (GSB), purple non-sulfur bacteria (PNSB), and green non-sulfur bacteria (GNSB), based on their aerobic/anaerobic characteristics, electron donor types, and Bacteriochlorophyll (Bchl) types (Talaiekhozani and Rezania 2017). Among those bacteria, purple non-sulfur bacteria (PNSB) are known to be the most metabolically versatile organisms among prokaryotes (Bergey 1994). *Rhodospirillum*, *Rhodocyclus*, *Rhodopseudomonas*, and *Rhodobacter* are representative species in a genus of the Rhodobacteraceae, PBSB (Ahn and Kim 2016). These microorganisms can grow under aerobic conditions using various organic carbon sources, such as organic acids, alcohols, carbohydrates, and aromatic compounds. In particular, *Rhodobacter* has various respiratory and metabolic activities, so it can live in various environments differently from other PNSB species. (Girija *et al*. 2010, Crouch and Jones, 2012). *Rhodobacter spheroides* and *Rhodobacter capsulatus* are the most studied species for bioremediation of wastewater (Madukasi *et al*. 2011, 2010, Merugu *et al*. 2011, Panwichian *et al*. 2011). However, although there have been several studies on bioremediation of wastewater containing heavy metals and nutrients by *Rhodobacter blasticus*, no research has been conducted on the application of this bacterium for domestic wastewater treatment (Yoo 2017).

This study was conducted to evaluate the removal efficiencies of nutrients, such as nitrogen and phosphorus,

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as well as heavy metals including copper and nickel, by *R. blasticus* in the synthetic wastewater in South Korea. Additionally, Scanning electron microscopy (SEM) analysis was performed to confirm the impact of copper and nickel on the cellular morphology of *R. blasticus*. Finally, the characteristics of heavy metal adsorption and removal such as changes in cell surface, elemental composition, and action mechanisms by both live and dead biomass were evaluated using SEM-EDX and FT-IR to investigate the removal mechanisms.

2. Materials and methods

2.1 Bacterial strain, cultivation and synthetic wastewater treatment

2.1.1 Bacterial strain and cultivation

Rhodobacter blasticus (KCTC No. 15056), PNSB used in this study was obtained from the Korean Collection for Type Cultures (KCTC). The cultures were stored at –80 degrees Celsius in a 20% glycerol solution until needed. After three subcultures, a loop of *R. blasticus* was transferred into 50 mL of Rhodospirillum (27M) broth with a pH 6.8. The cultures were then incubated under micro-aerobic conditions with light intensity of 3000 lux at 150 rpm and 30°C for 72 hrs (Panwichian *et al*. 2010).

The composition of the 27M medium is presented in Table 1 (Jeong *et al*. 2006). The bacterial culture broth was adjusted to an optical density of 0.5 at a wavelength of 660 nm (OD660) and uninoculated medium was used as the blank. A 10% (v/v) bacterial culture broth was innoculated into each 50 mL of the 27M broth containing nitrogen, phosphorus, and heavy metals to evaluate the bio-remediation of contaminants. The cultures were incubated at 30°C for 48 hrs under microaerobic light conditions.

2.1.2 Batch set-up for bioremediation of wastewater

A Batch test was established to evaluate the removal of nutrients (nitrogen and phosphorus) and heavy metals by cultivating *R. blasticus*. Ammonium chloride (NH_4Cl) were added as nitrogen sources to obtain different concentration of 0.935 mM, 1.869 mM, 4.674 mM, and 9.347 mM in the 27M broth. Potassium phosphate (KH_2PO_4) were added as nitrogen and phosphorus sources to obtain different concentration of 0.367 mM, 0.735 mM, 1.837 mM, and 3.674 mM in the 27M broth. Heavy metals stock solutions (5 mM) for Cu^{2+} and Ni^{2+} were also prepared with $CuCl_2{\cdot}2H_2O$ and $NiCl_2{\cdot}6H_2O$. The solutions were sterilized using a 0.22 μm membrane filter and stored at 4°C until needed.

2.2 Evaluation of bacterial growth and bioremediation of nutrients and heavy metals

2.2.1 Analysis of bacterial growth, nutrients (total nitrogen and total phosphorus)

Bacterial growth was measured using a UV/Vis spectrophotometer (UV Mini 1240 Shimadzu, Japan) at an optical density of OD660 nm (Panwichian *et al*. 2011). The culture solution to analyze total nitrogen (T-N) and total phosphorus (T-P) was centrifuged for 30 minutes at 3000 rpm to separate the cells. The obtained supernatant was analyzed with a UV/VIS spectrophotometer (Shimadzu, Japan). T-N analysis was conducted using the persulfate method, which simultaneously decomposes nitrogen compounds, with measurements taken at 220 nm. T-P was analyzed using a modified ascorbic acid-molybdenum blue method, with measurements taken at 880 nm.

Table 1 Composition of Rhodosporillum 27M medium (Jeong *et al*. 2006)

Compounds	Concentratrion(mM, mg/L) in 1 L of distilled water
Yeast extract	3.133 mM
99.5 % Ethanol	8.526 mM
Disodium succinate	6.171 mM
0.1 % Ferric citrate	0.039 mM
KH_2PO_4	0.367 mM ~ 3.674 mM
$MgSO_4{\cdot}7H_2O$	1.623 mM
NaCl	6.845 mM
NH_4Cl	0.935 mM ~ 9.347 mM
$CaCl_2{\cdot}2H_2O$	0.34 mM
Trace elements solution (SL-6)	1 mL
- $ZnSO_4{\cdot}7H_2O$	0.348 mM
- $MnCl_4{\cdot}4H_2O$	0.152 mM
- H_3BO_3	4.851 mM
- $CoCl_2{\cdot}6H_2O$	1.54 mM
- $CuCl_2{\cdot}2H_2O$	0.059 mM
- $NiCl_2{\cdot}6H_2O$	0.154 mM
- $Na_2MoO_4{\cdot}2H_2O$	0.124 mM
Sodium ascorbate	2.524 mM

Table 2 ICP-OES operation condition to analyze Copper and Nickel

Compound	λ(nm)	Correlation coefficient	Initial conc. (mg/L)
Copper	324.754	0.9999	6.35 (0.1 mM)
Nickel	231.604	0.9999	35.22 (0.6 mM)

2.2.2 Analysis of copper and nickel

The culture broth used for analysis of copper and nickel was centrifuged for 30 minutes at 3000 rpm to separate the cells. The supernatant was sterilized using a 0.22 μm membrane filter and stored at 4°C. The residual heavy metals concentrations were analyzed according to APHA standard method using inductively coupled plasma-optical emission spectroscopy (ICP-OES 720, Agilent, USA) as shown in Table 2.

The heavy metals removal efficiency (R (%)) was calculated by the following Eq. (1) (Mopoung and Kengkhetkit 2016, Yoo 2017):

$$R = \frac{\mathrm{Ci} - \mathrm{Cf}}{\mathrm{Ci}} \times 100(\%) \tag{1}$$

R : Removal efficiency (%)
Ci : Initial concentration of heavy metal
Cf : Final concentration of heavy metal

2.3 Evaluation of heavy metals adsorption on R. blasticus strain

2.3.1 Cell treatment and adsorption amount calculation

The cells of *R. blasticus* were obtained by centrifugation after 72 hrs of incubation, and the heavy metal adsorption by both live and dead biomass of *R. blasticus* was subsequently evaluated. Before exposure to heavy metals, all cells were washed several times with deionized water and were then freeze-dried for 72 hrs to be used as the dead biomass.

The 50 mL solution containing 0.3 mM Cu^{2+} and 1.0 mM Ni^{2+} was prepared in 100 mL Erlenmeyer flasks, and the initial pH was adjusted to 5.5 to prevent the precipitation of heavy metal ions. For the heavy metal adsorption experiment, 0.56 g of biomass (comparing both live and dead biomass) was added to the metal solution. The live and dead biomass of *R. blasticus* were utilized to assess the effects of bacterial activity on the adsorption rate and efficiency. To reach equilibrium, the solution was shaken and incubated at 30°C with a speed of 150 rpm. The concentration of heavy metals in the aqueous phase was periodically measured using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The amount of adsorption (q) was calculated using the following equation (2) (Volesky 2007):

$$q = \frac{V \times (Ci - Ce)}{S} \tag{2}$$

$\text{q : adsorption amount } \left(\frac{\text{mg}}{\text{g}}\right)$

V : Volume (L)

$\text{Cf : Final concentration of heavy metal } \left(\frac{\text{mg}}{\text{L}}\right)$

$\text{Ce : Equlibrium concentration of heavy metal } \left(\frac{\text{mg}}{\text{L}}\right)$

$\text{S : amount of biomass(g)}$

2.3.2 Analysis of FT-IR and SEM-EDX for heavy metal adsorption characteristics

The scanning electron microscopy (SEM, Hitachi S-2500C, Japan) was used to confirm whether heavy metal adsorption by *R. blasticus* affects cell morphology. *R. blasticus* was subcultured twice to obtain active cultures before the experiment and then adjusted to an optical density of 0.5 at 660 nm. The bacterial culture (10% v/v) was then inoculated into 27M broth in 50 mL Erlenmeyer flasks and adjusted to 0.1 mM Cu^{2+}, 0.6 mM Ni^{2+} and control without heavy metal treatment. Subsequently, the cells were centrifuged for 30 min at 3000 rpm, and the cell pellets were washed twice with sterilized 0.1% peptone water. The cell pellets were pre-fixed in a 2.5% glutaraldehyde solution buffered with 0.1 M PBS at pH 7.0 for 1 hr at 4°C and then washed three times with the solution. Then the cells were treated with 1% osmium tetraoxide for 90 min followed by washing with 0.1M sodium phosphate buffer. The samples were dehydrated in a series of ethanol solution (70-100%) for 15 min in each step. Finally, they were coated with platinum and observed using SEM (Panwichian *et al*. 2011).

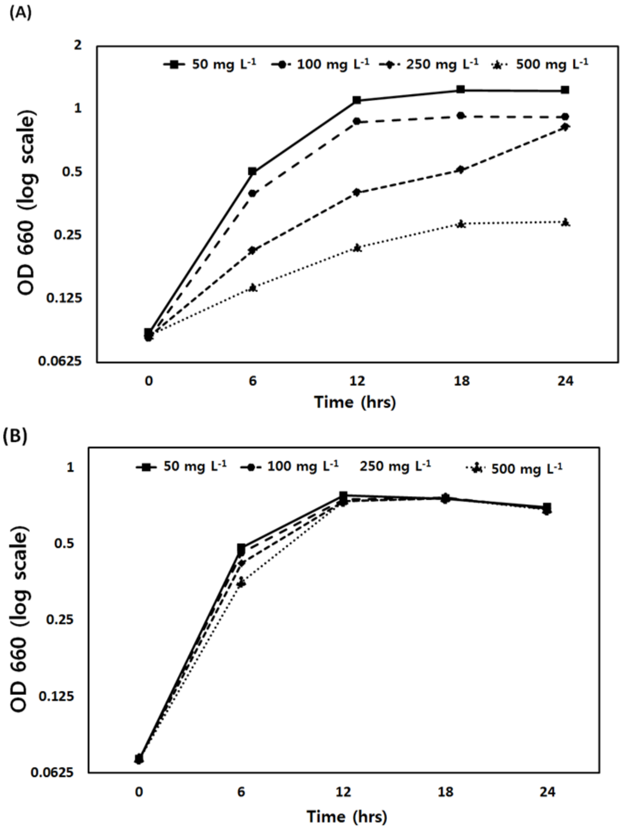

Fig. 1 The growth (OD660) of *Rhodobacter blasticus* in the 27M medium with ammonium (A) and phosphorus (B) at 30ºC and 150 rpm under microaerobic-light condition for 24 hrs

Before and after the adsorption of Cu^{2+} and Ni^{2+}, the surface structures of *R. blasticus* were confirmed using scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX). The functional groups present on the control of *R. blasticus* and the metal-loaded *R. blasticus* were identified through Fourier-transform infrared (FT-IR) analysis (Agilent Cary 670 FT-IR, USA). Infrared spectra were recorded in the attenuated total reflectance infrared (ATR) mode within the spectral range of 500 to 4000 cm-1 at room temperature.

2.3.3 Statistical analysis

All data were analyzed using SAS Version 9.1 (SAS Institute, Cary, NC, USA). These experiments were conducted in triplicate, and the results are presented as the mean with standard deviation from three determinations. The significance of mean differences was assessed using Fischer’s least significant difference (LSD) test. The level of significance among different groups exceeded 95% ($P < 0.05$).

3. Results and discussion

3.1 Removal of nitrogen and phosphorus by R. blasticus

In this study, the potential for *R. blasticus* to remove nitrogen and phosphorus was evaluated under the micro-

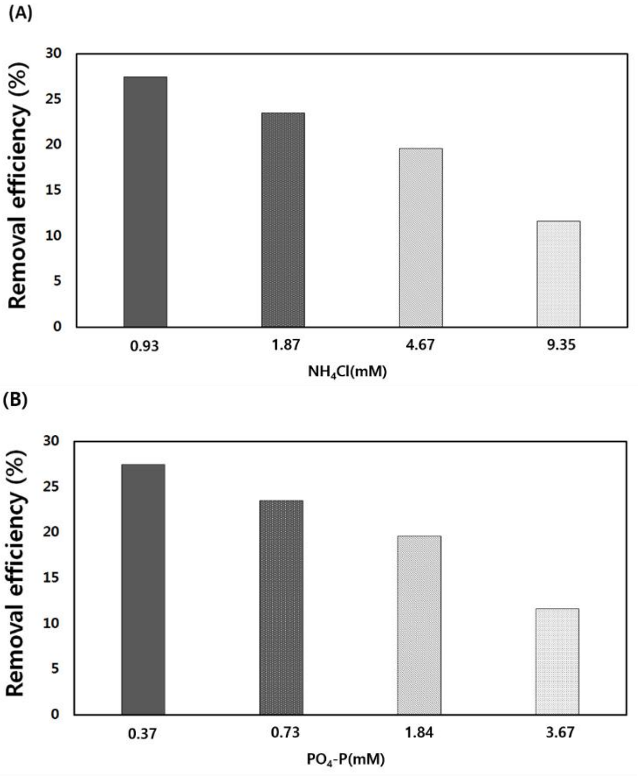

Fig. 2 The removal efficiencies (%) of ammonium (A) and phosphorus (B) by *Rhodobacter blasticus* cells when grown in 27M medium at 30ºC, 150 rpm under microaerobic-light conditions for 24 hrs (stationary phase)

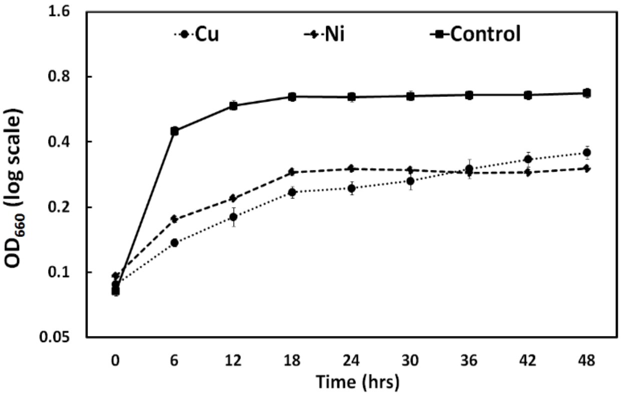

Fig. 3 The effects of heavy metals on the growth (OD660) of *Rhodobacter blasticus* at 30°C under the microaerobic-light condition for 48 hrs

Table 3 The removal efficiencies (%) of heavy metals by *Rhodobacter blasticus* when grown in 27M medium containing heavy metals (Cu^{2+} 0.1 mM and Ni^{2+} 0.6 mM) under microaerobic-light condition for 48 hrs

Compound	Initial conc. (mM)	Final conc. (mM)	Removal efficiency (%)
Copper	0.1±0.02	0.05±0.02	53
Nickel	0.6±0.01	0.27±0.02	55

Data are mean of three determinations and standard deviation

aerobic-light conditions. As the concentration of treated ammonium ions increased, bacterial growth decreased, however, phosphorus did not affect microbial growth (Fig. 1).

R. blasticus exhibited growth, as shown in Fig. 1 in all treatments containing different concentrations of nutrients. The growth of *R. blasticus* significantly decreased ($P < 0.05$) with increasing ammonia concentration (Fig. 1A). At lower phosphorous concentrations, the growth rate of initial *R. blasticus* was significantly faster than in other treatments (Fig. 1B). In other words, while increasing the concentration of treated phosphorus influenced the initial growth rate of *R. blasticus*, it did not affect the final optical density of the organism. Fig. 2 presents the removal efficiency of phosphorus and nitrogen from nutrient solutions at different concentrations: 50, 100, 200, and 500 mg/L. When *R. blasticus* was cultivated in 27M medium at different ammonia concentrations, the nitrogen removal efficiency and the concentration after removal at each level were as follows: 14 mg/L from initial concentration of 50 mg/L (27.46%), 23 mg/L from 100 mg/L (23.46%), 49 mg/L from 250 mg/L (19.54%), 58 mg/L from 500 mg/L (11.56%), as shown in Fig. 2A. The phosphorus removal rate exhibited a similar trend to the nitrogen treatment results (Fig. 2B).

Light intensity and inorganic compounds recognized as crucial factors in the growth of photosynthetic bacteria. In previous studies (Hill *et al*. 2009, Kebede-westhead *et al*. 2003, Sekar *et al*. 2002), nitrogen and phosphorus in water are transported into the PNSB cells and are offen considered limiting factors for bacterial growth. *R. blasticus* has also been shown to effectively remove the ammonium and phosphate (Liang *et al*. 2010). Liang (2010) indicated that PNSB are among the organisms that accumulate poly-phosphates (PAOs), with the capacity to store phosphorus amounting to 5-15% of the cell's dry weight. PAOs typically accumulate low levels of polyphosphate during the exponential growth phase, reaching maximum accumulation levels after entering the stationary phase (Tobin *et al*. 2007). In the stationary growth phase, purple non-sulfur bacteria (PNSB) produced polyphosphates under anaerobic-light conditions, and the ATP demand during this phase was lower than that in the exponential phase (Liang *et al*. 2010, Tobin *et al*. 2007). When PNSB grow photohetero-trophically, excess ATP generated from photosynthesis is utilized for polyphosphate production. Additionally, Hiraishi and Kitamura (1985) reported that although *R. blasticus* can accumulate significant amounts of phosphate, the efficiency of phosphate accumulation decreases under nitrogen-limiting conditions (Hiraishi and Kitamura, 1985). The nutrient removal efficiency of PNSB is influenced by the concentration of volatile fatty acid-COD in the wastewater (Hülsen *et al*. 2014). Therefore, when treating wastewater that requires advanced treatment or has a low carbon-to-nitrogen (C/N) ratio, it is believed that employing PNSB can lead to more stable and efficient nitrogen and phosphorus removal.

3.2 Removal and adsorption of heavy metals by R. blasticus

The bacterial growth (Fig. 3) and removal efficiencies

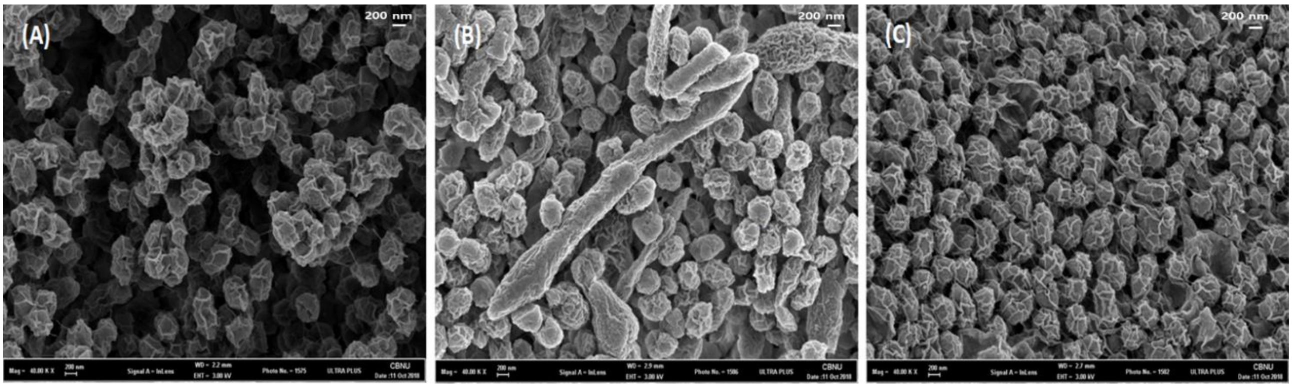

Fig. 4 Scanning electron microscopy (40,000X) of *Rhodobacter blasticus* cells when grown with 27M medium in the absence and presence of 0.1 mM for Cu^{2+} and 0.6 mM for Ni^{2+} at 30ºC under microaerobic-light condition for 48 hrs, *Rhodobacter blasticus* (A) control, (B) treated Cu^{2+}, and (C) treated Ni^{2+}

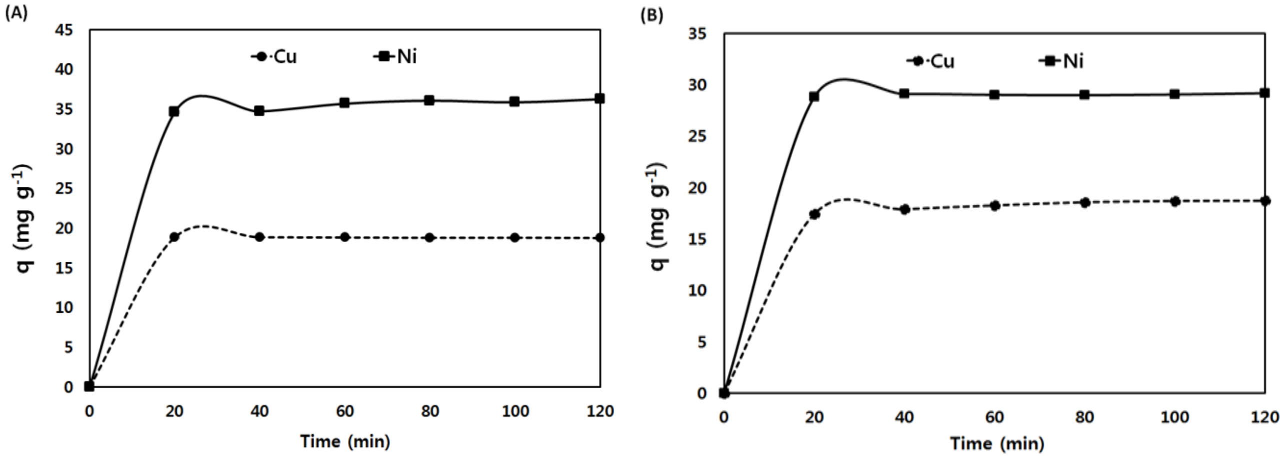

Fig. 5 Adsorption kinetics of heavy metals ions (Cu^{2+} and Ni^{2+}) on (A) live, and (B) dead biomass of *Rhodobacter blasticus* (*Rhodobacter blasticus* = 0.56 g/L, pH = 5.5, Temperature = 30ºC)

Table 4 Adsorption efficiencies of heavy metals by live and dead biomass of *R. blasticus*

Compound	Initial conc. (mM)	Final conc. (mM)	Removal efficiency (%)
Copper	0.3±0.02	(Live cell) 0.005±0.004 (Dead cell) 0.006±0.001	(Live cell) 98 (Dead cell) 98
Nickel	1.0±0.01	(Live cell) 0.343±0.01 (Dead cell) 0.471±0.01	(Live cell) 66 (Dead cell) 53

(Table 3) of heavy metals were measured to confirm the capacity of *R. blasticus* to remove heavy metals from synthetic wastewater containing 0.1 mM Cu^{2+} and 0.6 mM Ni^{2+}.

When cultured in 27M liquid medium for 48 hrs, the effects of Cu^{2+} and Ni^{2+} on bacterial growth is illustrated in Fig. 3. Compared to the control group, the growth of *R. blasticus* was inhibited under conditions of copper and nickel treatment. The removal efficiencies of Cu^{2+} and Ni^{2+} by *R. blasticus* were 53.38% and 54.93%, respectively (Table 3).

The impact of heavy metals on the cell morphology of *R. blasticus* strains was also assessed. The results comparing the cell morphology of microorganisms grown in control media (without heavy metal addition) and those treated with heavy metals are presented in Fig. 4.

In the Cu^{2+} treatment condition, *R. blasticus* exhibited filamentous cells (Fig. 4B). In contrast, under the Ni^{2+} treatment condition (Fig. 4C), the cell morphology remained unchanged compared to the control group (Fig. 4A). *R. blasticus* displayed filamentous forms in the presence of Cu^{2+} ions, whereas the control group consisted of rod-shaped cells. These findings align with previous studies indicating that the cell morphology of Rhodomium marinum NW16 and Rhodobacter sphaeroides KMS24 transitioned from rod to filamentous and dumbbell shapes in the presence of Cu^{2+} and Zn^{2+} ions. Consequently, *R. blasticus* may develop resistance by attempting to expel metal ions through an efflux mechanism, as excess intra-cellular metal ions can lead to morphological alterations (Panwichian *et al*. 2011). The results of this study demonstrated that *R. blasticus* is capable of removing both Cu^{2+} and Ni^{2+} ions. Research conducted by Talaiekhozani and Rezania (2017) indicated that PNSB can eliminate heavy

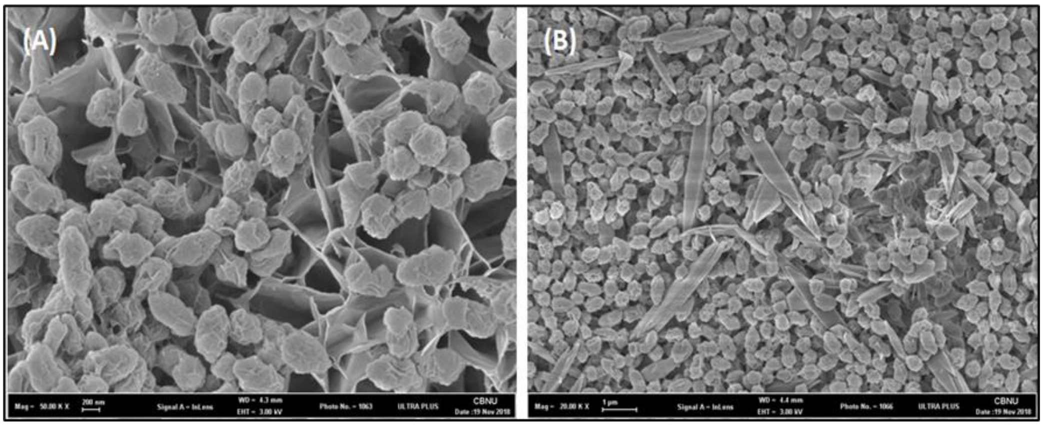

Fig. 6 Scanning electron microscopy images of *Rhodobacter blasticus* cells after the metal adsorption in the presence of (A) 0.3 mM for Cu^{2+} and (B) 1 mM for Ni^{2+} at 30ºC, initial pH 5.5, and 150 rpm

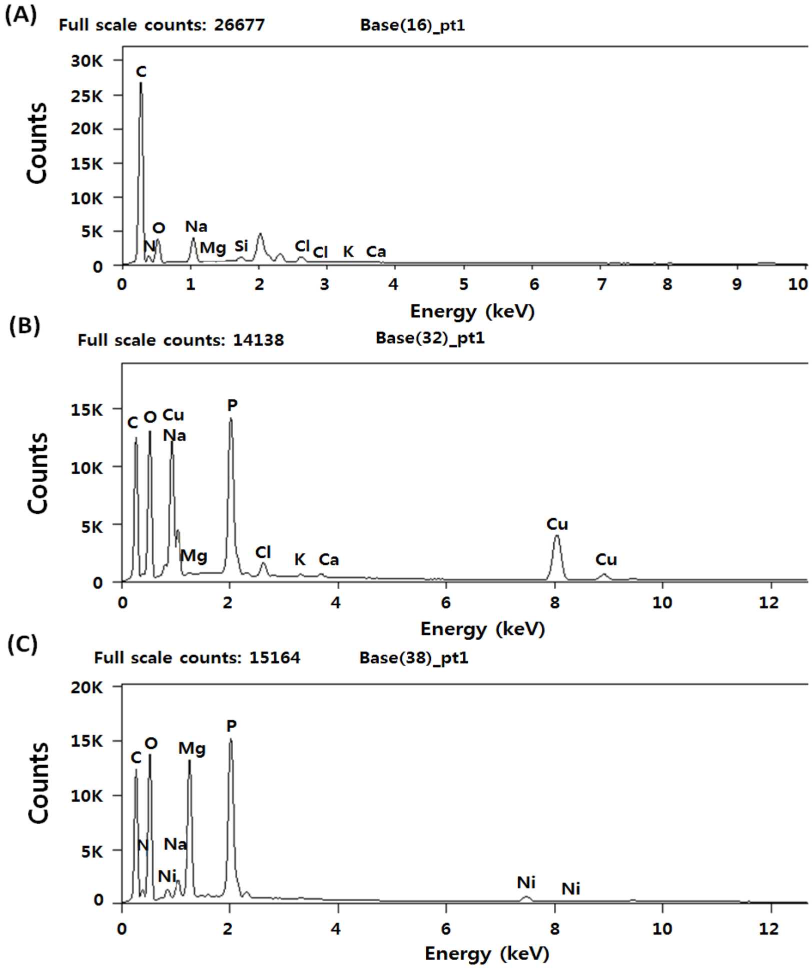

Fig. 7 Energy Dispersive X-ray spectroscopy spectrum of (A) control, (B) Cu^{2+}, and (C) Ni^{2+} detected in cells of *Rhodobacter blasticus* after the metal adsorption in the presence of 0.3 mM Cu^{2+} and 1 mM Ni^{2+} at 30ºC, initial pH 5.5, and 150 rpm

metals through various metabolic pathways, with the primary mechanisms of heavy metal removal being bio-accumulation and adsorption. Some PNSB utilize multiple mechanisms to remove heavy metal ions, and the specific mechanism employed by micro- organisms is influenced by the properties of the metal ions (Panwichian *et al.* 2011, Talaiekhozani and Rezania 2017).

3.3 Cell adsorption amount calculation of heavy metals on R. blasticus strain

The adsorption efficiencies of Cu^{2+} and Ni^{2+} by the live biomass of *R. blasticus* were 98.21% and 65.70%, respectively. The copper ion adsorption efficiency (98.11%) by the dead biomass of *R. blasticus* was approximately 1.9

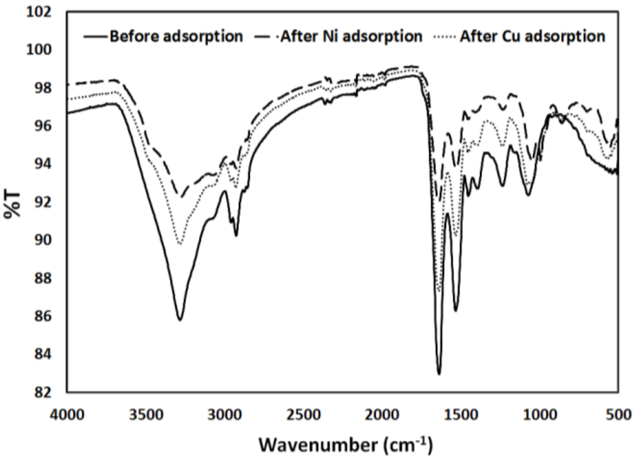

Fig. 8. FT-IR spectra of *Rhodobacter blasticus* before and after heavy metals (Cu^{2+} and Ni^{2+}) adsorption in the presence of 0.3 mM Cu^{2+} and 1 mM Ni^{2+} at 30ºC, initial pH 5.5, and 150 rpm

times higher than that of Ni^{2+} (52.93%) (Table 4). The amounts of heavy metals absorbed by the biomass were 1.67 mg/g for Cu^{2+} and 2.61 mg/g for Ni^{2+}.

The equilibrium rate of heavy metal adsorption by dead cells was nearly identical to that of live cells (Fig. 5). Previous research indicated that the adsorption rate was faster in dead cells, as the metabolic activity of living *R. blasticus* may influence the rate of heavy metal adsorption. Similar findings were reported by Volesky and May-Phillips (1995), who demonstrated that the activity of Saccharomyces cerevisiae affected the quantity of metal ions absorbed (Volesky and May-Phillips 1995). In this study, the adsorption capacity for heavy metals by live biomass of *R. blasticus* was greater than that of dead biomass. This suggests that living biomass employs both metabolic bioaccumulation and metabolic-independent adsorption processes to remove heavy metals. Likewise, research has shown that the live biomass of A. pullulans outperforms dead biomass in terms of lead (Pb) adsorption efficiency and rate (Suh *et al*. 1999). In contrast, Gabr *et al*. (2008) and Tunali *et al*. (2006) reported that the quantity of heavy metals absorbed on the cell surface exceeded the amount of metal ions present within the cells (Gabr *et al*. 2008, Tunali *et al*. 2006).

3.4 Adsorption characteristics of heavy metals

The surface changes before and after heavy metal adsorption were analyzed using SEM-EDX to determine the form of heavy metal adsorption by *R. blasticus*. SEM analysis revealed the presence of numerous electron-dense particles on the surface of *R. blasticus* cells after heavy metal adsorption (Fig. 6).

As a result of analyzing the components of electron-dense particles formed on the surface of the cell following heavy metal adsorption, it was confirmed that a complex was formed between *R. blasticus* and the heavy metals (Fig. 7).

Through a comparison of FT-IR spectra before and after heavy metal adsorption, the functional groups involved in the adsorption of heavy metal ions were identified. The functional groups associated with adsorption and their corresponding wavenumbers were determined in the biomass of *R. blasticus*, as illustrated in Fig. 8. The changes in the functional groups and surface properties of the biosorbent were confirmed through FT-IR spectrum analysis, which also explained the movement of certain functional groups to heavy metal adsorption. The main changes in peaks after Cu^{2+} adsorption were recorded at 3283, 3089, 2958, 1638, 1533, 1452 and 1394 cm^{-1}. Additionally, following Ni^{2+} treatment, changes in peaks were recorded at 3278, 3070, 2960, 2927, 1638, 1533, 1454, and 1400 cm^{-1}.

The change observed at 3283 cm^{-1} is attributed to stretching vibrations of –OH and –NH bonds (Guo & Zhang, 2004). Additionally, severtal functional groups were identified in this study, including the C-H stretch at 3070-3089 cm^{-1} and 2958-3089 cm^{-1}, C=O stretch at 1638 cm^{-1}, N-H bending at 1533 cm^{-1}, C-N stretch at 1452-1454 cm^{-1}, and COO- stretch and CH_2, CH_3 bending at 1394-1407 cm^{-1} (Lu *et al*. 2012). Furthermore, the presence of functional groups such as C-H stretching at 3070-3089 cm^{-1} and 2958-3089 cm^{-1}, C=O stretching at 1638 cm^{-1}, N-H bending at 1533 cm^{-1}, and C-N stretching at 1452~1454 cm^{-1}, COO-elongation and CH_2, CH_3 bending from 1394 to 1407 cm^{-1} were confirmed in this study (Lu *et al*. 2012).

After heavy metal adsorption, the bands at 3283, 2958, 2926, 1637, 1533, 1452, and 1394 cm^{-1} in the *R. blasticus* spectrum shifted toward higher wavenumbers. These changes indicate that functional groups such as amino, methyl, ester and carbonyl groups were involved in the adsorption of heavy metal by *R. blasticus*. Additionally, the permeability (%) of heavy metals increased after adsorption compared to before. A previous study by Argun and Dursun (2008) reported that the difference in transmittance (%) in FT-IR analysis was closely related to the quantitative change in functional groups. In other words, the increase in transmittance(%) after adsorption suggests that the amount of functional groups on the surface of *R. blasticus* is relatively smaller than before adsorption (Argun & Dursun, 2008). Therefore, it can be concluded that Cu^{2+} and Ni^{2+} ions can be absorbed by functional groups present on the surface of *R. blasticus* (Shim *et al*. 2016).

4. Conclusions

This study aimed to develop a biological removal method for nutrients and heavy metals from wastewater through microbial adsorption using *R. blasticus* in. To verify the removal of heavy metals through microbial adsorption, changes in the surface, inorganic elements, and functional groups before and after adsorption were analyzed using FT-IR. The following conclusions were drawn through this study: The nitrogen removal efficiency of *R. blasticus* decreased as the concentration of treated ammonium increased with removal efficiencies recorded at 50 mg/L (27.46%), 100 mg/L (23.46%), 250 mg/L (19.54%), and 500 mg/L (11.56%). Additionally, the phosphorus removal efficiencies were 50 mg/L (39.47%), 100 mg/L (26.42%), 250 mg/L (21.69%), and 500 mg/L

(16.57%), respectively. The removal efficiencies of Cu^{2+} and Ni^{2+} by *R. blasticus* were 53.38% and 54.93%, respectively. The heavy metal adsorption efficiency by *R. blasticus* live biomass was 98.21% for Cu and 65.70% for Ni^{2+}, while the adsorption efficiency for dead biomass was 98.11% for Cu^{2+} and 52.93% for Ni^{2+}. FT-IR analysis revealed that the increased transmittance (%) after heavy metal adsorption indicated that the adsorption of heavy metals into functional groups on the *R. blasticus* cell surface, with the main functional groups identified as amino, methyl, ester, and carbonyl groups. Based on the results of this study, it is evident that biological removal of nutrients and heavy metals from wastewater using *R. blasticus* will be feasible.

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