

## Microbial treatment of Pb(II) using a newly isolated Pb(II)-resistant *Methylobacterium* sp. MTS1 strain

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**Abstract**—Growing concerns about the adverse effects of Pb(II) on public health have drawn much attention to the development of efficient and environmentally friendly treatment methods. Here we report the isolation of a novel Pb(II)-resistant *Methylobacterium* sp. MTS1 strain from abandoned mine soil that has potential as a biosorbent for the removal of Pb(II). The isolated MTS1 strain not only showed high resistance even at 1,000 mg/L, but also showed excellent performance in removing Pb(II) through biosorption. The maximum removal capacity and removal efficiency of Pb(II) were found to be  $56.55 \pm 6.2$  mg/g and  $98.6 \pm 0.6\%$ , respectively, under optimized conditions (pH 7; biomass, 1 g/L; contact time, 60 min). Hydroxyl, amide, carboxyl, phosphate, nitro compound, and disulfide groups as well as various functional groups such as C, O, and P were identified as key factors for Pb(II) removal. In addition, equilibrium data obtained by biosorption and adsorption kinetic model were in agreement with Langmuir isotherm and pseudo-second-order models, indicating that the biosorption process involved monolayer chemisorption at uniformly distributed active sites on the surface of the MTS1 strain.

Keywords: Lead, *Methylobacterium*, Biosorption, Metal Resistance, Heavy Metal Treatment

### INTRODUCTION

Growing concerns about the leakage of toxic heavy metals in a variety of industries and their effect on humans are driving efforts to develop efficient and environmentally friendly heavy metal removal methods. Pb(II) is known as the most toxic heavy metal used in a variety of industries, including battery, metal smelting, and water pipe manufacturing industries [1]. Even trace amounts of Pb(II) can cause a variety of adverse effects, from intellectual disability and reproductive disorders to neurodegenerative disorders [2,3]. Therefore, there is an urgent need to develop an efficient and environmentally friendly method for the treatment of Pb(II).

There have been many studies on the development of physicochemical treatment methods for the removal of heavy metals, such as ion exchange, solvent extraction, adsorption, membrane filtration, precipitation, and reverse osmosis [4]. For example, the modified cationic resin Puroplite S-930 was effective in removing Pb(II) from drinking water by up to 90% [5]. The polyvinylidene difluoride (PVDF) membrane modified with zirconium and phosphate showed up to 121.2 mg-Pb/g of Pb(II) removal efficiency in wastewater treatment [6]. Additionally,  $\text{Ca}(\text{OH})_2$ , sodium di-(*n*-octyl) phosphinate (NaL), and 1,3-benzenediamidoethanethiol dianion ( $\text{BDET}^{2-}$ ) have also been used to treat Pb(II) from industrial wastewater [7,8]. However, these methods are not suitable for environmentally friendly water treatment due to their high energy costs and the generation of by-products that can cause secondary pollution.

Bioremediation through microbial treatment has long been regarded as a route with several advantages over the physicochemical treatment methods in terms of specificity, eco-friendliness, and adaptability to the contaminated area [9-13]. There have been several studies reporting the isolation of novel microorganisms with the potential for the treatment of environmental contaminants. Pb(II)-resistant *Bacillus* sp. PZ-1 with a maximum Pb(II) removal capacity of 93.01% was isolated from a lead-zinc mine in China [14]. Furthermore, the isolation of *Pseudomonas* sp. W6 strain capable of detoxifying Pb(II) by using extracellular polysaccharides has been reported by a case study conducted in North-East India [15].

Based on these observations, it inspired us to isolate a novel microorganism that has the potential to be used as a biosorbent for the treatment of Pb(II) from aqueous media. *Methylobacterium* sp. MTS1 strain newly isolated in this study showed remarkable performance in removing Pb(II) with a maximum removal capacity and removal efficiency of  $56.55 \pm 6.2$  mg/g and  $98.6 \pm 0.6\%$ , respectively.

### MATERIALS AND METHODS

#### 1. Soil Sampling and Isolation of *Methylobacterium* sp. MTS1

For the isolation of a lead-resistant bacterial strain, a soil sample was taken from an abandoned mine site in Republic of Korea (location at  $36^\circ 54' 10.19''\text{N}$ ,  $127^\circ 15' 17.72''\text{E}$ ). One gram of a soil sample was suspended in sterilized saline (0.85% NaCl, w/v). The suspension was agitated for 30 min at 100 rpm. The supernatant was serially diluted with sterilized saline and spread on TGY agar plates (0.5% tryptone, 0.1% glucose, 0.3% yeast extract, and 1.5% bactoagar) supplemented with 1 mM  $\text{PbCl}_2$  (Daejung, Korea). After the incubation of the agar plates at  $30^\circ\text{C}$  for one week, a single red-pig-

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mented colony was transferred to a fresh TGY agar medium to obtain a pure strain. A pure culture of the bacterial strain was mixed with 80% glycerol (v/v) in a 1:1 ratio and stored at  $-80^{\circ}\text{C}$  in a deep freezer until its analysis.

A 16s rRNA gene of length 1,534 bp was amplified by polymerase chain reaction (T100<sup>TM</sup> thermal cycler, Bio-Rad) by using the universal primers 27F (5'-AGAGTTTGTATCTTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTACGACTT-3'), and was then compared by using the EzTaxon-e server (<https://www.ezbiocloud.net/>) for bacterial identification. Phylogenetic analysis was performed for *Methylobacterium* sp. MTS1 by using the Mega 7.0 software [16], neighbor-joining (NJ) algorithm [17] with Kimura two-parameter model [18]. Furthermore, a bootstrap analysis with 1000 replicates was performed [19], and 16s rRNA sequences of closely related bacterial species were obtained from GenBank to construct a phylogenetic tree.

## 2. Phenotypic Characterization of *Methylobacterium* sp. MTS1

Biochemical tests were evaluated by the API 20NE kit (bioMérieux<sup>TM</sup>, France) and following the instructions of the manufacturer. Tetramethyl-*p*-phenylenediamine (1%, w/v) and 3% (v/v) of hydrogen peroxide (Sigma-Aldrich, St. Louis, MO, USA) were used for oxidase and catalase activities. Gram-staining of MTS1 strain was carried out as described by Gerhardt et al. [20]. The cell morphology was examined using an inverted light microscope (Nikon Eclipse Ti-U, Japan). The cell growth of the MTS1 strain was evaluated under different culture conditions, including different temperatures, different NaCl concentrations, and different pH.

## 3. Evaluation of Minimum Inhibitory Concentration (MIC) of Pb(II) for MTS1 strain

To test the resistance of the MTS1 strain to Pb(II), the minimum inhibitory concentration (MIC) was determined by plating assay using TGY agar. Briefly, a single colony was seeded in 3 mL of TGY broth. Seed culture (500  $\mu\text{L}$ ) was added to 50 mL of TGY broth and the mixture was incubated at  $30^{\circ}\text{C}$  for 24 h with shaking. It was then serially diluted with fresh TGY broth and spotted onto TGY agar plates supplemented with different  $\text{PbCl}_2$  concentrations (0–1,500 mg/L). The TGY agar plates were further incubated for seven days at  $30^{\circ}\text{C}$ .

## 4. Optimization of Pb(II) Removal Conditions

The isolated bacterial strain was assessed for Pb(II) removal under various biosorption parameters such as the initial Pb(II) concentration (10–400 mg/L), biomass amount (0.1–10 g/L), pH (3 to 9), and contact time (5 to 60 min). Fresh colonies of MTS1 strain were inoculated in 3 mL TGY broth at  $30^{\circ}\text{C}$  with 200 rpm agitation. Overnight cultures were transferred (1:100, v/v) into 50 mL of fresh TGY broth and grown until they entered a stationary phase. Cells were then harvested and washed three times with deionized water. The sample was lyophilized and stored at  $-80^{\circ}\text{C}$  before used in Pb(II) removal test. The pH was adjusted using 0.1 M HCl or 0.1 M NaOH liquid solution. Pb(II) removal was tested using 10 mL of Pb(II) solution in a 250 mL Erlenmeyer flask. The other parameters, such as agitation (200 rpm) and temperature ( $30^{\circ}\text{C}$ ), were maintained constant. The amount of Pb(II) was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, iCAP<sup>TM</sup> 7000 Series, Thermo Scientific<sup>TM</sup>, USA). All experiments were performed in triplicate and all data are ex-

pressed as mean and  $\pm$  standard deviation. The Pb(II) removal efficiency (in percentage) and removal capacity (in milligrams per gram) were determined by the following equations:

$$\text{Removal efficiency (\%)} = \frac{C_i - C_e}{C_i} \times 100 \quad (1)$$

$$\text{Removal capacity (q}_e\text{)} = \frac{C_i - C_e}{X} \quad (2)$$

where  $C_i$  and  $C_e$  are the initial and final concentrations of Pb(II) (in milligrams per liter) in the aqueous solution, respectively,  $q_e$  (in milligrams per gram) is Pb(II) concentration that reached equilibrium on the biomass, and  $X$  is the biomass amount (in grams of dry cell per liter) [16].

## 5. Biosorption Isotherms and Kinetics Studies

Experiments for evaluating biosorption isotherms were conducted using 1 g/L of biomass at pH 7, with an initial Pb(II) concentration ranging from 10 mg/L to 400 mg/L; the duration of the experiments was 1 h. The Langmuir and Freundlich adsorption models were used to determine the adsorption isotherm of MTS1 strain for Pb(II). The Langmuir equation is

$$\frac{C_{eq}}{q_{eq}} = \frac{1}{Q_{max}K_L} + \frac{C_{eq}}{Q_{max}} \quad (3)$$

where  $C_{eq}$  is the equilibrium concentration of Pb(II) (in milligrams per liter),  $Q_{max}$  is the saturated adsorption capacity of the biosorbent (in milligrams per gram), and  $K_L$  (in liters per milligram) is the Langmuir constant [21]. The Freundlich model is

$$\ln q_e = \ln K_F + \frac{1}{n} \times \ln C_e \quad (4)$$

where  $K_F$  (in liters per gram) and  $n$  are an empirical parameter and the Freundlich constant, respectively [22].

In the case of biosorption kinetic experiments, the pH of the aqueous solution was fixed at pH 7 and the initial Pb(II) concentration was 10 mg/L. The biomass amount was fixed at 1 g/L. Samples were taken at different time intervals. The pseudo-first-order and pseudo-second-order kinetic models were used to fit the biosorption kinetic data. The equations of these kinetic models are as follows:

$$\ln(q_e - q_t) = \ln q_e - K_1 t \quad (5)$$

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \left(\frac{1}{q_e}\right)t \quad (6)$$

where  $q_e$  and  $q_t$  are the amounts of absorbed Pb(II) ions (in milligrams per gram) on the biomass at equilibrium and at time  $t$  (in minutes),  $K_1$  is the pseudo-first-order rate constant (in liters per minute), and  $K_2$  is the pseudo-second-order rate constant (in grams per milligram per minute) [16,23].

## 6. Analytical Methods

The surface morphology of *Methylobacterium* sp. MTS1 was analyzed using a field-emission scanning electron microscope (FE-SEM) (Inspect F50, FEI, USA), and the chemical elements were analyzed using an energy-dispersive spectrophotometer (EDAX Apollo XL, AMETEK). Before analysis, the samples were coated with platinum (Pt).

Fourier-transform infrared (FT-IR) spectroscopy (Nicolet iS10,

Thermo Scientific, USA) was used to investigate the surface functional groups of *Methylobacterium* sp. MTS1 before and after Pb(II) removal. Each sample was mixed with 2% KBr and compressed into a translucent sample disk, and infrared spectra were recorded in the region of 500–4,000  $\text{cm}^{-1}$  at a resolution of 1  $\text{cm}^{-1}$ .

## RESULTS AND DISCUSSION

### 1. Identification of Pb(II)-resistant Bacterium *Methylobacterium* sp. MTS1

The lead-resistant bacterium was screened using a TGY agar plate supplemented with 1 mM  $\text{PbCl}_2$  (278 mg/L). Then, the physiological and biochemical characteristics of an isolated strain were determined using the standard method [24] and 20NE strip kit.

Table 1. Physiological and biochemical characteristics of *Methylobacterium* sp. MTS1 strain

Characteristics	<i>Methylobacterium</i> sp. MTS1
<b>Gram staining</b>	–
<b>Colony color</b>	Red
<b>Cell shape</b>	Rod
<b>Cell dimensions (<math>\mu\text{m}</math>)</b>	
Cell length	4.1 ( $\pm 0.8$ )
Cell width	1.6 ( $\pm 0.18$ )
<b>Motility</b>	+
<b>Temperature range (<math>^{\circ}\text{C}</math>)</b>	20–40
<b>NaCl range (%)</b>	0–1
<b>Range of pH</b>	6–8
<b>Production of acid from glucose</b>	–
<b>Production of indole</b>	–
<b>Nitrate reduction</b>	+
<b>Enzyme activity</b>	
Catalase	+
Oxidase	w
Arginine dihydrolase	–
Urease	+
$\beta$ -Glucosidase	–
$\beta$ -Galactosidase	–
Protease	–
<b>Assimilation</b>	
D-glucose	–
L-arabinose	+
D-mannose	–
D-mannitol	–
N-acetyl-glucosamine	–
D-maltose	–
Potassium gluconate	+
Capric acid	–
Adipic acid	–
Maleic acid	w
Trisodium citrate	–
Phenyl acetic acid	–

w, weak; –, negative; +, positive

As shown in Table 1, the isolated bacterial strain was categorized as gram-negative, motile, aerobic, rod-shaped, and red-pigmented bacterium, and it could grow optimally at pH 7.0 and 30  $^{\circ}\text{C}$  in a TGY culture medium without salt. From the 20NE test, the bacterium was found to show positive reactions for catalase, oxidase, urease, and the reduction of nitrate. It could also utilize L-arabinose, potassium gluconate, and maleic acid as the sole carbon source. In addition, the isolated strain was identified as belonging to the genus *Methylobacterium* through phylogenetic analysis based on the 16S rRNA gene (Fig. 1) and showed the highest similarity to *M. curvus* (GenBank accession: MH158285) (98.2%). Based on these results, the newly isolated bacterium in this study was named *Methylobac-*

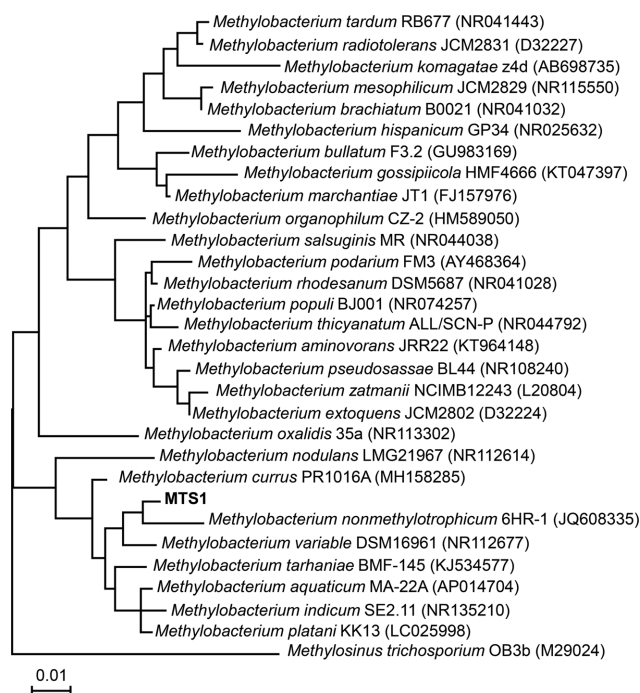


Fig. 1. Phylogenetic tree of *Methylobacterium* sp. MTS1 strain constructed using the Neighbor Joining algorithm (bar: 0.01 substitutions per nucleotide position).

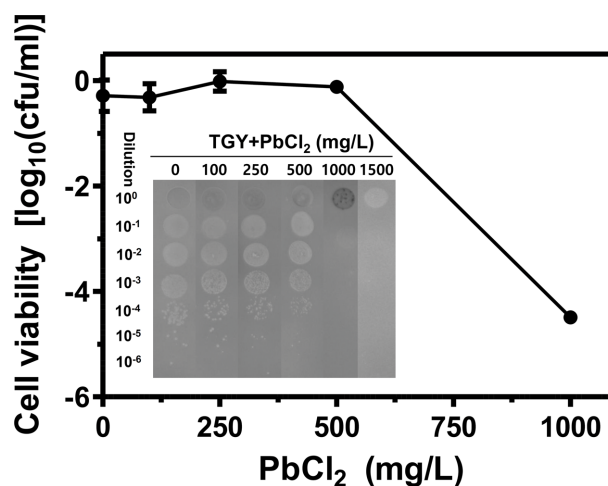


Fig. 2. Pb(II) resistance of *Methylobacterium* sp. MTS1 strain.

*terium* sp. MTS1.

## 2. Removal of Pb(II) using *Methylobacterium* sp. MTS1 Strain

Several studies have reported that microorganisms that are highly resistant to certain heavy metals also show excellent performance in removing those heavy metals [25–31]. As shown in Fig. 2, the MTS1 strain showed strong resistance against Pb(II) up to 500 mg/L, despite being decreased sharply with increased concentration up to 1,000 mg/L. Therefore, it could be hypothesized that the Pb(II)-resistant bacterial strain MTS1 isolated in this study has the potential to be used for biological treatment of Pb(II)-contaminated sites. Thus, we investigated the performance of the *Methylobacterium* sp. MTS1 strain for the removal of Pb(II).

Before examining the Pb(II) removal performance of the *Methylobacterium* sp. MTS1 strain, we first optimized various parameters that could give the best performance in Pb(II) removal, including pH, biomass amount, contact time, and initial concentration. As shown in Fig. 3, the maximum Pb(II) removal efficiency of the MTS1 strain was observed at pH 7 ( $99.3 \pm 2.2\%$ ), 1 g/L biomass ( $98.5 \pm 3.2\%$ ), within 60 min ( $99 \pm 3.1\%$ ), and initial concentration of 10 mg/L Pb(II) ( $98.6 \pm 0.6\%$ ). Interestingly, the *Methylobacterium* sp. MTS1 strain showed rapid kinetics in the pH 4–5 range and within 10 minutes after the start of the reaction. It seems to be due to the active adsorption sites available on the biomass surface and the degree of protonation of the functional groups involved in the adsorption of metal ions. These results are consistent with the previous studies reporting microbial treatment of cationic heavy metals through biosorption [32,33]. Next, to determine the maximum

Pb(II) removal capacity of MTS1 strain, a batch adsorption experiment was carried out using varying concentrations of Pb(II) (10–400 mg/L) under optimized conditions (1 g/L of biomass, 7.0 of pH, and 1 h of contact time) (Fig. 3(d), inset). At lower initial concentration, the Pb(II) removal capacity ( $q_e$ ) gradually increased by increasing the initial concentration of Pb(II). This is probably because higher initial Pb(II) concentrations can increase the collisions between Pb(II) and available active sites of the biomass, thereby promoting the sorption of Pb(II) ions from the aqueous solution [34]. With continuously increasing initial concentration of Pb(II) up to 400 mg/L, the Pb(II) removal capacity reached an equilibrium status between Pb(II) and the biomass, and the maximum removal capacity for Pb(II) was achieved to be  $56.55 \pm 6.2$  mg/g, which is higher than that of other reported microbial biosorbents (Table 3).

## 3. SEM-EDX and FT-IR Analysis

For the elemental identification and determination of the chemical components involved in the MTS1 strain-mediated Pb(II) removal, scanning electron microscopy-energy dispersive X-ray (SEM-EDX), and FT-IR analysis were performed. As shown in Fig. 4, Pb(II) was confirmed to be adsorbed on the cell surface, and a rough and wrinkled form was observed after Pb(II) adsorption. This is probably because the cationic Pb(II) ions and the negatively charged functional groups strongly cross-link to the cell wall [14, 33,40]. In addition, as depicted in the inset of Fig. 4, the atomic percentage (at%) of Pb(II) was 1.16%, and after Pb(II) adsorption, carbon (C) significantly decreased from 82.41% to 80.31%, while

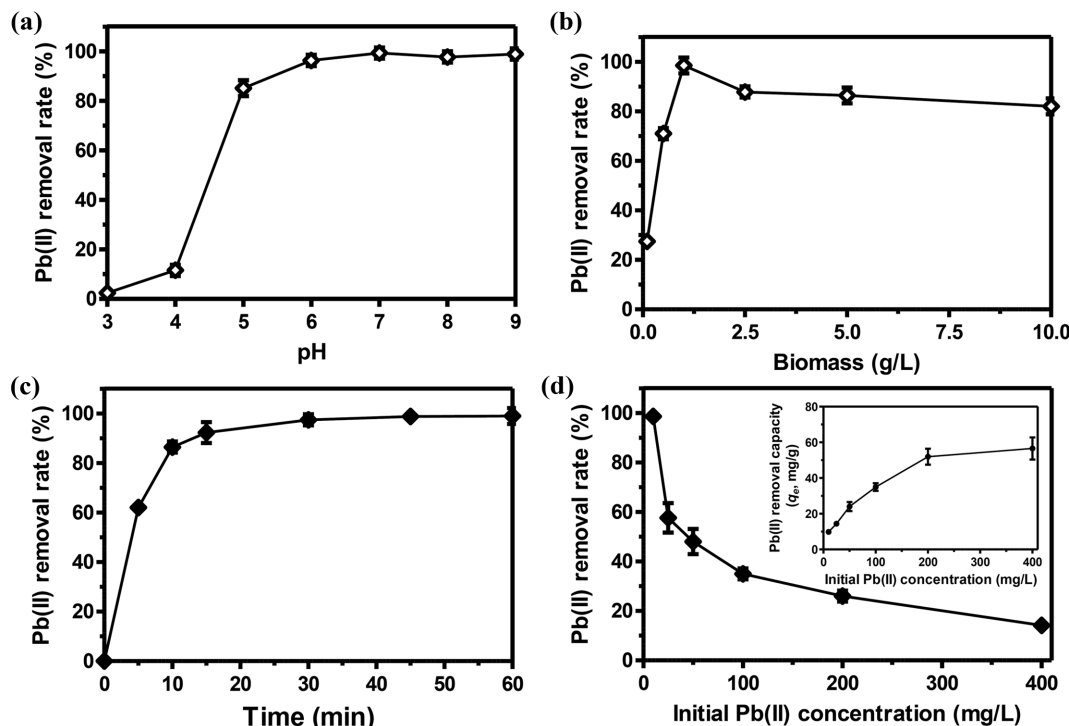


Fig. 3. Effect of different parameters on Pb(II) biosorption by MTS1 strain: (a) pH, (b) biomass, (c) contact time, and (d) initial Pb(II) concentration. Each experimental condition: for pH, 1 g/L of biomass, 1 h of contact time, and 10 mg/L of initial Pb(II) concentration; for biomass, 1 h of contact time, 10 mg/L of initial Pb(II) concentration, and pH 7; for contact time, 1 g/L of biomass, 10 mg/L of initial Pb(II) concentration, and pH 7; for initial Pb(II) concentration, 1 g/L of biomass, 1 h of contact time, and pH 7.

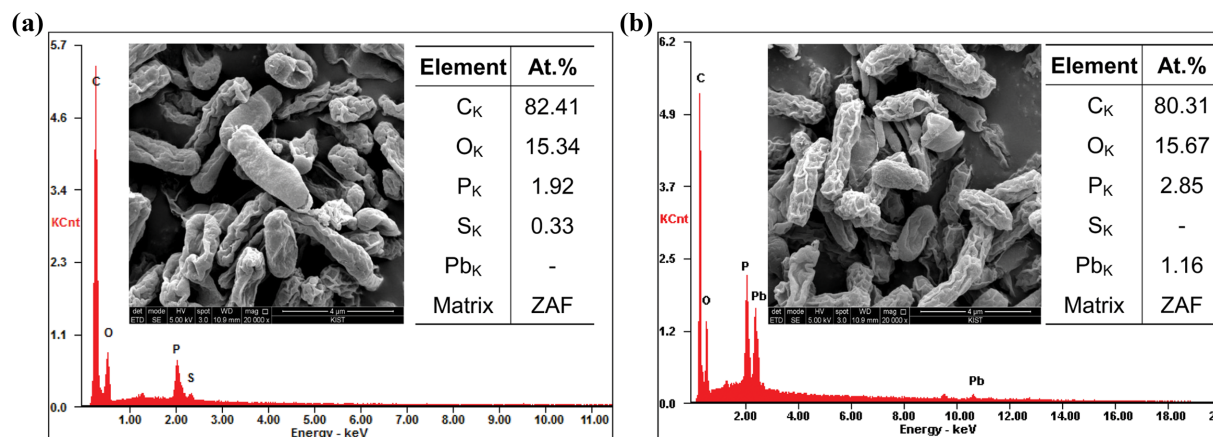


Fig. 4. SEM-EDX analysis of MTS1 cell surface (4  $\mu\text{m}$ , magnification=20,000 $\times$ ) (a) before and (b) after Pb(II) treatment. The percentage of atomic ratios is tabulated in each graph.

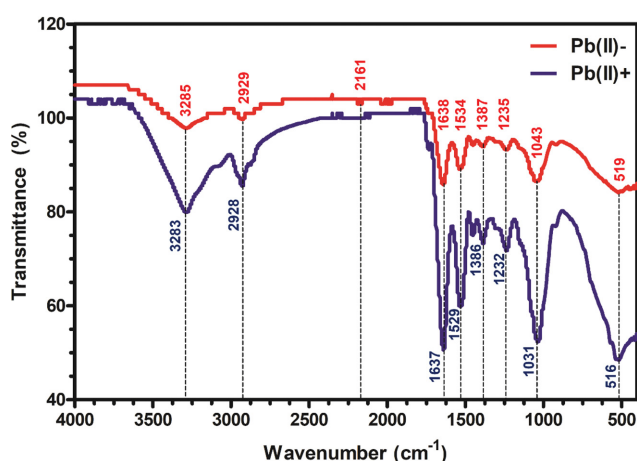


Fig. 5. FT-IR spectra of MTS1 before (red line) and after (blue line) Pb(II) treatment.

oxygen (O) and phosphate (P) elements slightly increased from 15.34% to 15.67% and 1.92% to 2.85%, respectively. These results indicate that Pb(II) ions can be captured by C, O, and P-containing functional groups on the cell surface. Similar results were confirmed in heavy metal removal studies using microorganisms, suggesting that functional groups in the biofilm, including carboxylate, hydroxyl, amino, and phosphate, are mainly involved in metal

adsorption [41-43].

Next, FT-IR analysis in the range of 500-4,000  $\text{cm}^{-1}$  was performed to determine the functional groups involved in Pb(II) removal. As shown in Fig. 5, the adsorption peak was shifted from 3,285 to 3,283  $\text{cm}^{-1}$  for hydroxyl (O-H) and amine (N-H) groups. Small shifts from 2,929 to 2,924  $\text{cm}^{-1}$  (C-H stretching vibrations), 1,387 to 1,386  $\text{cm}^{-1}$  (amide III group), and 1,638 to 1,637  $\text{cm}^{-1}$  (C=O stretching and N-H deformation of amide I band) were observed. The peak at 2,161  $\text{cm}^{-1}$  that was attributed to an alkyne ( $\text{C}\equiv\text{C}$ ) group completely disappeared after Pb(II) adsorption. Furthermore, significant shifts from 1,534 to 1,529  $\text{cm}^{-1}$  (N-H bending in amide II and C-N stretching in  $-\text{CO}-\text{NH}-$ ), 1,235 to 1,232  $\text{cm}^{-1}$  (phosphate group), 1,043 to 1,031  $\text{cm}^{-1}$  (C-OH stretching), and 519 to 516  $\text{cm}^{-1}$  (nitro compounds and disulfide groups) were also observed. Taken together, these observations reveal that hydroxyl, amide, carboxyl, phosphate, nitro compounds, and disulfide groups play an important role in the biosorption of Pb(II) by the MTS1 strain.

#### 4. Kinetics and Isotherm Modeling

To understand the mechanisms underlying the biosorption of Pb(II) by the MTS1 strain, the biosorption isotherm and kinetics were further investigated. The Langmuir and Freundlich models were employed to calculate the isotherm parameters and maximum adsorption capacity (Figs. 6(a) and 6(b)). The detailed parameters of adsorption isotherm calculated by two isotherm models are listed in Table 2. According to correlation coefficient values ( $R^2$ ) calcu-

Table 2. Parameters calculated using the equations of adsorption isotherms and kinetic models

Experimental value (isotherms)		Langmuir isotherm			Freundlich isotherm		
$q_{\text{exp}}$ (mg/g)	$Q_{\text{max}}$ (mg/g)	$K_L$ (L/mg)	$R^2$	$K_F$ (L/g)	$n$	$R^2$	
55.65 $\pm$ 6.2	60.35	0.034	0.985	6.081	2.472	0.962	
Experimental value (kinetics)		Pseudo-first-order			Pseudo-second-order		
$q_{\text{exp}}$ (mg/g)	$q_{\text{cal}}$ (mg/g)	$K_1$ (L/min)	$R^2$	$q_{\text{cal}}$ (mg/g)	$K_2$ (g/mg/min)	$R^2$	
9.898 $\pm$ 0.004	5.07	0.127	0.989	10.35	0.041	0.999	

Table 3. Various bacterial strains used for biosorption of Pb(II)

Bacterial strain	Experimental conditions						Reference
	pH	Temperature (°C)	Initial concentration (mg/L)	Biomass dosage (g/L)	Contact time (h)	Adsorption capacity (mg/g)	
<i>Methylobacterium</i> sp. MTS1	7	30	400	1	1	56.55	This study
<i>Bacillus</i> sp. PZ-1	5	15	400	40	0.3	15.38	[14]
<i>Alcaligenes</i> sp.	5	35	100	1.5	0.5	56.8	[35]
<i>Arthrobacter</i> sp. GQ-9	5.5	35	100	1.2	4	17.56	[36]
<i>Geobacillus thermodenitrificans</i>	4.5	65	175	0.3	12	32.26	[37]
<i>Bacillus cereus</i>	6	25	80	1	1.34	22.1	[38]
<i>Pectobacterium</i> sp. ND2	5	60	300	1	0.33	31.2	[39]

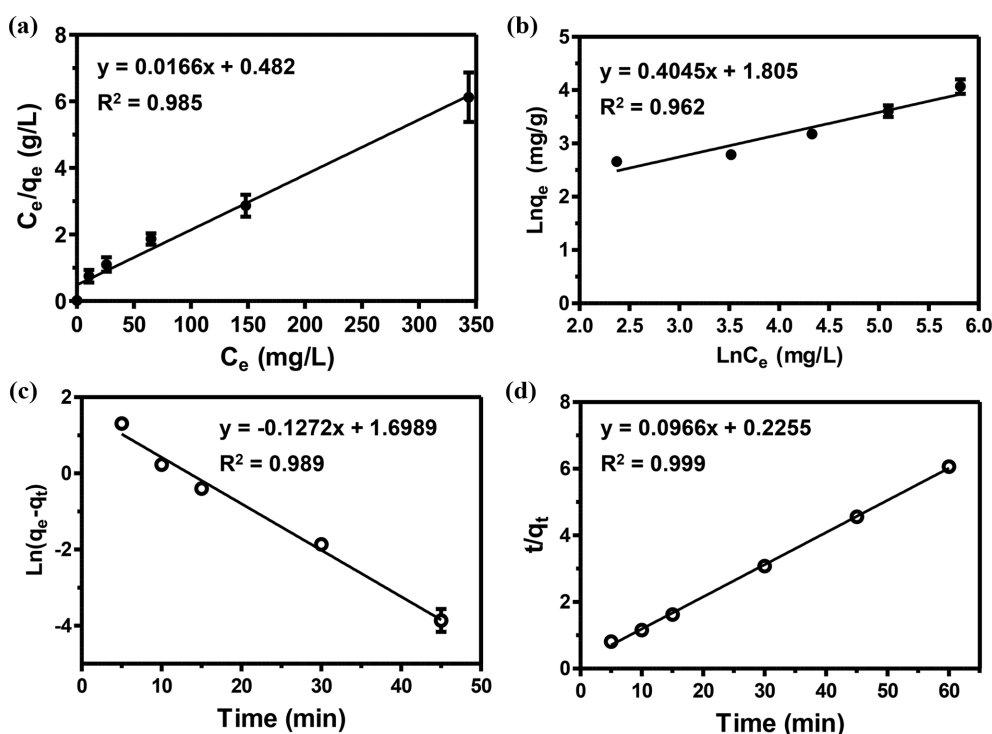


Fig. 6. Pb(II) biosorption isotherm and kinetic study results (a) Langmuir model, (b) Freundlich model, (c) pseudo-first-order model, and (d) pseudo-second-order model.

lated by two isotherm models, the Pb(II) adsorption process by the MTS1 strain can be better explained by the Langmuir model ( $R^2=0.985$ ) than by the Freundlich model ( $R^2=0.962$ ). Moreover, the maximum biosorption capacity for Pb(II) was  $56.55 \pm 6.2$  mg/g, in good agreement with the maximum theoretical capacity calculated by the Langmuir model (60.35 mg/g; Table 2). These observations suggest that biosorption of Pb(II) ions by MTS1 strain occurs through monolayer adsorption process onto a homogeneous surface by [44,45]. This result is consistent with previous reports demonstrating the potential use of microorganisms as a biosorbent for the removal of Pb(II) [14,46,47]. Kinetic analysis of Pb(II) biosorption showed that the experimental  $q_{exp}$  value for Pb(II) biosorption ( $9.898 \pm 0.004$  mg/g) matched the  $q_{cal}$  value of the pseudo-second-order kinetic model (10.35 mg/g) better than that of the pseudo-first-order kinetic model (5.07 mg/g) (Table 2). Furthermore, the

$R^2$  value of the pseudo-second-order model (0.999) was higher than that of the pseudo-first-order kinetic model (0.989) (Fig. 6(c), 6(d)). These results indicate that Pb(II) biosorption by *Methylobacterium* sp. MTS1 strain can be well explained by the pseudo-second-order kinetic model and that the biosorption is followed by a chemisorption reaction [48].

## CONCLUSIONS

We report the isolation of a Pb(II)-resistant *Methylobacterium* sp. MTS1 strain and its potential for use as biosorbent for the removal of Pb(II) from aqueous media. The MTS1 strain showed a remarkable removal efficiency of  $98.6 \pm 0.6\%$  and a maximum biosorption capacity of  $56.55 \pm 6.2$  mg/g under optimized conditions, including pH 7, 1 g/L biomass, 10 mg/L initial Pb(II) concentra-

tion, and 60 min contact time. The mechanism of Pb(II) removal was found to be biosorption, in which hydroxyl, amide, carboxyl, phosphate, nitro compounds, and disulfide groups, which were identified by SEM-EDX and FT-IR analysis, play an important role. Furthermore, the biosorption of Pb(II) was well explained by the Langmuir isotherm and pseudo-second-order kinetic model. In conclusion, the newly isolated *Methylobacterium* sp. MTS1 strain has a high potential for use as a biosorbent for Pb(II) removal, and it could be valuable for *in situ* restorations of Pb(II)-contaminated environments.

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