

## Mercury Absorption by *Pseudomonas fluorescens* BM07 Grown at Two Different Temperatures

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### Abstract

*Pseudomonas fluorescens* BM07 was characterized as a producer of cold-induced biopolymer by decreasing the temperature down to as low as 10°C. It was previously shown that the synthesis of BM07 biopolymer was inhibited at 30°C. The present study was conducted to investigate the biosorption of mercury ( $\text{Hg}^{2+}$ ) ions on the BM07 cells grown on M1 minimal medium at two temperatures (10°C and 30°C). The effects of various factors including pH, contact time, initial concentration of metal and cell biomass on the biosorption yield were also studied. Study of the effect of pH on mercury removal indicated that the metal biosorption increased with increasing pH from 3.0 to 7.0. The optimum adsorption pH value was found to be 7.0. Our results showed that, at optimum pH, BM07 cells were able to uptake the mercury up to 102 and 60 mg  $\text{Hg}^{2+}$ /g dry biomass for 10°C and 30°C grown cells respectively. The removal capacity of cells increased when the cell biomass concentrations increased. The maximum removal efficiency was obtained when cells concentration was 0.83 mg dry biomass/ml for both conditions. The initial metal ion concentration significantly influenced the equilibrium metal uptake and adsorption yield. The equilibrium data were analyzed using Langmuir adsorption model. The  $q_{\text{max}}$  was 62.9 and 82.25 mg  $\text{Hg}^{2+}$ /g dry biomass for cells grown at 30°C and 10°C respectively. The results suggest that, the existence of residual cold-induced biopolymer on the external surface of cells may play an important role in biosorption efficiency, as *P. fluorescens* BM07 cells which were grown at 10°C under similar conditions showed higher efficiency to biosorb mercury than non-polymer producing cells grown at 30°C

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**Key words:** *Pseudomonas fluorescens* BM07, biosorption, mercury

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### Introduction

Contamination of soil, water and groundwater by heavy metals in many areas in the world is a matter of high concern and constituting serious environmental problems. Among these heavy metals, mercury is well known for its high toxicity and strong affinity toward the thiol groups in proteins. Purification of areas polluted by heavy metals such as mercury is difficult because they cannot be transformed to harmless elements. Conventional methods such as: chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies are generally used for removing metals from aqueous solutions (Grau and Bisang, 1995; Gray, 1999). These chemical pro-

cesses may be ineffective or extremely expensive and need enormous input of chemicals leading to secondary pollution (Habashi, 1978). Therefore removal of toxic heavy metals by an environmentally friendly manner is of great importance.

Far works has been done on the heavy metals absorption by microorganisms. The microbial processes for bioremediation of toxic metals from waste streams employ living cells, non-living biomass or biopolymers as biosorbents (Volesky and Holan, 1995). Different bioproducts and non-living biomass types have been used to adsorb heavy metal ions from the environment. Chitosan, wool, peanut skins, seaweed, mold, bacteria, crab shells and yeast are among the different kinds of biomass, which have been tested for metal biosorption or removal (de Rome and Gadd,

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1991; Friedman and Waiss, 1972; Peniche-Covas *et al.*, 1992; Tiemann *et al.*, 1999; Volesky and Holan, 1995; Volesky, 1990).

*Pseudomonas fluorescens* is nonpathogenic, saprophyte which can be found in soil, water and plant surface environments. This bacterium has simple nutritional requirements and grows well in mineral salt media supplemented with various types of carbon sources (Palleroni, 1989). BM07 strain was capable to produce a lot of slime material when the temperature was decreasing down to as low as 10°C. Maximum production of the cold-induced biopolymer was obtained when cells were grown aerobically at 10°C and its synthesis was inhibited at 30°C (Noghabi, unpublished). It has been reported the sorption of mercury ion by inactivated cells of mercury resistant bacterium, *Pseudomonas aeruginosa* PU21 (Chang and Hong, 1994). No reports have been mentioned about the mercury biosorption by *P. fluorescens*. The current study was undertaken to assess the mercury sorption efficiency of living biomass of *P. fluorescens* BM07 which was grown at two different temperatures (10°C and 30°C) and the probable role of residual biopolymer on the external surface of cells grown at 10°C as well as its efficacy for Hg<sup>2+</sup> ions absorption rate.

## Experimental

### Materials and Methods

**Microorganism and culture conditions.** *Pseudomonas fluorescens* BM07 was isolated from activated sludge in a municipal wastewater treatment plant in the south of Korea and maintained in nutrient rich agar medium containing 1% yeast extract, 1.5% nutrient broth, 1% ammonium sulfate and 2% agar, at 4°C (Lee *et al.*, 2001). A 5 ml NR broth medium was inoculated with a single colony of *P. fluorescens* BM07 and incubated at 30°C, 180 rpm for 12 h as seed culture and then transferred to a 2-liter flask containing 500 ml of modified M1 minimal medium of the same composition as reported earlier (Choi and Yoon, 1994). Fructose (70 mM) and ammonium sulfate (1 g/l) were used as carbon and nitrogen sources and initial pH of the medium was adjusted to 7.0. *P. fluorescens* BM07 was cultivated in M1 minimal medium at two different temperature, 30°C and 10°C. Bacterial cells at late exponential phase (144 h and 72 h for cells grown at 10°C and 30°C respectively) were harvested by centrifugation (10 000 × g, 20 min). Afterward the cells were washed with distilled water twice and freeze dried. Time course samples of culture medium were withdrawn in appropriate time intervals and monitored for optical density at 660 nm (OD<sub>600</sub>).

**Metal sorption assay.** A batch equilibrium method was used to determine sorption of mercury by *P. fluorescens* BM07. A set of 100 ml Erlenmeyer flasks containing 30 ml of the tested mercury solution was used in the experiments. Powdered dried cells (10 mg) were exposed to metal solution for 2 hours at 25°C on a rotary shaker at 170 rpm. The dried powdered cells were separated by centrifugation at 12 000 × g for 10 min, and supernatants were analyzed for residual mercury concentration on ICP-AES model Optima 4300 DV. Metal absorption by the tested dried cells (mg metal/g dry cells biomass) was calculated according to the Volesky and May-Phillips method (Volesky, 1990).

The mercury sorption efficiency of the dried cells was determined by the following equation in all of the experiments, unless otherwise stated:  $Q = V(C_i - C_f) / 1000 M$ ; where:  $Q$  is specific mercury uptake (mg/g biosorption),  $V$  is volume of mercury solution (ml),  $C_i$  is initial concentration of mercury in the solution (mg/l),  $C_f$  is final concentration of mercury in the solution (mg/l),  $M$  is mass of the powdered dried cells (g).

The Langmuir model as sorption model was used to evaluate the sorption behavior of BM07 cells grown at two different temperatures. It served to estimate the maximum mercury uptake values. Its constant  $b$  can serve as an indicator of the isotherm which reflects quantitatively the “affinity” between the sorbent and the sorbate (equations) (Hughes and Poole, 1989).

$$Q = \frac{Q_{max} bC}{1 + bC}; \quad Q = \frac{Q_{max} bC}{K + C};$$

$Q$  is the amount of mercury bound per unit weight biomass,  $b$  is the equilibrium constant and  $K$  (mg/l) is dissociation constant related to the stability of metal-biosorbent complexes, reciprocal to the equilibrium constant  $b$ .  $C$  is the concentration of mercury remaining in solution at equilibrium (free),  $Q_{max}$  (mg Hg<sup>2+</sup>/g biosorbent) is the maximum metal uptake under the given conditions.

**Sorption study as a function of pH.** In order to evaluate the effect of pH on Hg<sup>2+</sup> uptake, 10 mg of dried powdered living cells of *P. fluorescens* BM07 were suspended in a volume of 30 ml of mercury solution in a 100 ml conical flask for 2 hours on a rotary shaker at 170 rpm. The pH of solution was adjusted to be in the range 3–8. After shaking the flasks for 2 h, the suspension was centrifuged at 12 000 × g for 10 min. The supernatant was collected in separate clean test tubes and analyzed for residual mercury content.

**Sorption study as a function of metal concentration.** To determine the absorptive capacity of *P. fluorescens* BM07, the initial metal ion concentrations varied from 7–750 mg/l while the dry cell weight in each sample was constant at 0.33 mg/l. Equilibrium batch experiments resulted in points which were approximated by the Langmuir model. This model was used

to evaluate the sorption behavior of the materials examined and served to estimate the maximum level of metal uptake values ( $q_{\max}$ ) when they could not be reached in the experiments. A volume of 30 ml of metal ion solution  $\text{Hg}^{2+}$  (as  $\text{HgCl}_2$ ) was placed in a 100 ml conical flask with varying initial metal ion concentrations in duplicates. An accurately weighed *P. fluorescens* BM07 biomass sample (10 mg) was then added to the solution to obtain a suspension. The suspensions were adjusted to pH 7.0. A series of such conical flasks was then shaken at a constant speed 170 rpm at temperature of 25°C. After shaking the flasks for 2 hours, the suspension was centrifuged at  $12\,000 \times g$  for 10 min. The supernatants were collected in separate clean test tubes. To evaluate the biosorption rate of mercury as a function of cell biomass quantities, various dried cells weight of 0.16 to 0.83 mg/l was used. The residual metal content at each condition was determined using ICP-AES.

**Effect of exposure time.** To better examine the mercury biosorption mechanism, 10 mg of dried powdered cells of bacteria were contacted with 30 ml of aliquots of mercury solution (52 mg/l) in 100 ml of conical flasks. Shaking flasks were incubated at 25°C for different time intervals (0 to 120 min) and analyzed for residual mercury content.

**Scanning electron microscopic analysis of BM07 cells grown at 10°C.** *P. fluorescens* BM07 cells in the late exponential phase were centrifuged at 5000 rpm

for 20 min and washed sufficiently with distilled water. The cells were fixed with 0.1 M phosphate buffer (pH 7.2) containing 1% glutaraldehyde for 2 h, washed with distilled water. Fixed cells were then dehydrated through a graded ethanol series (25, 50, 75, 95 and 100%) for 5 min each. The final dehydration process was repeated two times. The dried cells were spatter-coated with gold. SEM observation was carried out using a JEOL JEM-2010 scanning electron microscope.

## Results and Discussion

**Effect of pH value.** Removal of metals from aqueous solutions is significantly influenced by pH of the medium. Experimental results are presented in Figure 1. In both conditions the maximum mercury biosorption occurred at around neutral pH. The amount of adsorbed mercury on dry biomass at pH 7 were respectively about 3% and 58% for cells grown at 30°C and 10°C. There was an increase in  $\text{Hg}^{2+}$  adsorption per unit weight of biomass with pH 7.

**Biosorption kinetics and effect of biomass quantity on mercury uptake.** The uptake of mercury takes place at a high reaction rate and is completed after few minutes. A comparison between maximum amounts of uptake concentration and theoretical maximum amounts with a total saturation of the

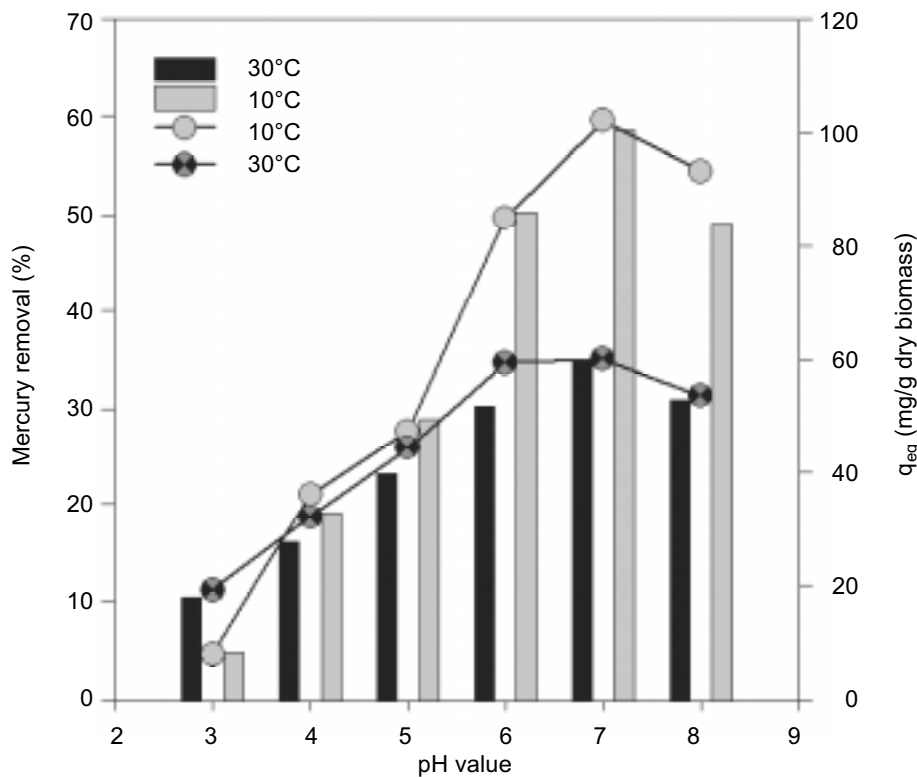


Fig. 1. Effect of initial pH on equilibrium absorption capacity of mercury ion and its removal by dried cells of *P. fluorescens* BM07 grown on M1 minimal medium at 30°C and 10°C ( $C_0$ : 63 mg/l, temperature 25°C)

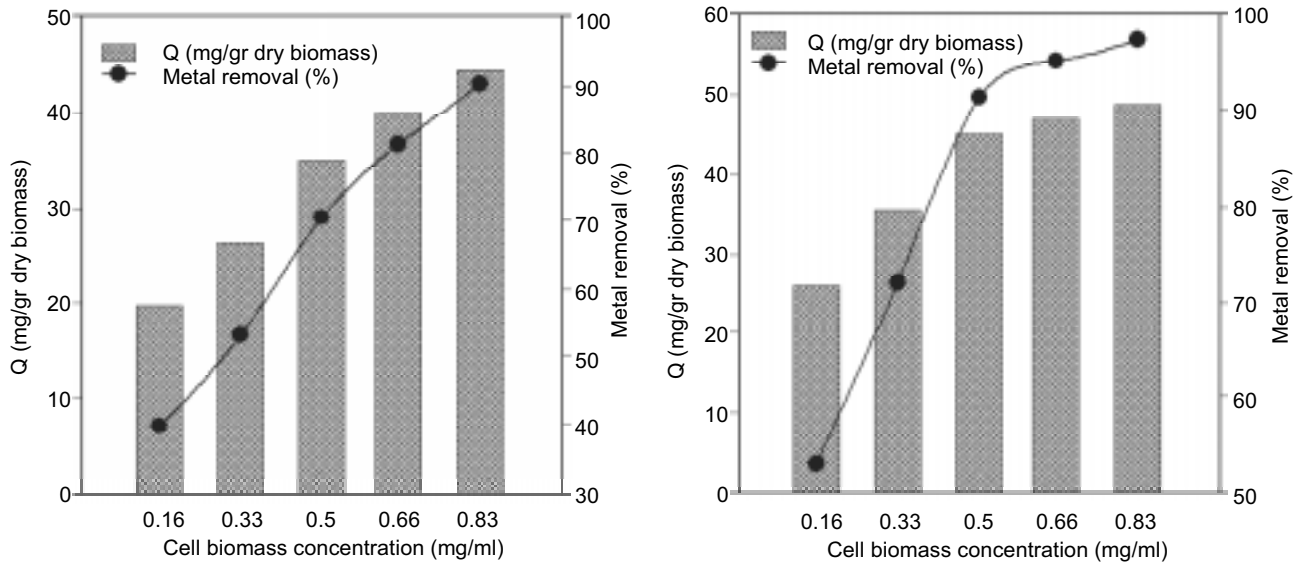


Fig. 2. Effect of BM07 cell biomass quantity on specific uptake and mercury removal at 30°C (A) and 10°C (B).

surface leads to the assumption that mercury is bound to definite sites.

The biomass concentration remarkably influenced the equilibrium metal uptake and specific uptake of mercury. With an increase in the concentration of BM07 cell biomass the larger amount of mercury was taken up. Maximum removal and specific uptake of  $Hg^{2+}$  was achieved using 0.83 mg/ml of cell biomass quantity for both conditions (Fig. 2).

Time of contact of adsorbent and adsorbate is of great importance in biosorption, because it depends on the nature of system used. Mercury uptake by BM07

living cells was a rapid process and occurred within a few minutes. This has well supported our observation of mercury-bacterium adsorption system equilibrium where maximum adsorption was achieved within 20 and 40 min respectively for cells grown at 10°C and 30°C (Fig. 3).

**Effect of the initial mercury concentration on biosorption rate.** The concentration of both the metal ions and biosorbent is a significant factor to be considered for effective biosorption. It determines the sorbent/sorbate equilibrium of the system. The rate of adsorption is a function of the initial concentration of

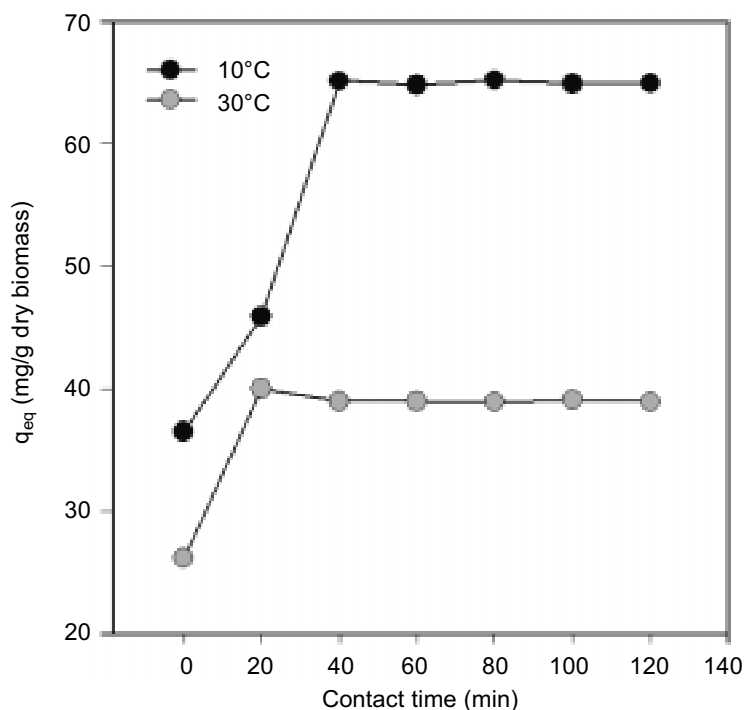


Fig. 3. Time course of mercury uptake by dried cells of *P. fluorescens* BM07 ( $C_0$ : 52 mg/l, temperature 25°C).

Table I

Equilibrium adsorbed quantities and adsorption yields of mercury ion obtained at different initial metal ion concentrations\*

Cells grown at 10°C			Cells grown at 30°C		
$C_0$ (mg/l)	Ad%	$q_{eq}$ (mg/g)	$C_0$ (mg/l)	Ad%	$q_{eq}$ (mg/g)
7.04	2.84	0.60	7.04	30.57	6.46
29.15	34.30	30.0	29.15	27.65	24.2
110.3	9.50	31.5	110.3	9.32	30.9
721	3.64	85.5	721	3.50	74.5

\* Temperature 25°C; agitation rate 170 rpm, at pH 7.0.

metal ions. The initial mercury ion concentration remarkably influenced the equilibrium metal uptake and absorption yield as shown in Table I. When the initial mercury ion concentration varied from 7 to 721 mg/l, the loading capacity of dried cells of *P. fluorescens* BM07 increased from 1 to 85 and 6 to 78 mg/g dry biomass of cells grown at 10°C and 30°C respectively. The increase of loading capacity of biosorbents with the increase of metal ion concentration is probably due to higher interaction between metal ions and each of biosorbent. As can be deduced from Table I, higher adsorption yields were observed at lower concentrations of metal ion.

**Isotherms of mercury biosorption by cells.** Basing on the data obtained here, the mercury ion uptake capacity of the living dried cells of *P. fluorescens* BM07 grown at two different temperatures (30°C and 10°C) was calculated using the Langmuir isotherms at fixed biosorbent mass of 0.33 mg/ml. As mentioned above the  $Q_{max}$  (mg/g dry biosorbent) is the maximum metal uptake under the given conditions,  $b$  is the equi-

librium constant and  $K$  (mg/l) is dissociation constant related to the stability of metal-biosorbent complexes, reciprocal to the equilibrium constant,  $b$ . Thus, a plot of  $1/Q$  versus  $1/C$  was used to obtain the  $Q_{max}$  (intercept), and  $K$  (slope) as shown in Fig. 4. The data on conversion to Langmuir adsorption isotherms model resulted in a straight line (Fig. 4). The values of  $q_{max}$  calculated from the linearized Langmuir plot for *P. fluorescens* BM07 cells. These values are very close to the experimental values. This shows that the experimental biosorption data perfectly fit the Langmuir isotherms equation. The regression coefficient ( $r^2$ ) for cells was respectively 0.988 and 0.922 for cells grown at 30°C and 10°C which further support the goodness of fit to the Langmuir model. The Langmuir parameters of living biomass of *P. fluorescens* BM07 grown at 30°C and 10°C were estimated and  $Q_{max}$  was 62.9 and 82.25 mg/g dry biomass and  $K$  value was 1.49 and 0.45 mg/l for 30°C and 10°C respectively.

Scanning electron micrograph (Fig. 5) shows cells of *P. fluorescens* BM07 grown to late exponential phase at 10°C under condition of induced extracellular biopolymer production with a uniquely structured sheath surrounding them.

**Conclusions.** It is very important to study the metal-removing characteristics of biomass to identify possible individual differences and exploit them. Therefore we aimed to investigate the biosorption characteristic of *P. fluorescens* BM07 strain in the removal of mercury ions with emphasis on its unique ability of producing biopolymer which is thought to affect its sorption abilities. *P. fluorescens* BM07 were found to be efficient for adsorption of mercury from solutions. The characterization of mercury uptake

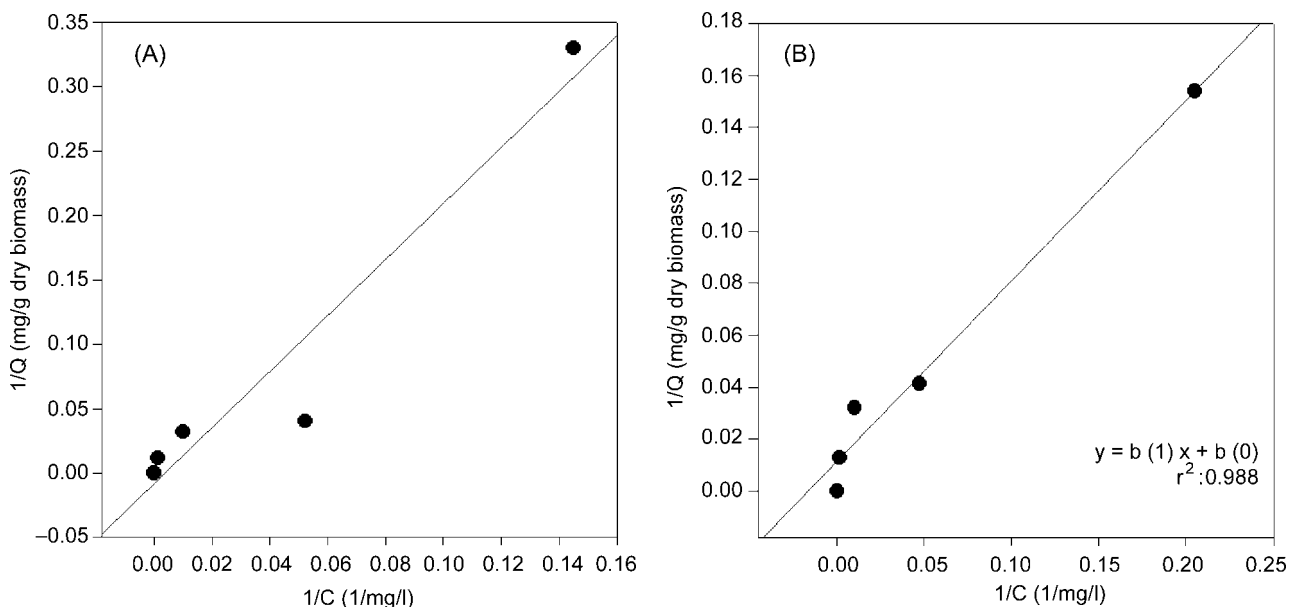


Fig. 4. Langmuir adsorption isotherm for mercury biosorption by living BM07 cells at 10°C (A) and 30°C (B).

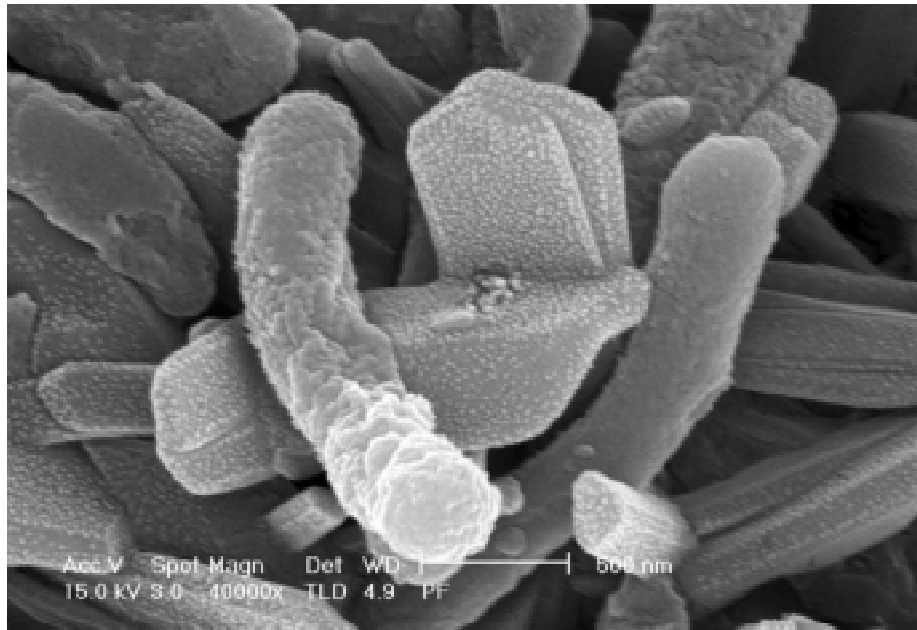


Fig. 5. Scanning electron micrograph of *P. fluorescens* BM07 cells grown in fructose-containing M1 minimal medium for 144 h (late exponential phase) at 10°C under condition of induced extracellular biopolymer production.

Micrographs showing groups of cells with a uniquely structured sheath surrounding them. Magnification 40 K, Bar 500.

showed that, mercury biosorption is dependent on initial pH, initial mercury ion concentration and biomass quantity. The Langmuir model of mercury biosorption by *P. fluorescens* BM07 was successfully applied to describe the biosorption equilibrium. Under similar conditions mercury adsorption rate by cells grown at 10°C was higher than those which were grown at 30°C grown cells. Considering that the biopolymer is synthesized at 10°C and with respect to the presence of a variety of many functional groups such as carboxyl, amine, hydroxyl, phosphate and sulfhydryl groups in the biopolymer, which potentially are capable of ion exchanging with metal cations, it seems reasonable to ascribe the elevated mercury adsorption to the production of the biopolymer. These metal ions may be adsorbed with negatively charged reaction sites on the cell surface (Beveridge and Murray, 1980; Gadd, 1990; Gupta *et al.*, 2000).

On the other hand, BATH test data showed that the surface of cells grown at 10°C was more hydrophobic than the surface of cells grown at 30°C (unpublished data). It might be due to the release of cold-induced biopolymer to out of the cells. Furthermore the role of van der Waals interaction between the cations and hydrophobic biopolymer can not be ignored (Cotton and Wilkinson, 1972; Frausto da Silva and Williams, 1991). Microorganisms generally produce an extracellular network of polysaccharides and proteins, such as capsules, slime, and more-structured sheaths. A coating of extracellular biopolymer may provide some capacity to adsorb cat-

ionic metal species, and its ability to sequester such cations will be provided by the cell wall, in which case the cationic species must be able to migrate through the sheath. The sheath's permeability is sufficient to allow a flow of metal ions. The existence of residual biopolymer on the external surface of BM07 cells grown at 10°C may be a pivotal factor to augment the mercury biosorption rate in comparison with 30°C grown cells.

Considering all of the parameters, it seems likely that the cold-induced biopolymer production plays an important role in biosorption efficiency, as *P. fluorescens* BM07 cells which were grown at 10°C under similar condition showed higher efficiency to biosorb mercury than non-polymer producing cells grown at 30°C. Further investigations are required to reveal how the dual-layered distribution of reactive sites of BM07 cells grown at 10°C will affect its ability to adsorb mercury more efficiently than non-polymer producing cells grown at 30°C.

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