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Article

Biological Treatment of Nutrients and Heavy Metals in Synthetic Wastewater Using a Carrier Attached to *Rhodobacter blasticus*

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Abstract

The removal efficiencies of nutrients (N and P) and heavy metals (Cu and Ni) by *Rhodobacter blasticus* and *R. blasticus* attached to polysulfone carriers, alginate carriers, PVA carriers, and PVA + zeolite carriers in synthetic wastewater were compared. In the comparison of the nutrient removal efficiency based on varying concentrations (100, 200, 500, and 1000 mg/L), *R. blasticus* + polysulfone carrier treatment showed removal efficiencies of 98.9~99.84% for N and 96.92~99.21% for P. The *R. blasticus* + alginate carrier treatment showed removal efficiencies of 88.04~97.1% for N and 90.33~97.13% for P. The *R. blasticus* + PVA carrier treatment showed removal efficiencies of 18.53~44.25% for N and 14.93~43.63% for P. The *R. blasticus* + PVA + zeolite carrier treatment showed removal efficiencies of 26.65~64.33% for N and 23.44~64.05% for P. In addition, at the minimum inhibitory concentration of heavy metals, *R. blasticus* (dead cells) + polysulfone carrier treatment showed removal efficiencies of 7.77% for Cu and 12.19% for Ni. *Rhodobacter blasticus* (dead cells) + alginate carrier treatment showed removal efficiencies of 25.83% for Cu and 31.12% for Ni.

Keywords: *Photosynthetic bacteria, Carrier, Nutrient, Heavy metal, Biological treatment*

1. Introduction

Industrial wastewater contains large amounts of toxic substances, such as heavy metals and dyestuffs; thus, discharge of wastewater into nature adversely affects the aquatic environment[1,2]. Livestock wastewater has a high chemical oxygen demand and high nitrogen, phosphorus, and heavy metal contents. Eutrophication, groundwater pollution, and air pollution can occur if wastewater remains untreated[3-5].

Since the 1900s, researchers have studied methods for treating wastewater. Physical, chemical, and biological techniques for wastewater treatment include flotation, precipitation, oxidation, solvent extraction, evaporation, carbon adsorption, ion exchange, membrane filtration, electrochemistry, biodegradation, and phytoremediation[6-11].

Biological treatment involves the removal of organic matter, nutrients, and heavy metals by microorganisms, such as algae, bacteria, and protozoa in wastewater. Biological treatment is an eco-friendly method and, unlike chemical treatment, can overcome concerns related to secondary pollution. In addition, it has high adsorption power, selectivity, and economic efficiency depending on the type of microorganism used[12].

For example, photosynthetic bacteria are highly efficient and inexpensive[13]. Photosynthetic bacteria utilize various nutrients from carbon and energy sources and are widely distributed in rice fields, swamps, lakes, and seas. Among photosynthetic bacteria, *Rhodobacter* showed a high efficiency in removing high concentrations of NH^{4+} and low concentrations of C and N from wastewater[14]. Additionally, *Rhodobacter* can remove and has a high tolerance to heavy metals [15]. The functional group of the cell wall of *Rhodobacter* acts as an ion exchanger that adsorbs heavy metal ions by desorbing cations[16].

The removal efficiency by microorganisms at the laboratory scale has been widely evaluated. However, in actual contamination sites, the biological treatment method exhibits a low removal efficiency, limiting its field application. The anammox[17], Sharon[18], and sequence batch reactor processes[19] have been examined for treating biological wastewater. However, in Korea, microbial input is applied only in the sequence batch reactor process because of issues related to the microbial culture time and concentration maintenance[20].

To overcome these limitations, a carrier capable of attaching to microorganisms along with the bioreactor burial method have been examined[21]. Using a microbial carrier in the wastewater treatment process can reduce the influence of the environment on microorganism growth [22,23]. In addition, as the adsorption reaction proceeds rapidly, several contaminants can be quickly removed[24]. Therefore, using micro-

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organisms and carriers concurrently can prevent the loss of microorganisms to maintain their high concentrations during wastewater treatment[25].

A microorganism-attached carrier used to treat wastewater is known as a membrane bioreactor (MBR). MBR combines the activated sludge process of biological treatment with the membrane process but is problematic because biofilm contaminates the separator. In addition, chemical stability and physical strength are essential because carriers should remain stable in wastewater[26].

Materials are typically classified as metals, organic polymers, ceramics, and nanocomposites; however, metals are not suitable as microbial carriers because of their crystal structure. Therefore, organic polymers, ceramics, and nanocomposites are primarily used as carrier materials. Thus, substances such as polysulfone[27], alginate[28], polyvinyl alcohol (PVA)[29], and zeolite[30] have been studied as microbial carriers.

Polysulfone has extremely high dimensional stability and excellent chemical durability against inorganic acids, alkalis, oxidizing agents, surfactants, and hydrocarbon oils over a wide pH range of 2~13[31].

Alginate was the first hydrophilic polysaccharide to be extracted from brown algae[32,33]. In the alginate structure, α-L guluronate and β-D mannuronate are combined[34]. Alginate beads are formed by repeated crosslinking upon addition of a divalent cation[35-37]. Ca^{2+} is the most widely used divalent cation in these carriers because these carriers are strong and easy to manufacture[26].

PVA is a hydrophilic material containing many hydroxide groups, which form a complex chain network by cross-linking with boric acid. The PVA carrier shows minimal deformation following exposure to heat and organic solvents and has plasticity because of heat[38,39]. Moreover, these carriers are neither biodegradable nor toxic and have an average pore diameter of 20.3 μm[40].

Zeolites are composed of refined crystal grains. Because zeolites float or are suspended in water, separating and reusing the solid from the liquid after wastewater treatment is challenging. In addition, a pressure decrease may occur when the column is filled[41]. Recently, studies have been conducted to treat wastewater using a carrier in which a polymer material and zeolite are mixed to compensate for the disadvantages of zeolites[42].

Microorganisms effectively removed contaminants from wastewater when attached to the materials described above. In addition, wastewater treatment methods using the adsorption mechanism of a microorganism carrier with dead cells have been evaluated. Dead cells are easier to manage in the field, have excellent field applicability, and shorten the contaminant removal time compared with studies using live bacteria [43-46].

The removal of pollutants from wastewater by microorganisms attached to carriers has been widely examined. However, studies comparing the removal efficiencies of microorganisms attached to different carriers are lacking. Therefore, we compared the efficiency of pollutant removal from wastewater by *Rhodobacter blasticus* attached to polysulfone carriers, alginate carriers, PVA carriers, and PVA + zeolite carriers.

Table 1. Components of Van Niel's Medium

Component	Amount in distilled water (1 L)
$MgSO_4$	0.1 g
EDTA	0.002 g
Yeast extract	10.0 g
Trace elements	10.0 mL
4% K_2HPO_4	2.5 mL

2. Experimental procedures

2.1. Bacterial culture

Rhodobacter blasticus (KCTC No. 15056) was supplied by the Korean Collection for Type Cultures (Jeollabuk-do, Korea). The cells were incubated in a shaking incubator at 30 °C, pH 7, 14:10 h photoperiod (light: dark cycle), h3000 lux, and 200 rpm shaking.

2.2. Carrier manufacture

To prepare a polysulfone carrier, 10% polysulfone was prepared by mixing 90 g *N*, *N*-dimethylformamide and 10 g polysulfone. Next, 10% polysulfone was transferred into 80% methanol using a syringe to cause hardening into a bead shape. The cured beads were washed twice with distilled water and dried at ambient room temperature for 48 h.

To prepare PVA carriers, 8% (w/v) PVA was entirely dissolved in an autoclave. PVA [8% (w/v)] was transferred into a solution of 6% (w/v) boric acid and 25% glutaraldehyde using a syringe for curing into the bead shape. The cured beads were then washed twice with distilled water.

To prepare the PVA-zeolite carrier, 4 g of PVA, 0.675 g of sodium alginate, and 3 g of zeolite were entirely dissolved in 50 mL distilled water. The mixed solution was transferred into 6% (w/v) boric acid using a syringe to form the bead shape. The cured beads were washed twice with distilled water.

Sodium alginate [2% (w/v)] was prepared and transferred into 0.1 M $CaCl_2$ using a syringe to cure the bead shape. The cured beads were washed twice with distilled water.

2.3. Synthetic wastewater manufacture

The growth and removal efficiency of *R. blasticus* according to the concentration of nutrients (N and P) in Van Niel's medium (Table 1) were evaluated. To remove nutrients, nitrogen treatment plots were prepared at concentrations of 50, 100, 200, and 500 mg/L using NH_4Cl in medium. Phosphorus treatment plots were prepared at concentrations of 50, 100, 200, and 500 mg/L using KH_2PO_4 in medium.

The removal efficiency by the *R. blasticus* + carrier according to the concentration of nutrients (N and P) in Van Niel's medium was evaluated. To remove nutrients, nitrogen treatment plots were prepared at NH_4Cl concentrations of 100, 200, 500, and 1000 mg/L in medium. KH_2PO_4 solutions were prepared at concentrations of 100, 200, 500, and 1000 mg/L in medium.

The minimum inhibitory concentration (MIC) of *R. blasticus* according to heavy metals (Cu and Ni) in 27M medium (see Table 2) was

Table 2. Components of 27M

Component	Amount in distilled water (1 L)
Yeast extract	1.0 g
Ethanol	0.5 mL
Disodium succinate	1.0 g
0.1% Ferric citrate	5.0 mL
KH_2PO_4	0.5 g
$MgSO_4{\cdot}7H_2O$	0.4 g
NaCl	0.4 g
NH_4Cl	0.4 g
$CaCl_2{\cdot}2H_2O$	0.05 g
Trace Element Solution SL-6	1.0 g
Sodium ascorbate	0.5 g

measured. The heavy metal removal efficiency was analyzed in Van Niel's medium prepared at the MIC (Ni 35 mg/L, Cu 6 mg/L). A heavy metal stock solution (5 mM) was prepared using copper chloride ($CuCl_2 \cdot 2H_2O$) and nickel chloride ($NiCl_2 \cdot 6H_2O$), which was sterilized through a filter membrane with a pore size of 0.22 μm.

2.4. Analysis methods

Nutrients were analyzed by measuring total nitrogen (T-N) and total phosphorus (T-P) according to the water pollution test method (MOE 2011).

T-N was analyzed at 220 nm based on oxidation, and T-P was measured at 880 nm using ultraviolet/visible spectroscopy.

Heavy metals were analyzed using an Inductively Coupled Plasma Optical Emission Spectrometer (PerkinElmer, Waltham, MA, USA) after pretreating the samples with nitric acid-sulfuric acid according to the water pollution standard method (MOE 2011). The analysis conditions of inductively coupled optical emission spectrometry were as follows: RF (Radio Frequency) power 1,300 KW, nebulizer spray, plasma flow of 15 L/min, auxiliary flow of 0.2 L/min, and nebulizer flow of 0.65 L/min.

2.5. Statistical analysis

The results were analyzed using SAS software (Statistical Analysis System, version 9.1, SAS Institute, Inc., Cary, NC, USA). A *t*-test, analysis of variance, and Tukey's honestly significant difference test were performed to detect significant differences in the experimental results. The confidence interval was set to 95%, and all experiments were performed thrice.

3. Results and discussion

3.1. Carrier shape

Four types of carriers were prepared: polysulfone, PVA, PVA + zeolite, and alginate. The carriers were in the form of spherically shaped beads (Figure 1).

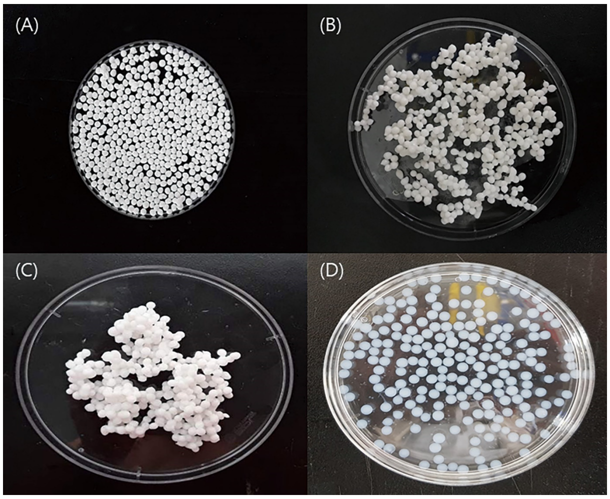

Figure 1. Shape of the polysulfone carrier (A), polyvinyl alcohol (PVA) (B), PVA + zeolite carrier (C), and alginate carrier (D).

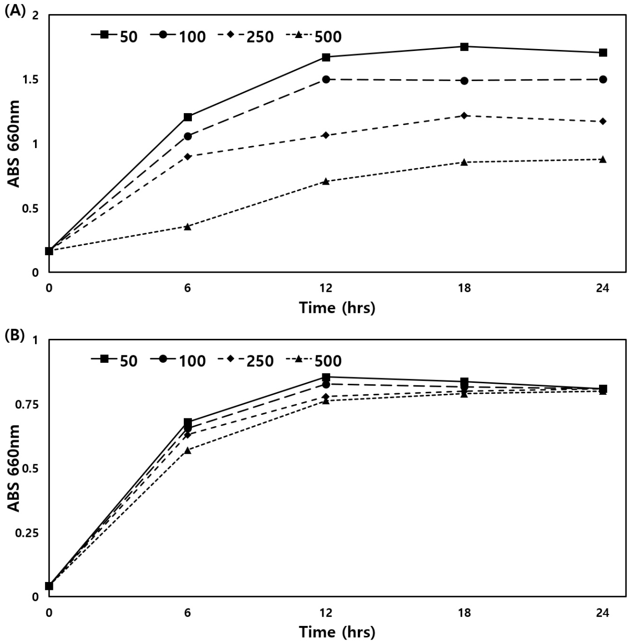

Figure 2. Growth of *Rhodobacter blasticus* at different concentrations of ammonium (A) and phosphate (B).

The polysulfone carrier had a diameter of approximately 3~4 mm and specific gravity of 0.933 g/cm^3. The PVA carrier had a diameter of approximately 3 mm and specific gravity of 1.031 g/cm^3. The PVA + zeolite carrier had a diameter of approximately 3~4 mm and specific gravity of 0.933 g/cm^3. The alginate carrier had a diameter of approximately 3~4 mm and specific gravity of 0.933 g/cm^3.

3.2. Growth of *R. blasticus* depending on the nutrient (N and P) concentration in synthetic wastewater

This experiment was conducted to confirm the growth of *R. blasticus* by nutrients (N, P). The experiment was performed in synthetic wastewater containing ammonium and phosphate (at concentrations of 50, 100, 250, and 500 mg/L) (see Figure 2).

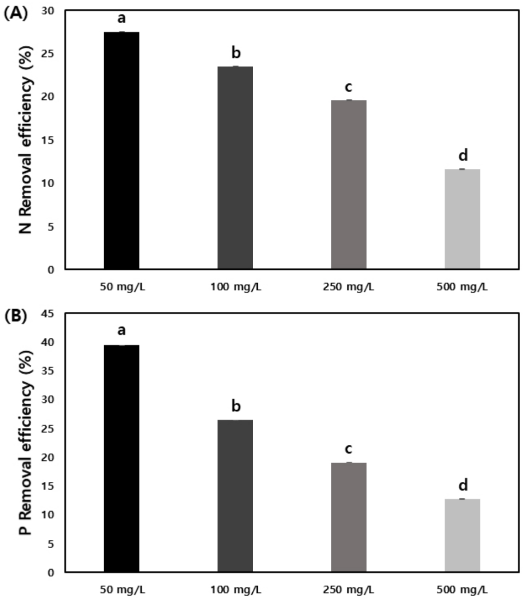

Figure 3. Removal efficiencies of N (A) and P (B) by *R. blasticus* for 24 h (stationary phase). Means followed by letter were significantly different according to analysis of variance at the 0.05 level.

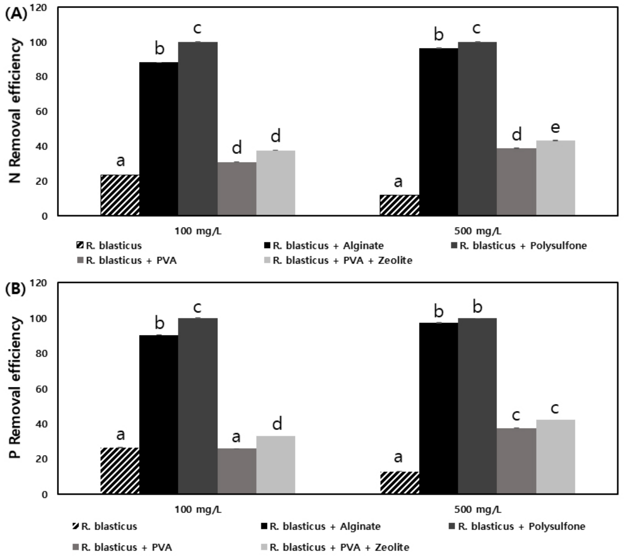

Figure 4. Removal efficiencies of N (A) and P (B) by *Rhodobacter blasticus*, *R. blasticus* + carrier (alginate, polysulfone, PVA, and PVA + zeolite) for 24 h (stationary phase). Means followed by letter were significantly different according to analysis of variance at the 0.05 level.

The growth of *R. blasticus* decreased significantly with increasing ammonium concentrations ($p < 0.05$). Moreover, the initial growth of *R. blasticus* decreased with increasing phosphate concentrations; however, the growth of *R. blasticus* after 24 h did not significantly differ among the tested concentrations ($p < 0.05$). Most microbial surfaces are negatively charged, and thus are greatly affected by positive ions than by negative ions. Cationic substances can destroy cell membranes and inhibit bacterial growth[47]. Therefore, the nitrogen concentration had greater effects than the phosphorous concentration on the growth of *R. blasticus*.

3.3. Removal efficiency of *R. blasticus* depending on nutrient (N and P) concentration in synthetic wastewater

We examined the ability of *R. blasticus* to efficiently remove nutrients from synthetic wastewater containing ammonium and phosphate (see Figure 3). The removal efficiencies in the nitrogen treatment plot were 28.37, 22.29, 18.44, and 12.03% in the presence of 50, 100, 250, and 500 mg/L ammonium, respectively. The removal efficiencies in the phosphorus treatment plot were 39.34, 26.33, 19.00, and 12.67% in the presence of 50, 100, 250, and 500 mg/L phosphate, respectively ($p < 0.05$). Therefore, *R. blasticus* can remove some nitrogen and phosphorus from wastewater.

The N and P removal efficiency by R. *blasticus* decreased as the concentration of ammonium or phosphate increased. Photosynthetic bacteria grow by assimilating ammonium and phosphate ions[48,79]; Nitrogen must also be assimilated and accumulated in photosynthetic bacteria. Phosphorus can accumulate through both assimilation and in the form of polyphosphate[50]. A higher removal efficiency was observed in the phosphorus treatment plot than in the nitrogen treatment plot because phosphoric acid ions accumulated in *R. blasticus* via a method other than assimilation.

3.4. Removal efficiency of *R. blasticus* with each carrier depending on the nutrient (N and P) concentrations in synthetic wastewater

We compared the removal efficiency of nutrients from single treatment plots of *R. blasticus* and mixed treatment plots of *R. blasticus* + carrier. The experiment was conducted in synthetic wastewater containing ammonium and phosphate (concentrations: 100 and 500 mg/L) (see Figure 4). The removal efficiency of nutrients did not significantly differ between the *R. blasticus* single treatment plot and *R. blasticus* + PVA carrier mixed treatment plots at 100 mg/L phosphate. However, other *R. blasticus* + carrier mixture treatment plots showed higher removal efficiencies compared to that of *R. blasticus* single treatment plots at all concentrations. Therefore, attaching *R. blasticus* to the carrier increased its nutrient removal efficiency in wastewater.

We also compared the nutrient removal efficiency of *R. blasticus* attached to the different carriers. Experiments were conducted in synthetic wastewater containing ammonium and phosphate concentrations of 100, 200, 500, and 1000 mg/L (see Figure 5).

The removal efficiencies in the *R. blasticus* + polysulfone carrier mixture treatment plots were 99.84, 99.55, 99.72, and 98.9% at ammonium concentrations of 100, 200, 500, and 1000 mg/L, respectively, and 99.21, 97.84, 97.33, and 96.92% at the same phosphate concentrations, respectively ($p < 0.05$). The removal efficiencies in the *R. blasticus* + alginate carrier mixture treatment plots were 88.04, 92.54, 96.22, and 97.1% at ammonium concentrations of 100, 200, 500, and 1000 mg/L, respectively, and 90.33, 95.25, 97.13, and 95.32% at the same phosphate concentrations, respectively ($p < 0.05$). The removal efficiencies in the *R. blasticus* + PVA carrier mixture treatment plots

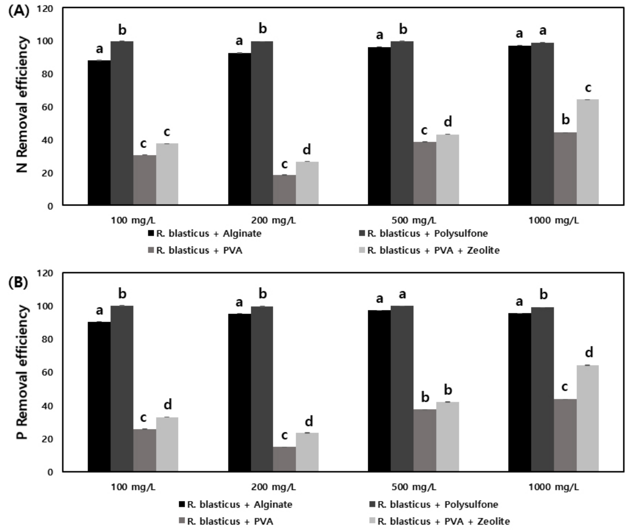

Figure 5. Removal efficiencies of N (A) and P (B) by *Rhodobacter blasticus* + carrier (alginate, polysulfone, PVA, and PVA + zeolite) for 24 h (stationary phase). Means followed by letter were significantly different according to analysis of variance at the 0.05 level.

were 30.75, 18.53, 38.63, and 44.25% at ammonium concentrations of 100, 200, 500, and 1000 mg/L, respectively, and was 25.72, 14.93, 37.42, and 43.63% at the same phosphate concentrations, respectively ($p < 0.05$). The removal efficiencies in the *R. blasticus* + PVA + zeolite carrier mixture treatment plots were 37.53, 26.65, 43.15, and 64.33% at ammonium concentrations of 100, 200, 500, and 1000 mg/L, respectively, and 32.94, 23.44, 42.03, and 64.05% at the same phosphate concentrations, respectively ($p < 0.05$).

Rhodobacter blasticus + polysulfone carrier mixture treatment plots showed the highest removal efficiencies, whereas *R. blasticus* + PVA carrier mixture treatment plots showed the lowest removal efficiencies.

The surface properties of the four carriers were evaluated. The membrane of the polysulfone carrier was close to hydrophobic, whereas the membranes of the PVA carrier, PVA + zeolite carrier, and alginate carrier were close to hydrophilic[51-54]. Hydrophobic carriers adsorb microorganisms faster and have a higher adsorption rate compared to that in hydrophilic carriers. In addition, the hydrophobic membrane, not the hydrophilic membrane, adsorbs microorganisms irreversibly, resulting in a low desorption rate[55].

The polysulfone carrier was prepared by mixing polysulfone with *N*, *N*-dimethylformamide solution. The adsorption of existing polysulfone carriers was mostly limited to the outer surface. Previous studies involving scanning electron microscopy and transmission electron microscopy analyses confirmed that adsorption of the polysulfone carrier mixed with *N*, *N*-dimethylformamide was also performed internally [56]. Because the polysulfone carrier is hydrophobic, it has a stronger ability to adsorb *R. blasticus* compared with the abilities of other hydrophilic carriers. In addition, *R. blasticus* attached to the polysulfone carrier showed the highest wastewater removal efficiency likely because it is not strongly influenced by the environment.

Alginate is a seaweed polysaccharide that possesses hydrophilic functional groups, such as hydroxyl groups (-OH), carboxyl groups (-COOH), amino groups (-NH_2), keto groups (-CO), and sulfone groups (-SO_3H). During the manufacture of alginate carriers, the carboxyl groups of alginate bind to Ca^{2+} to increase the hydrophobicity of the alginate carrier[57]. Therefore, the alginate carrier showed the second-highest nutrient removal efficiency, possibly because of its increased hydrophobicity.

The membrane of the PVA + zeolite carrier is more porous than that of the PVA carrier[58]. As the number of pores in a carrier increases, the microorganism's adherence to the carrier also increases[59]. Because of the unique crystal structure and hydrophilicity of zeolites, they exhibit high water permeability. As zeolite particles are negatively charged, excluding negative ions may increase because of the charge effect[60]. Therefore, the PVA + zeolite carrier mixture treatment plot exhibited a higher nutrient removal efficiency than that of the PVA carrier mixture treatment because of its larger number of pores and higher permeability. The negative charges of PVA and zeolite led to a low removal efficiency of negatively charged nitrate and phosphate ions. In a previous study, the removal efficiency of cations as adsorbents was concluded to be high when the PVA + zeolite carrier was used to treat wastewater[61].

3.5. Growth of *R. blasticus* depending on heavy metal (Cd, Ni, and Zn) concentrations in synthetic wastewater

We examined the growth of *R. blasticus* in synthetic wastewater containing the heavy metals cadmium, nickel, and zinc (concentrations of each were: 50, 10, 20, and 80 mg/L) (see Figure 6). The growth of *R. blasticus* decreased with increasing Cd^{2+} concentrations, whereas growth of *R. blasticus* at 20 and 80 mg/L Cd^{2+} showed no significant difference ($p < 0.05$). The growth of *R. blasticus* decreased with increasing Ni^{2+} concentrations ($p < 0.05$). The growth of *R. blasticus* decreased with increasing Zn^{2+} concentrations but did not significantly differ at 5 and 10 mg/L Zn^{2+} ($p < 0.05$). Therefore, *R. blasticus* can remove different heavy metals with efficiencies in the order of Zn, Ni, and Cd.

Based on these results, *R. blasticus* is resistant to high concentrations of heavy metal ions. These results are consistent with those of studies demonstrating that high concentrations of nutrient (N, P) ions do not affect R. *blasticus* during photosynthesis and those revealing large delays in growth at high concentrations of heavy metals, unlike high concentrations of nutrients (see Figure 2 and 6)[62].

3.6. Removal efficiency of *R. blasticus* depending on heavy metal (Cd, Ni, and Zn) concentrations in synthetic wastewater

We examined the ability of *R. blasticus* to efficiently remove heavy metals from synthetic wastewater containing cadmium, nickel, and zinc (concentrations of each were: 10, 20, 50 and 80 mg/L) (see Figure 7). The removal efficiencies were 92.03, 84.69, 58.88, and 47.45% in the Cd treatment plot; 60.91, 58.99, 51.47, and 54.79% in the Ni treatment plot; and 11.14, 11.80, 8.7, and 2.33% in the Zn treatment plot containing 50, 10, 20, and 80 mg/L of each ion, respectively ($p < 0.05$). Therefore, *R. blasticus* is suitable for removing Cd, Ni, and Zn from

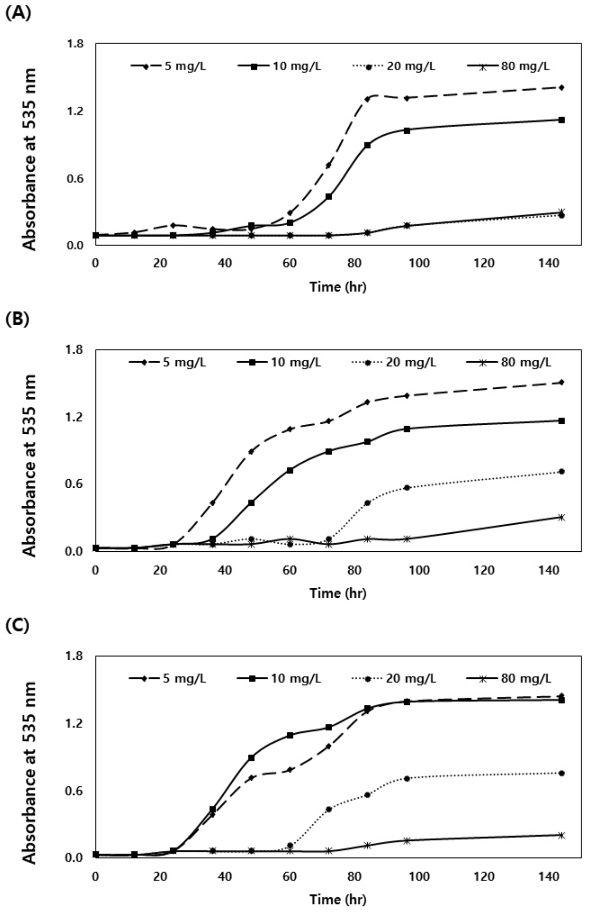

Figure 6. Growth of *Rhodobacter blasticus* at different concentrations of Cd^{2+} (A), Ni^{2+} (B), and Zn^{2+} (C).

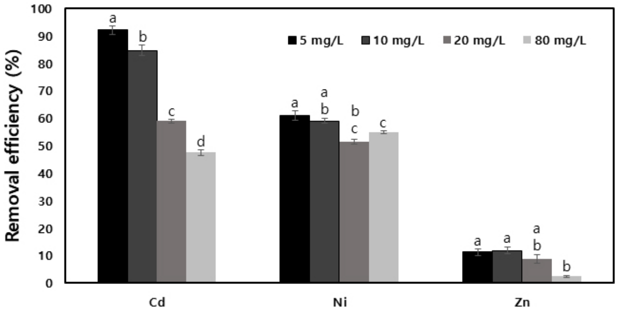

Figure 7. Removal efficiencies of Cd^{2+} (A), Ni^{2+} (B), and Zn^{2+} (C) by *Rhodobacter blasticus* for 24 h (stationary phase). Means followed by letter were significantly different according to analysis of variance at the 0.05 level.

wastewater.

The removal efficiency of *R. blasticus* decreased with increasing Cd^{2+} concentrations ($p < 0.05$) and decreased with increasing Ni^{2+} concentrations but did not significantly differ at 5 and 10 mg/L, 10 and 20 mg/L, and 20 and 80 mg/L Ni^{2+}, respectively ($p < 0.05$). The removal efficiency of *R. blasticus* decreased with increasing Zn^{2+} concentrations but did not significantly differ between 5, 10, and 20 mg/L and between 20 and 80 mg/L Zn^{2+}, respectively ($p < 0.05$).

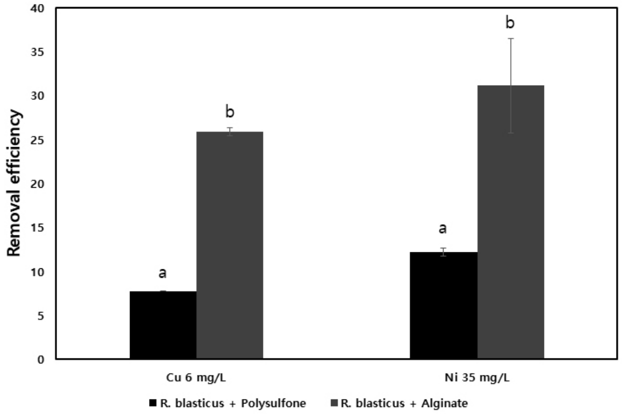

Figure 8. Removal efficiencies of Cu^{2+} and Ni^{2+} by *Rhodobacter blasticus* + alginate carrier and *R. blasticus* + polysulfone carrier for 24 h (stationary phase). Means followed by letter were significantly different according to *t*-test at the 0.05 level.

Heavy metal ions react with functional groups on the *Rhodobacter* surface, which can disrupt the plasma membrane[63]. *Rhodobacter* can accumulate metal ions, which is considered as a mechanism of heavy metal resistance[64]. In this study, different concentrations of Zn^{2+} showed minimal effects compared with those of other ions on the growth of *R. blasticus*. However, the removal efficiency was lower than those in the other heavy metal treatment plots. The growth of *R. blasticus* in the Cd^{2+} treatment plot showed the largest decrease with increasing concentrations, but the removal efficiency was highest compared to those in other heavy metal treatment plots. Therefore, in contrast to nutrient removal by *R. blasticus*, heavy metal removal is correlated with resistance rather than with growth.

3.7. MIC of *R. blasticus* depending on heavy metal (Cu and Ni) concentrations in synthetic wastewater

The MIC of *R. blasticus* was measured to establish the removal efficiency of dead *R. blasticus* cells with each carrier in synthetic wastewater containing heavy metals (Cu and Ni). After culturing *R. blasticus* at Cu and Ni concentrations of 0~1.5 mM for 48 h under microaerobic conditions, the MIC of *R. blasticus* in the Cu^{2+} treatment plot was 6 mg/L (0.12 mM) and that in the Ni^{2+} treatment plot was 35 mg/L (0.78 mM).

3.8. Removal efficiency by dead *R. blasticus* cells with each carrier depending on heavy metal (Cu and Ni) concentrations in synthetic wastewater

The removal efficiencies of heavy metals (Cu^{2+}, Ni^{2+}) were compared between the dead *R. blasticus* cell + polysulfone carrier mixture treatment plot and dead *R. blasticus* cell + alginate carrier mixture treatment plot in terms of the MIC of heavy metals. The experiment was performed using synthetic wastewater containing 6 mg/L Cu^{2+} or 35 mg/L Ni^{2+} (see Figure 8). The removal efficiencies in the *R. blasticus* (dead cells) + polysulfone carrier treatment plots were 7.77% for Cu^{2+} and 12.19% for Ni^{2+}. The *R. blasticus* (dead cells) + alginate carrier treatment plots showed 25.83% removal efficiencies for Cu^{2+} and 31.12% for Ni^{2+}. These results showed that the removal efficiency of Cu^{2+} and Ni^{2+} significantly differed between the *R. blasticus* (dead cell) + polysulfone mixed treatment plot and *R. blasticus* (dead cell) + alginate mixed treatment plot.

The membrane of the polysulfone carrier was hydrophobic, whereas that of the alginate carrier was hydrophilic. Moreover, the alginate carrier membrane was negatively charged[65]. Therefore, the removal efficiency of heavy metal ions by the negatively charged alginate carrier was higher in the *R. blasticus* (dead cells) + alginate mixed treatment plot than in the *R. blasticus* (dead cells) + polysulfone mixed treatment plot. Alginate carriers adsorbed by dead photosynthetic bacteria can desorb adsorbed heavy metals[66]. Therefore, the alginate carrier to which *R. blasticus* is attached can be reused by separating the attached heavy metals.

4. Conclusions

In the N and P treatment groups (50, 100, 250, and 500 mg/L), the nitrogen removal efficiency by *R. blasticus* was 12.03~28.37% and phosphorus removal efficiency was 12.67~39.34%. In each heavy metal treatment group (5, 10, 20, and 80 mg/L), the Cd^{2+} removal efficiency by *R. blasticus* was 47.45~92.0 3%, Ni^{2+} removal efficiency was 54.79~60.91%, and Zn^{2+} removal efficiency was 2.33~11.14%. Thus, *R. blasticus* can remove nutrients and heavy metals from wastewater.

Regardless of the carrier type, adding a carrier to synthetic wastewater increased the removal efficiency of nutrients (N and P) by *R. blasticus*. The hydrophobic polysulfone carrier increased the ability of *R. blasticus* to remove nutrients by more than the hydrophilic alginate, PVA, and PVA + zeolite carriers. Additionally, hydrophobic carriers have a higher microbial adsorption capacity compared with those of hydrophilic carriers.

Because alginate contains a carboxyl group (-COOH) and thus is negatively charged, it has a strong capacity to remove heavy metals from wastewater. The efficiency of heavy metal removal was greatly increased by the alginate carrier than by the polysulfone carrier. A microorganism attached to a negatively charged carrier has a high capacity to remove heavy metals.

Acknowledgements

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