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Bioaccumulation and biosorption of Cd^{2+} and Zn^{2+} by bacteria isolated from a zinc mine in Thailand

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ABSTRACT

The three bacteria, *Tsukamurella paurometabola* A155, *Pseudomonas aeruginosa* B237, and *Cupriavidus taiwanensis* E324, were isolated from soils collected from a zinc mine in Tak Province, Thailand. Among these bacteria, *P. aeruginosa* B237 and *C. taiwanensis* E324 were tolerant of both cadmium and zinc, while *T. paurometabola* A155 was highly tolerant of zinc only. Bioaccumulation experiment revealed that Cd^{2+} and Zn^{2+} were mainly adsorbed on the cell walls of these bacteria rather than accumulated inside the cells. During Cd^{2+} and Zn^{2+} biosorption, *P. aeruginosa* B237 and *T. paurometabola* A155 showed the highest removal efficiencies for Cd^{2+} and Zn^{2+}, respectively. The maximum biosorption capacities of *P. aeruginosa* B237 and *T. paurometabola* A155 biomasses for Cd^{2+} and Zn^{2+} biosorptions were 16.89 and 16.75 mg g^{-1}, respectively, under optimal conditions. The experimental data of Cd^{2+} and Zn^{2+} biosorptions fitted well with Langmuir isotherm model, suggesting that Cd^{2+} and Zn^{2+} adsorptions occurred in a monolayer pattern on a homogeneous surface. Furthermore, the pseudo-second order and pseudo-first order kinetic models best described the biosorption kinetics of Cd^{2+} and Zn^{2+} adsorptions, respectively, suggesting that the Cd^{2+} and Zn^{2+} adsorptions took place mainly by chemisorption (Cd^{2+}) and physisorption (Zn^{2+}).

1. Introduction

Heavy metal pollution is a serious environmental problem reported worldwide, especially in developing countries (Duffus, 2001). Heavy metal contamination of the environment may result from natural processes such as volcanic emissions, forest fires, and leaching by acid rain (Robards and Worsfold, 1991). While mining and industrial manufacturing are the major sources of human-induced contamination (Gray, 1997). The contamination of paddy fields and soils by cadmium (Cd) and zinc (Zn) has been widely reported (Simmons et al., 2003). Such contamination of soil and water leads to the accumulation of heavy metals in plants and, (especially aquatic) animals (ATSDR, 2005). Physical and chemical approaches such as filtration, ion-exchange, and chemical precipitation are widely used to remove heavy metals from the environment (Mulligan et al., 2001). However, most of these methods are expensive, non-specific, and of limited effectiveness, especially when the concentrations of polluting materials are below 100 mg L^{-1}. Moreover, they often generate toxic secondary wastes (Volesky, 2001). In recent years, biological methods of heavy metal clean-up such as microbial remediation have stimulated increased interest for because they are not only environmentally friendly but also cost-effective (Volesky and Holan, 1995). Commonly, biosorbents derived from microorganisms such as fungi, yeast, and algae, are suitable for removal of heavy metals because of their high surface to volume ratios, large available quantities, and low cost (Gadd, 1990).

Microbial remediation generally takes place either by bioaccumulation or biosorption. Both processes normally occur in microorganisms, depending on type of microorganisms and the heavy metal species (Ledin, 2000). Bioaccumulation is an active process, in which heavy metal particles are transported to cells, where they accumulate, using energy from the cell metabolism (Ledin, 2000). Biosorption, on the other hand, is a passive process, in which heavy metal particles are passively adsorbed on to the surface of biological materials (Volesky, 1999). The bacterial cell wall is the first effective compartment for adsorbing heavy metal

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particles because it contains many anionic functional groups capable of binding to heavy metals, such as peptidoglycan, teichoic acids, phospholipids and lipopolysaccharides (Beveridge and Murray, 1976). Microorganisms therefore have a high potential for use in bioaccumulation and biosorption processes to remove heavy metals from polluted environments.

The purpose of the present work was to investigate the ability of three cadmium- and/or zinc-resistant bacteria, i.e. *Tsukamurella paurometabola* A155, *Pseudomonas aeruginosa* B237, and *Cupriavidus taiwanensis* E324 in bioaccumulation and biosorption of Cd^{2+} and Zn^{2+}. The accumulations of Cd^{2+} and Zn^{2+} in cell walls and intracellular spaces of these bacteria were compared. In addition, the environmental factors affecting biosorption efficiency were optimized.

2. Materials and methods

2.1. Isolation and identification of cadmium- and/or zinc-resistant bacteria

Cadmium- and/or zinc-resistant bacteria used in this study were isolated from soil samples collected from the zinc mine in Tak Province, Thailand. One gram of soil samples was dissolved in sterile deionized water. 100 μL of soil solution were then inoculated into nutrient broth (0.3% beef extract, 0.5% gelatin peptone) supplemented with 5 mM $ZnCl_2$ or 2 mM $CdCl_2$ and incubated at 30 °C for 24 h. The culture was spread on nutrient agar (nutrient broth with 1.5% agar) containing 5 mM $ZnCl_2$ or 2 mM $CdCl_2$. After incubation at 30 °C for 3 days, colonies were purified by restreaking 3–4 times in fresh media of the same composition. The purified colonies were physically evaluated by Gram staining, and genetically identified, by partial 16S rRNA gene sequencing. PCR was performed in a GeneAmp PCR system 9600 (Perkin Elmer) using bacterial genomic DNA as template and the eubacterial universal primers, p27f and p1495r referred to *Escherichia coli* nucleotide gene sequence (Weisburg et al., 1991) as forward and reverse primers, respectively (Nilsson et al., 2003). The PCR conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 7 min. The PCR products were purified by Gel/PCR DNA extraction Kit (RBC bioscience), and sequenced at The Research Center of Ramathibodi Hospital (Bangkok, Thailand). The nucleotide sequences were aligned with those in the GenBank database using the Advanced BLAST search program of the National Center for Biotechnology Information (NCBI), and the partial 16S rRNA sequences available in Ribosomal Database Project (Maidak et al., 1994; Cole et al., 2005) from NCBI.

2.2. Heavy metal resistance of the isolated strains

2.2.1. Minimal inhibitory concentration (MIC)

MIC is the lowest concentration that completely inhibits the visible growth of microorganisms (Andrews, 2001). The MICs of arsenic (As^{5-}), cadmium (Cd^{2+}), cobalt (Co^{2+}), mercury (Hg^{2+}), nickel (Ni^{2+}), and zinc (Zn^{2+}) for the bacterial isolates were determined by the agar dilution method (Wiegand et al., 2008). Bacterial cells grown to log phase in nutrient broth at 30 °C were diluted to an $O.D._{600}$ of 0.1, and 100 μL of cell suspensions were spread onto nutrient agar (pH 6.8) plates containing various concentrations of $HAsNa_2O_4$, $CdCl_2$, $CoCl_2$, $HgSO_4$, $NiCl_2$, and $ZnCl_2$. Growth of these isolates was assessed after 3 days of incubation. The heavy metal-resistant ability of these isolated bacteria was compared to those of *Bacillus cereus* ATCC11778 as a Gram-positive control, and *E. coli* ATCC25922 as a Gram-negative control.

2.2.2. Effect of Cd^{2+} and Zn^{2+} on bacterial growth

The growth of cadmium- and/or zinc-resistant bacteria in the presence of Cd^{2+} and Zn^{2+} was evaluated in liquid cultures. The bacterial strains pre-cultivated to log phase in nutrient broth at 30 °C were inoculated into nutrient broth (pH 6.8) containing 1 mM $CdCl_2$ or 3 mM $ZnCl_2$ with an initial $O.D._{600}$ of 0.1, and incubated at 30 °C with shaking at 200 rpm. The growth of bacterial cultures was monitored every 2 h until 24 h by measuring $O.D._{600}$.

2.3. Bioaccumulation experiment

The log-phase *T. paurometabola* A155, *P. aeruginosa* B237, and *C. taiwanensis* E324 cells were inoculated into nutrient broth (pH 6.8) containing 2 mM $CdCl_2$ or 5 mM $ZnCl_2$ with an initial $O.D._{600}$ of 0.1, and incubated at 30 °C with shaking at 200 rpm. The bacterial cells were harvested at 15 min, 1, 3, 6, and 12 h after inoculation by centrifugation, and then washed with sterile deionized water to remove free heavy metal ions. The concentrations of Cd^{2+} and Zn^{2+} remaining in the bacterial cells were measured by flame atomic absorption spectrometry (FAAS, Varian SpectrAA 55B).

To examine the Cd^{2+} and Zn^{2+} accumulations in the cell walls and intracellular spaces of these bacteria, the cell pellets were treated with 10 mM EDTA (pH 8) at 30 °C with agitation for 30 min (Wei et al., 2009). The bacterial cell suspensions were separated by centrifugation into supernatants and cell pellets, which were used for the determination of heavy metal contents on bacterial cell walls and intracellular spaces, respectively, by FAAS as mentioned above.

2.4. Biosorption experiment

2.4.1. Biomass preparation

T. paurometabola A155, *P. aeruginosa* B237, and *C. taiwanensis* E324 grown to log phase at 30 °C were harvested by centrifugation at 4 °C and washed twice with sterile deionized water. The cell pellets were oven dried at 60 °C for 3 days. Thereafter, the dried cells were ground and sieved before use.

2.4.2. Cd^{2+} and Zn^{2+} removal efficiency

One gram per liter of *T. paurometabola* A155, *P. aeruginosa* B237, and *C. taiwanensis* E324 biomass was added to sterile deionized water containing 1 mM $CdCl_2$ or 1 mM $ZnCl_2$ at pH 6 and incubated at 30 °C with agitation prior to harvesting by centrifugation. The percentage of Cd^{2+} and Zn^{2+} removal efficiency was calculated by using the following equation:

$$\%\ \text{adsorption} = \frac{(C_0 - C_{eq})}{C_0} \times 100$$

where C_0 and C_{eq} are the initial and equilibrium metal concentration in the solution (mg L^{-1}), respectively.

2.4.3. Biosorption profile

The factors that affect the biosorption efficiencies of *T. paurometabola* A155 and *P. aeruginosa* B237 were examined in a batch experiment. Except where stated, the conditions for biosorption experiments were: biomass dosage = 1 g L^{-1}, initial metal concentration = 25 mg L^{-1}, pH = 6, temperature = 30 °C, and contact time = 12 h. The effect of pH range (3–9) on the biosorption efficiency of *P. aeruginosa* B237 and *T. paurometabola* A155 biomasses for Cd^{2+} and Zn^{2+}, respectively, was evaluated. Similar experiments were performed to investigate the effect of biomass dosage (0.25–5 g L^{-1}), initial metal concentration (5–100 mg L^{-1}), contact time (0–180 min), and temperature (10, 30, 50 °C). After incubation, the biomasses were harvested by centrifugation and filtrated with filter paper (Whatman No. 42), and the supernatant

fractions were used to determine Cd^{2+} and Zn^{2+} contents by FAAS.

After the biosorption experiments, Cd^{2+} and Zn^{2+} concentrations remaining in the solutions were calculated according to the following equation:

$$Q = \frac{V(C_0 - C_{eq})}{M}$$

where Q is the metal biosorption (mg g^{-1}); C_0 and C_{eq} are the initial and equilibrium metal concentration in the solution (mg L^{-1}), respectively; V is the volume of solution (L); and M is the mass of biosorbent (g).

2.4.4. Biosorption isotherms

Langmuir and Freundlich isotherms are widely applied to describe the adsorption mechanism between adsorbate and adsorbent. The Langmuir isotherm is valid for monolayer adsorption onto a completely homogeneous surface, which is eventually limited by the number of active sites on the biomass surface (Langmuir, 1918), whereas the Freundlich isotherm is an empirical isotherm to evaluate the heterogeneous biosorbents with various adsorption sites (Freundlich, 1906).

The linearized Langmuir isotherm model is described by the following equation (Langmuir, 1918):

$$\frac{C_{eq}}{q_{eq}} = \frac{1}{b \cdot Q_{max}} + \frac{C_{eq}}{Q_{max}}$$

where q_{eq} (mg g^{-1}) is the metal ion uptake per unit weight of biomass (mg of metal ion adsorbed/g biomass); C_{eq} (mg L^{-1}) is the concentration of metal ions in solution at equilibrium (mg L^{-1}); Q_{max} (mg g^{-1}) and b (l mg^{-1}) are the maximum adsorption capacity, and a constant related to adsorption energy of adsorption, respectively (Langmuir, 1918).

Moreover, the Langmuir isotherm model can also be used to predict the potential adsorption probability relationship between solid and liquid, which can be represented by the following equation (Horsfall Jr and Spiff, 2005):

$$R_L = \frac{1}{1 + bC_0}$$

where R_L is separation factor or equilibrium parameter; b is a constant related to adsorption energy of adsorption (l mg^{-1}), and C_0 is an initial metal concentration in the solution (mg L^{-1}). The parameter R_L can indicate the shape of the isotherm and nature of the biosorption process ($R_L > 1$: unfavorable; $R_L = 1$: linear; $0 < R_L < 1$: favorable; $R_L = 0$: irreversible).

The Freundlich equation is as follows (Freundlich, 1906):

$$\log q_{eq} = \log K_F + \frac{1}{n} \log C_{eq}$$

where q_{eq} (mg g^{-1}) is the metal ion uptake per unit weight of biomass (mg of metal ion adsorbed g biomass^{-1}); C_{eq} (mg L^{-1}) is the concentration of metal ions in solution at equilibrium (mg L^{-1}); K_F corresponds to the binding capacity; and n indicates the affinity of the sorbent towards the biomass.

2.4.5. Biosorption kinetics

Kinetic studies are necessary to investigate the adsorption mechanism and the potential of rate-limiting steps including mass transfer and chemical reaction processes to obtain the optimum conditions for full-scale batch processes (Loukidou et al., 2004). The adsorption kinetics are generally described by pseudo-first order and pseudo-second order kinetic models to predict the adsorption rate and mechanism. These kinetic models are described based on the rate-controlling step of adsorption sites for solid–liquid phase, which are the physical and chemical interactions, respectively, between adsorption sites and heavy metal ions (Ho and McKay, 1999; Fan et al., 2008). The pseudo-first order kinetic model is described by the following equation (Fan et al., 2008):

$$\log(q_{eq} - q_t) = \log q_{eq} - \frac{k_1}{2.303} t$$

The pseudo-second order kinetic model is described by the following equation (Ho and McKay, 1999):

$$\frac{t}{q_t} = \frac{1}{k_2 q_{eq}^2} + \frac{1}{q_{eq}} t$$

where k_1 is the rate constant of pseudo-first order adsorption (1 min^{-1}) and k_2 is the rate parameter of pseudo-second-order equation (g $mmol^{-1}$ min^{-1}). q_{eq} and q_t are the amounts of metal adsorbed at equilibrium and time t (mg g^{-1}), respectively.

2.5. Statistical analysis

All experiments were performed in triplicate and expressed as means with standard deviation. Analysis of variance was conducted by one-way analysis of variance (ANOVA) using least significant difference method (LSD) on the SPSS statistical package (version 18.0 for Windows, SPSS Inc.). The level of statistical significance was set at $p < 0.05$.

3. Results

3.1. Isolation and identification of cadmium- and/or zinc-resistant bacteria

Based on the analysis of partial 16S rDNA sequences, the A155, B237, and E324 strains isolated from the soil samples collected from the Tak zinc mine were identified as *T. paurometabola* (GenBank accession no. KP993466, 100% identity), *P. aeruginosa* (GenBank accession no. KP993467, 100% identity), and *C. taiwanensis* (GenBank accession no. KP993468, 99% identity), respectively.

3.2. Determination of heavy metal resistance

3.2.1. MICs of arsenic, cadmium, cobalt, Mercury, nickel, and zinc

Compared with the control strains, *P. aeruginosa* B237 and *C. taiwanensis* E324 exhibited high MIC values for cadmium and zinc, indicating their resistance to both cadmium and zinc (Table 1). On the other hand, *T. paurometabola* A155, which showed the highest MIC value for zinc, was not strongly resistant to cadmium (Table 1). Since the MIC values for the other metals tested, i.e., arsenic, cobalt, mercury, and nickel (apart from mercury in *C. taiwanensis* E324) were similar to those of the control strains (Table 1), it is likely that the metal tolerance capacity of these isolated bacteria was mainly restricted to only cadmium and/or zinc.

3.2.2. Effect of Cd^{2+} and Zn^{2+} on bacterial growth

As expected, neither control strain, i.e., *B. cereus* ATCC11778 and *E. coli* ATCC25922, was able to grow in the presence of Cd^{2+} and Zn^{2+} (Fig. 1A and B). Consistent with the results of MIC assay, all three isolates grew well in the nutrient broth containing $ZnCl_2$, whereas only *P. aeruginosa* B237 and *C. taiwanensis* E324 were able to grow in the presence of $CdCl_2$ (Fig. 1A).

3.3. Cd^{2+} and Zn^{2+} accumulations

All three bacterial strains rapidly accumulated Cd^{2+} and Zn^{2+}

Table 1
The minimum inhibitory concentrations (MICs) of As^{5+}, Cd^{2+}, Co^{2+}, Hg^{2+}, Ni^{2+}, and Zn^{2+} for *T. paurometabola* A155, *P. aeruginosa* B237, and *C. taiwanensis* E324.

Strain	MIC											
	Zn^{2+}		Cd^{2+}		As^{5+}		Co^{2+}		Hg^{2+}		Ni^{2+}	
	mM	mg L^{-1}	mM	mg L^{-1}	mM	mg L^{-1}	mM	mg L^{-1}	mM	mg L^{-1}	mM	mg L^{-1}
T. paurometabola A155	22	2999	3	550	3	936	1	130	0.2	59	3	389
P. aeruginosa B237	19	2590	8	1467	2	624	1	130	0.2	59	3	389
C. taiwanensis E324	19	2590	5	917	3	936	2	260	0.7	208	4	518
B. cereus ATCC11778	2	273	1	183	3	936	2	260	0.2	59	3	389
E. coli ATCC25922	2	273	1	183	2	624	3	390	0.1	30	4	518

after exposure (Fig. 2A and B). After prolonged incubation, the levels of Cd^{2+} and Zn^{2+} that accumulated in the *P. aeruginosa* B237 and *C. taiwanensis* E324 decreased, whereas those of the *T. paurometabola* A155 did not change significantly (Fig. 2A and B). Among these three isolates, *T. paurometabola* A155 showed the highest levels of Cd^{2+} and Zn^{2+} accumulation (Fig. 2A and B).

Since the metal accumulation reached its maximum level in a very short time, it is likely that Cd^{2+} and Zn^{2+} were absorbed on the bacterial cell walls rather than accumulated inside the cells. Post-exposure, the Cd^{2+} and Zn^{2+} contents in cell walls and intracellular spaces of these bacteria were then determined. The results showed that most Cd^{2+} and Zn^{2+} accumulated on the cell walls of the isolated bacteria (Fig. 2C and D), within 15 min after inoculation (Fig. 2A and B). Among these three bacteria, *T. paurometabola* A155 showed the highest levels of Cd^{2+} accumulation on cell walls (Fig. 2C). In the case of Zn^{2+} accumulation on cell walls, *C. taiwanensis* E324 showed the highest levels after short-term exposure, within 60 min, whereas *T. paurometabola* A155 showed the highest levels after long-term exposure for 12 h (Fig. 2D).

3.4. Cd^{2+} and Zn^{2+} biosorption

3.4.1. Removal efficiency

The biomasses of *P. aeruginosa B237* and *T. paurometabola* A155 exhibited the highest capacities for removal of Cd^{2+} (74.2%) and Zn^{2+} (78.3%), respectively (Fig. 3). We thus used the biomasses of *P. aeruginosa* B237 and *T. paurometabola* A155 to examine the biosorption kinetics of Cd^{2+} and Zn^{2+}, respectively.

3.5. Biosorption experiment

3.5.1. Effect of pH

The maximum biosorption efficiencies for both Cd^{2+} (17.7 mg g^{-1}) and Zn^{2+} (18.9 mg g^{-1}) were attained at pH 6.0 (Fig. 4A and B). Furthermore, for pH values up to 6, the adsorption efficiencies for Cd^{2+} and Zn^{2+} increased with increasing pH, possibly due to the deprotonation of metal binding sites on cell walls (Yang and Volesky, 1999). Since metal cations form hydroxide complexes under alkaline conditions (Ku and Chiou, 2002), it is likely that the high Q_e values obtained at high pH (> 7) were due to an increase of metal-hydroxide precipitates rather than an increase of biosorption efficiencies of bacterial biomasses.

3.5.2. Effect of biomass dosage

The maximum biosorption efficiencies for both Cd^{2+} and Zn^{2+} were attained at 1 g L^{-1} of biomass (Fig. 4C and D). For biomass dosages within the range of 0.25–1 g L^{-1}, the biosorption efficiencies for both Cd^{2+} and Zn^{2+} rapidly increased with increasing biomass dosages to 16.09 and 16.91 mg g^{-1}, respectively (Fig. 4C and D). However, when the biomass dosage was higher than 1 g L^{-1}, the biosorption efficiencies for both Cd^{2+} and Zn^{2+} decreased drastically (Fig. 4C and D).

3.5.3. Effect of initial metal concentration

Up to concentrations of 25 mg L^{-1}, the biosorption efficiencies for both Cd^{2+} and Zn^{2+} increased with increasing initial metal concentrations (Fig. 4E and F). When the initial metal concentrations were higher than 25 mg L^{-1}, there was no further change in the biosorption efficiencies for either Cd^{2+} or Zn^{2+} (Fig. 4E and F).

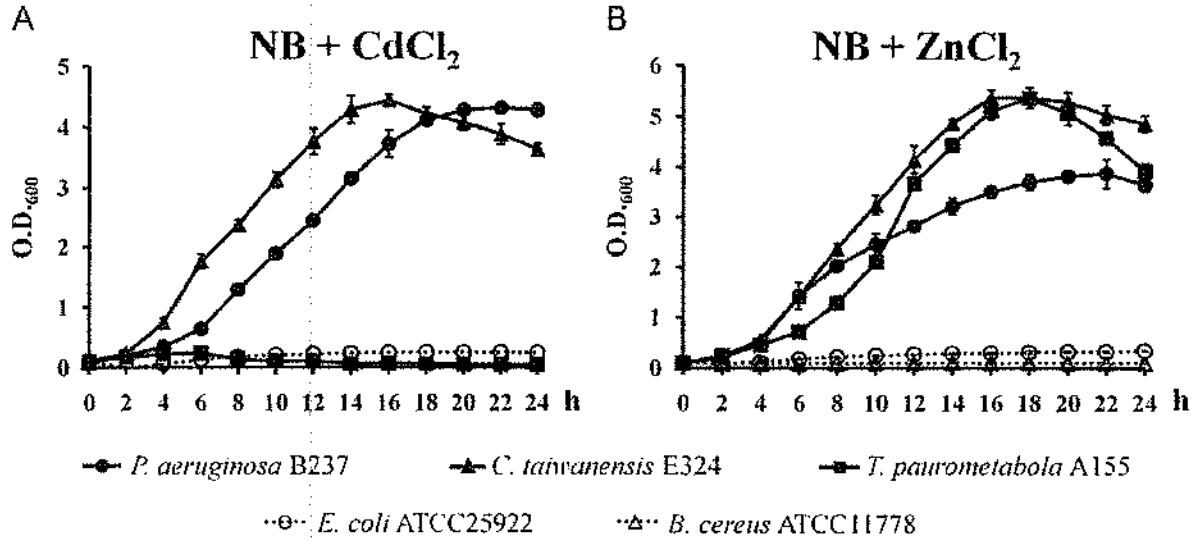

Fig. 1. Growth profiles of *T. paurometabola* A155, *P. aeruginosa* B237, *C. taiwanensis* E324, Gram-positive control *B. cereus* ATCC11778, and Gram-negative control *E. coli* ATCC25922 in nutrient broth containing 1 mM $CdCl_2$ (A) or 3 mM $ZnCl_2$ (B) at 30 °C. The $O.D._{600}$ was measured at 2-h intervals for 24 h. Error bars represent ± S.D.

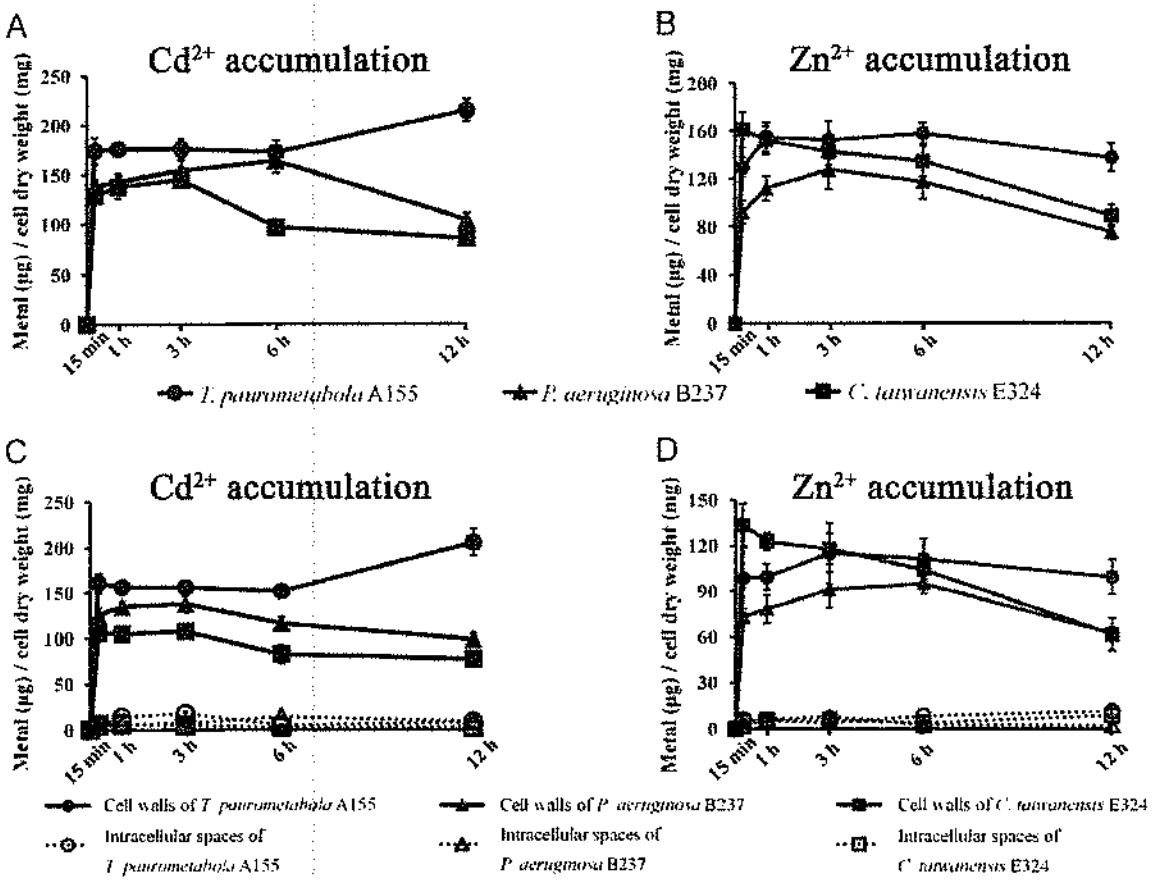

Fig. 2. Time-course accumulation profiles for Cd^{2+} and Zn^{2+} by *T. paurometabola* A155, *P. aeruginosa* B237, and *C. taiwanensis* E324 grown in nutrient broth containing 2 mM $CdCl_2$ or 5 mM $ZnCl_2$ at 30 °C. Total accumulations of Cd^{2+} (A) and Zn^{2+} (B), and the comparison of Cd^{2+} (C) and Zn^{2+} contents (D) accumulated in cell walls and intracellular spaces were determined. Error bars represent ± S.D.

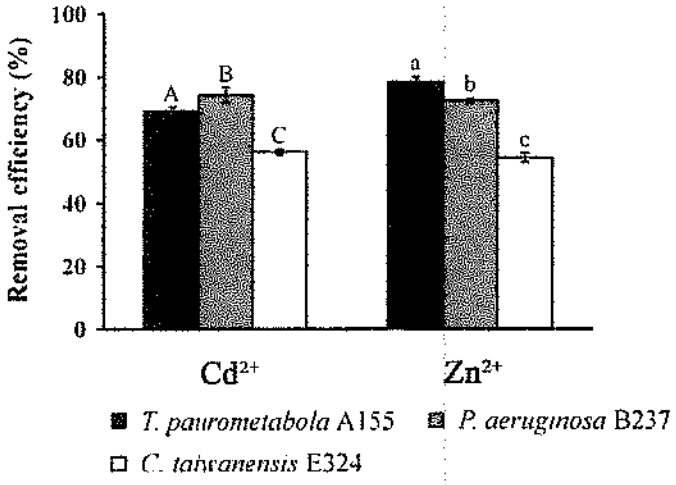

Fig. 3. Cd^{2+} and Zn^{2+} removal efficiencies of biomasses of *T. paurometabola* A155 (■), *P. aeruginosa* B237 (▩), and *C. taiwanensis* E324 (□), after incubation with 1 mM $CdCl_2$ or 1 mM $ZnCl_2$ at 30 °C for 12 h. The means with different letters indicate significant differences (One-way ANOVA, $p < 0.05$). Error bars represent + S.D.

Based on these results, we therefore used an initial metal concentration of 25 mg L^{-1} for further experiments.

3.5.4. *Effect of contact time*

The biosorption of Cd^{2+} onto *P. aeruginosa* B237 biomass was very rapid, reaching equilibrium within 15 min (Fig. 4G). On the other hand, although Zn^{2+} was also rapidly adsorbed by *T. paurometabola* A155 biomass, it took 120 min to reach equilibrium (Fig. 4H). Therefore, the optimal contact times for Cd^{2+} and Zn^{2+} biosorption were attained at 15 and 120 min, respectively.

3.5.5. *Effect of temperature*

The biosorption efficiencies at 10, 30, and 50 °C were not significantly different (Fig. 4I and J), suggesting that the Cd^{2+} and Zn^{2+} biosorptions by these bacterial biomasses were stable over a wide range of temperatures.

3.6. *Biosorption isotherms*

To describe the model of *P. aeruginosa* B237 and *T. paurometabola* A155 biomass adsorption for Cd^{2+} and Zn^{2+}, respectively, the experimental data obtained from a separate experiment conducted using the optimum parameters were fitted with the linearized models of Langmuir and Freundlich isotherms. The adsorption constants of Langmuir and Freundlich isotherm models were evaluated. The correlation coefficient (R^2) values obtained from Langmuir isotherm of both Cd^{2+} biosorption by *P. aeruginosa* B237 biomass (0.9822), and Zn^{2+} biosorption by *T. paurometabola* A155 biomass (0.974), were higher than those obtained from the Freundlich isotherm, which were 0.526 and 0.712, respectively (Table 2). These results clearly indicate that the experimental data fitted the Langmuir isotherm model better than the Freundlich isotherm model, suggesting that the Cd^{2+} and Zn^{2+} biosorptions by *P. aeruginosa* B237 and *T. paurometabola* A155 biomasses, respectively, were of the monolayer type.

To determine the capacities of these biomasses for Cd^{2+} and Zn^{2+} biosorptions, the maximum biosorption capacities (Q_{max}) were calculated from Langmuir isotherms (Table 2). The Q_{max} of *P. aeruginosa* B237 biomass for Cd^{2+} adsorption and *T.*

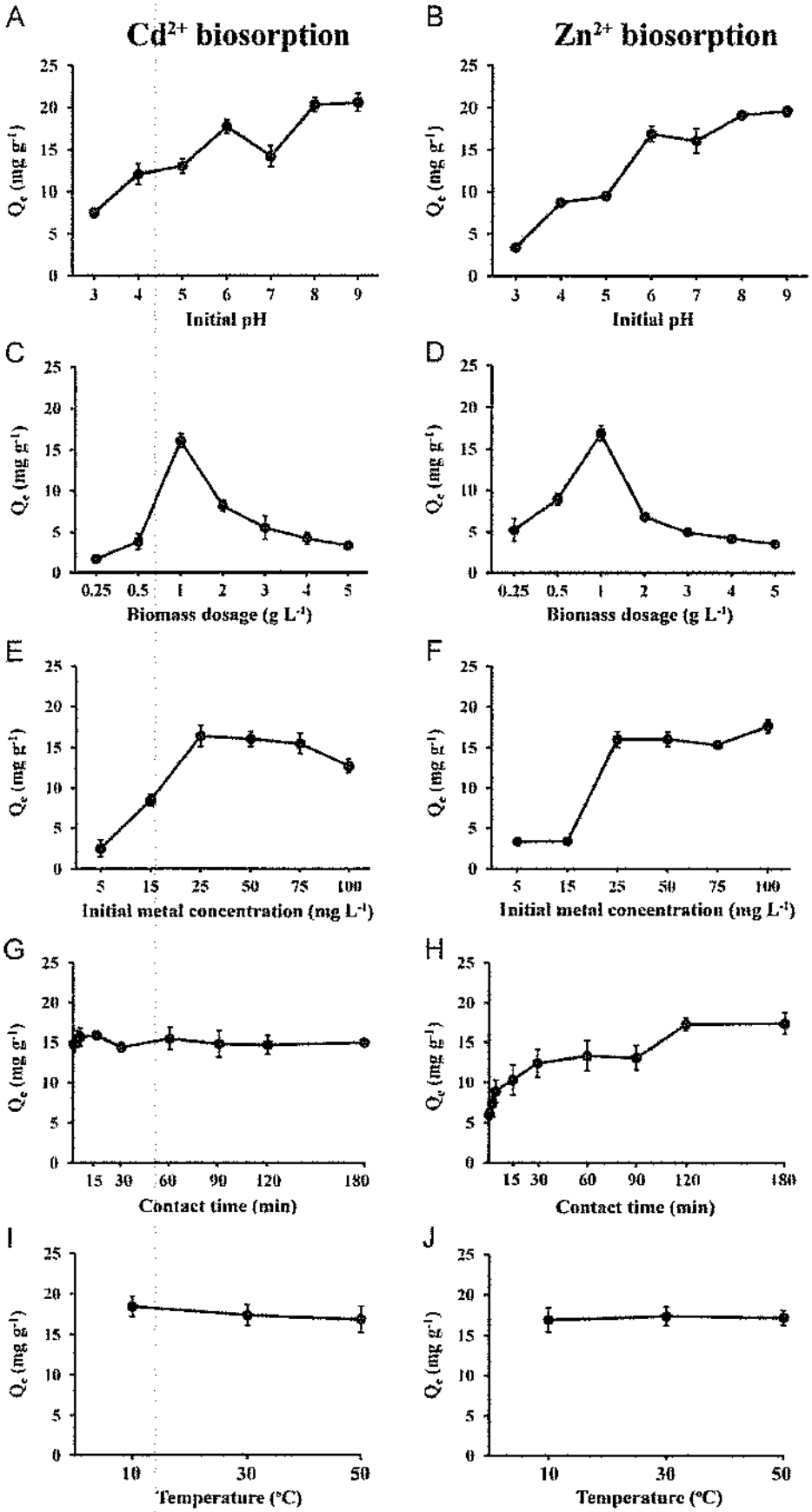

Fig. 4. Effect of initial pH (3–9) (A and B), biomass dosage (0.25–5 g L^{-1}) (C and D), initial metal concentration (5–100 mg L^{-1}) (E and F), contact time (0–180 min) (G and H), and temperature (10–50 °C) (I and J) on Cd^{2+} biosorption by *P. aeruginosa* B237 biomasses (A, C, E, G and I) and Zn^{2+} biosorption by *T. paurometabola* A155 biomasses (B, D, F, H, and J). Except where stated, the conditions for biosorption experiments were: pH=6, biomass dosage=1 g L^{-1}, initial metal concentration=25 mg L^{-1}, contact time=12 h, and temperature=30 °C. Error bars represent ± S.D.

Table 2
Langmuir and Freundlich isotherm parameters for biosorption of Cd^{2+} and Zn^{2+} by biomasses of *P. aeruginosa* B237 and *T. paurometabola* A155, respectively.

Metal	Langmuir isotherm				Freundlich isotherm		
	Q_{max}(mg g^{-1})	b (l mg^{-1})	R^2	R_L	K_f (l g^{-1})	n	R^2
Cd^{2+}	16.48	17.05	0.982	0.002	3.79×10^{-7}	0.282	0.526
Zn^{2+}	17.67	14.62	0.974	0.003	1.50×10^{-11}	0.366	0.712

paurometabola A155 biomass for Zn^{2+} adsorption (16.48 and 17.67 mg g^{-1}, respectively; Table 2), were consistent with the experimental data (q_{eq}) for Cd^{2+} and Zn^{2+} adsorptions, which were 16.89 and 16.75 mg g^{-1}, respectively (Fig. 4E and F). These results further confirmed that the Langmuir isotherm model was suitable to describe the Cd^{2+} and Zn^{2+} adsorptions by these bacterial biomasses.

Another essential factor of the Langmuir isotherm model is R_L, which predicts the potential adsorption probability relationship between solid and liquid (Horsfall Jr and Spiff, 2005). In our study, the R_L values of Cd^{2+} and Zn^{2+} adsorptions were 0.002 and 0.003, respectively, indicating that Cd^{2+} and Zn^{2+} biosorptions were favorably adsorbed by *P. aeruginosa* B237 and *T. paurometabola* A155 biomasses, respectively.

3.7. Biosorption kinetics

To describe the adsorption kinetics of *P. aeruginosa* B237 and *T. paurometabola* A155 biomasses for Cd^{2+} and Zn^{2+} adsorption, respectively, the experimental data obtained from a separate experiment conducted using the optimum parameters were fitted to the pseudo-first order and the pseudo-second order kinetic models. The R^2 values for pseudo-second order kinetic model of Cd^{2+} biosorption by *P. aeruginosa* B237 biomass (1.000) was higher than that for pseudo-first order kinetic model (0.995; Table 3). Our Cd^{2+} biosorption was therefore better described by the pseudo-second order kinetic model, suggesting that the rate controlling step in the mechanism of Cd^{2+} biosorption by *P. aeruginosa* B237 biomass was the chemical interactions between functional groups present on the biosorbent surface and Cd^{2+} ions (Ho and McKay, 1999; Bulgariu and Bulgariu, 2012). In contrast, in the case of Zn^{2+} biosorption by *T. paurometabola* A155 biomass, the R^2 values for pseudo-first order kinetic model (0.952) was higher than that for pseudo-second order kinetic model (0.864) (Table 3). Since it is clear that our Zn^{2+} biosorption data fitted better with the pseudo-first order kinetic model, we conclude that the Zn^{2+} biosorption by *T. paurometabola* A155 biomass is physical adsorption depending on the physical characteristics of biosorbent (Fan et al., 2008). Furthermore, the calculated values ($q_{eq,cal}$) of Cd^{2+} and Zn^{2+} adsorptions obtained from both kinetic models were close to the experimental data ($q_{eq,exp}$) (Table 3), thus confirming that the pseudo-second order and pseudo-first order kinetic models were suitable for describing the adsorption kinetics of Cd^{2+} and Zn^{2+}, respectively, by these bacterial biomasses.

4. Discussion

4.1. Mechanism of cadmium and zinc tolerance

Among three bacterial strains isolated from soils of the Tak zinc mine, *P. aeruginosa* B237 and *C. taiwanensis* E324 were tolerant to both cadmium and zinc, while *T. paurometabola*A155 was tolerant only to zinc. Several *Pseudomonas* sp. and *Cupriavidus* sp. have previously been reported to be resistant to cadmium and/or zinc (Raja et al., 2006; Chen et al., 2008). The resistance of *Tsukamurella* sp. to both organic (e.g. pesticide and oil spill) and inorganic pollutants (e.g. dyes) has been demonstrated (Hassanshahian et al., 2013), though there are no previous reports of its resistance to heavy metals. Other than *C. taiwanensis* E324, which also exhibited an Hg-tolerant phenotype, in MICs determination, none of these three bacteria showed tolerance to other metals tested, suggesting that the cellular mechanisms for metal tolerance in these bacteria were mainly specific for cadmium and/or zinc. Bacterial mechanisms previously reported to be involved in metal detoxification include efflux systems, intracellular and extracellular sequestration, and detoxifying enzymes (Bruins et al., 2000). In both the Gram-positive *Staphylococcus aureus* and the Gram-negative *E. coli*, P-type ATPase CadA and ZntA have been shown to be involved in the active efflux of cadmium and zinc, respectively (Nies and Silver, 1995; Beard et al., 1997). In *Pseudomonas* sp. and *Cupriavidus* sp., the production of metal-chelating proteins such as metallothionein and glutathione is required for cadmium sequestration so as to reduce cadmium toxicity (Lima et al., 2006; Siripornadulsil and Siripornadulsil, 2013). In addition, modification of the cell surface in response to metal stress is another cellular strategy important for protecting microorganisms from metal toxicity. In the red macroalga *Gelidium floridanum*, increased thickness of the cell wall consisting of sulfated compounds, which may promote metal chelation, was observed after exposure to Cd^{2+}, Cu^{2+}, and Pb^{2+} (dos Santos et al., 2013, 2014). In *Pseudomonas* sp., the production of extracellular polymeric substances (EPS) containing anionic functional groups (carboxyl, phosphoric, amine, and hydroxyl groups) plays an important role in cadmium tolerance via ion exchange reactions (Zhang et al., 2006; Aguilera et al., 2008). Since most Cd^{2+} and Zn^{2+} ions in the present study were rapidly adsorbed on cell walls of these cadmium- and/or zinc-resistant bacteria, it is possible that the anionic functional groups present on the cell surfaces are involved in the extracellular sequestration of Cd^{2+} and Zn^{2+} ions (Lima et al., 2006; Vijayaraghavan and Yun, 2008). Carboxyl, hydroxyl, and amino groups on the cell surface of *Lysinibacillus* sp. BA2 have been shown to be responsible for binding with Ni^{2-} ions (Prithviraj et al., 2014). While hydroxyl, carbonyl, and carboxyl groups on the cell walls of *Bacillus* sp. PZ-1 are involved in Pb^{2+} adsorption (Ren et al., 2015). Moreover, Ag^{+} was adsorbed onto *Saccharomyces cerevisiae* cells by binding with O-, P- or S-containing functional groups on the cell surface (Chen et al., 2014). Although the precise cellular mechanisms of cadmium and/or zinc tolerance in these isolated bacteria are still unclear, it is possible that the rapid adsorption of metal ions onto the cell walls, possibly through covalent binding and/or ionic binding with functional groups on the

Table 3
Pseudo-first order and pseudo-second order parameters for biosorption of Cd^{2+} and Zn^{2} by the biomasses of *P. aeruginosa* B237 and *T. paurometabola* A155, respectively.

Metal	$q_{eq,exp}$ (mg g^{-1})	Pseudo-first order kinetic			Pseudo-second order kinetic		
		$q_{eq,cal}$ (mg g^{-1})	k_1 (1 min^{-1})	R^2	$q_{eq,cal}$ (mg g^{-1})	k_2 (g $mmol^{-1}$ min^{-1})	R^2
Cd^{2+}	16.89	15.88	0.453	0.995	16.95	0.057	1.000
Zn^{2+}	16.75	17.26	1.152	0.952	17.28	1.970	0.864

cell walls, may prevent the entry of metal ions into the cells, thereby partially contributing to metal tolerance in these strains. However, the role of intracellular detoxification mechanisms in cadmium and/or zinc tolerance cannot be excluded. In case of *G. floridanum*, increased vacuole volume and deposition of metals in the vacuole were observed after treatment with Cd^{2+}, Cu^{2+}, and Pb^{2+}, suggesting that intracellular sequestration plays a role in metal detoxification (dos Santos et al., 2014). Since only *T. paurometabola* A155, which accumulated the highest levels of Cd^{2+} on its cell walls, was intolerant of cadmium, it is assumed that the Cd^{2+} adsorption to its cell walls was insufficient to confer cadmium tolerance, and that the intracellular mechanisms for zinc detoxification in this strain were ineffective in reducing cadmium toxicity.

4.2. Mechanisms of Cd^{2+} and Zn^{2+} biosorption

The pH value strongly influences both the solubility of heavy metals and the availability of functional groups on biosorbent surfaces (Yang and Volesky, 1999). The optimal initial pH for Cd^{2+} and Zn^{2+} biosorption by biomasses of *P. aeruginosa* B237 and *T. paurometabola* A155, respectively, was 6. At low pH (<6), the available metal binding sites on the biomasses may be insufficient, possibly due to an increased association with hydrogen ions (Yang and Volesky, 1999). At pH higher than 7, it is likely that any increased metal contents in the precipitates is a consequence of increased formation of metal-hydroxide complexes, which are formed at alkaline pH (Ku and Chiou, 2002), rather than due to increased biosorption efficiencies of bacterial biomasses. At pH 7, the low biosorption efficiencies may be due to the hydrolysis of metal cations, which in turn causes the release of hydronium ions (H_3O^+) into the metal solutions, resulting in a reduction of metal binding sites on the biomass surfaces (Bayramoğlu et al., 2003). It is therefore likely that the pH-dependent biosorption efficiency of these bacterial biomasses for Cd^{2+} and Zn^{2+} was influenced by the availability of metal ions and metal binding sites on bacterial biomasses.

An initial biomass concentration within the range of 0.25–1 g L^{-1} resulted in an increase in Cd^{2+} and Zn^{2+} biosorption efficiency, possibly due to an increase of surface area and metal binding sites (Vijayaraghavan et al., 2006). Although the initial metal concentration provides a driving force to overcome mass transfer resistances of metal ions between the aqueous and solid phase (Vijayaraghavan et al., 2006), increased biomass dosage has previously been shown to induce an electrostatic interaction among biosorbents. It is therefore possible that the reduced biosorption efficiency at biomass dosage higher that 1 g L^{-1} in the present study was caused by the aggregation of biomasses, leading to a decrease of metal binding sites (Tangaromsuk et al., 2002). In addition, Cd^{2+} and Zn^{2+} biosorption efficiencies were not significantly greater at metal concentrations higher than 25 mg L^{-1}, possibly due to an insufficiency of metal binding sites on biomasses (Aksu, 2005).

In most cases, the biosorption of heavy metal by biosorbents reaches equilibrium within an hour (Kapoor and Viraraghavan, 1997). Although the biosorption of Cd^{2+} and Zn^{2+} onto bacterial biomasses occurred rapidly in the present study, Zn^{2+} biosorption required longer time to reach equilibrium than Cd^{2+} biosorption. Cadmium ions were shown to be strongly bound to sulfhydryl, carboxyl, and phosphoryl ligands on bacterial cell walls (Mishra et al., 2010). In addition, Cd^{2+}, which has larger ionic radius than Zn^{2+}, may be fixed more firmly in the polysaccharide network of bacterial cell walls than Zn^{2+} (Loaëc et al., 1997). Consistent with these suggestions, the correlation coefficients (R^2) of Cd^{2+} biosorption from pseudo-first order and pseudo-second order kinetic models were much higher (0.995 and 1.000, respectively). This therefore indicates that both chemical and physical interactions between Cd^{2+} ions and *P. aeruginosa* B237 biomass are required for the Cd^{2+} adsorption process, although the chemisorption process seemed to be more important. Since the data for Zn^{2+} biosorption were better described by the pseudo-first order kinetic model, physisorption adsorption seems to play the major role in Zn^{2+} biosorption.

Temperature is an important factor that increases the thermodynamic and kinetic of the metal–microorganism interaction (Sağ and Kutsal, 2000). High temperature enhances biosorption efficiency through increased surface activity and kinetic energy of the solute (Sağ and Kutsal, 2000; Vijayaraghavan and Yun, 2008). In the present study, however, temperature, at least between 10–50 °C, did not significantly affect the Cd^{2+} and Zn^{2+} biosorption efficiencies of our bacterial biomasses.

Our experimental data for both Cd^{2+} and Zn^{2+} biosorption fitted more closely to the Langmuir isotherm than the Freundlich isotherm. This suggests that the adsorptions of Cd^{2+} and Zn^{2+} onto the surface of the bacterial biomasses were by monolayer biosorption, the capacity of which is limited by the number and binding affinity of active sites on the biomass surfaces (Langmuir, 1918). The maximum biosorption capacities (Q_{max}) calculated from the Langmuir isotherm for Cd^{2+} and Zn^{2+} biosorption by *P. aeruginosa* B237 and *T. paurometabola* A155 biomasses, respectively, were consistent with the experimental data (q_{eq}). In addition, the affinity constant (b) values for both Cd^{2+} and Zn^{2+} biosorptions from the Langmuir isotherm model were very high, suggesting a high affinity of bacterial biomasses for Cd^{2+} and Zn^{2+} ions (Loukidou et al., 2004; Li et al., 2010). The results of this study indicated that *P. aeruginosa* B237 and *T. paurometabola* A155 biomasses displayed high efficiency for Cd^{2+} and Zn^{2+} adsorption, respectively. These biomasses therefore have high potential for use as bacteria-based biosorbents for the removal and recovery of metals from industrial wastewater.

5. Conclusions

The bacteria, *P. aeruginosa* B237 and *C. taiwanensis* E324, tolerant to both cadmium and zinc, and *T. paurometabola*A155, tolerant only to zinc, were able to rapidly accumulate Cd^{2+} and Zn^{2+} on their cell walls. The Langmuir isotherm model was the most appropriate to describe Cd^{2+} and Zn^{2+} adsorption isotherms of *P. aeruginosa* B237 and *T. paurometabola* A155 biomasses, respectively. Cd^{2+} and Zn^{2+} adsorption kinetics were best described by pseudo-second order and pseudo-first order kinetic models, respectively. The biomasses of *P. aeruginosa* B237 and *T. paurometabola* A155 showed high potential as biosorbents for remediating Cd^{2+} and Zn^{2+} from wastewaters.

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