

Full Length Research Paper

Biosorption of mercury by capsulated and slime layer-forming Gram -ve bacilli from an aqueous solution

Saleh M. Al-Garni*, Khaled M. Ghanem and Ahmed S. Ibrahim

Biological Sciences Department, Faculty of Sciences, King Abdul-Aziz University, P.O. Box, 80203, Jeddah 21589, Saudi Arabia.

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The biosorption of mercury by two locally isolated Gram-ve bacilli: *Klebsiella pneumoniae* ssp. *pneumonia* (capsulated) and slime layer forming *Pseudomonas aeruginosa*, was characterized. Mercury adsorption was found to be influenced by the pH value of the biosorption solution, initial metal concentration, amount of the dried biomass and contact time. The optimum biosorption capacity of *K. pneumoniae* (about 15%) was recorded at pH 5, initial mercury concentration of 0.1 g/L and when contacted for less than 60 min with 1.0 g dried cells/L. While, the highest biosorption capacity of *P. aeruginosa* (about 25%) was reached at pH 5.8, initial mercury level of 0.15 g/L and for less than 60 min contacted with 1.0 g dried biomass/L. The efficiency average of slime layer forming *P. aeruginosa*, of high negatively charged components, showed more than 1.5 fold increase as compared to capsulated *K. pneumoniae* of low negatively charged constituents, under all the tested characteristics of mercury biosorption from aqueous solution.

Key words: Biosorption, mercury, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, capsulated and slime forming bacilli.

INTRODUCTION

Mercury is one of the most toxic heavy metals released in the environment (Shaolin and David, 1997; Zilloux et al., 1993). The major effects of mercury poisoning are neurological and renal disturbances, as well as impairment of pulmonary function (Manohar et al., 2002; Saglam et al., 2002). This metal is released to the environment from anthropogenic activities that include agriculture, battery production, fossil fuel burning, mining and metallurgical processes, paint and chloralkali industries, and wood pulping (Boening 2000). In addition, activities such as solid waste combustion, industrial developing and petrochemical activities discharge mercury to the environment, especially aquatic ecosystem (WHO, 1998). Finding an effective method of removal of toxic heavy metals from industrial waste water is essential from the stand point of environmental pollution control (Green-Ruiz, 2006; Khoramabadi et al., 2008; Say et al., 2003; Svecova et al., 2006).

Conventional techniques for the removal of heavy metals from wastewater, such as chemical precipitation, ion exchange, activated carbon adsorption and separation processes have limitations and become inefficient and expensive especially when the heavy metal concentration is less than 100 ppm (Yan and Viraraghavan, 2001). The use of inexpensive biological materials, such as microorganisms, for removing and accumulating heavy metals from contaminated aqueous solutions has been widely reported (Al-Garni et al., 2009; EL-Sherif et al., 2008; Green-Ruiz, 2006; Khoramabadi et al., 2008; Lopez-Errasquin and Vazquez, 2003; Svecova et al., 2006; Zamil et al., 2009). The uptake of metals by biomass can take place actively, by means of metabolic activity-dependent process (bioaccumulation) or by means of a passive and usually rapid (several minutes) metabolism-independent process called biosorption (Godlewska-Zykiewicz and Kozowska, 2005). Inactivated, nonliving microbial biomass seems to be more advantageous than using living cells (Al-Garni et al., 2009; Gadd, 1992) and can serve as a basis for the development of potent biosorbent materials of strategic or valuable heavy metals

*Corresponding author. E-mail: algarni@kau.edu.sa.

and has a potential application in environmental control of these toxicants (Volesky, 1990). The mechanism and kinetics of metal biosorption on biomass depends on the experimental conditions particularly, medium pH, initial metal ion concentration, biomass concentration and affinity of the binding sites for the metal (Converti et al., 2006; Zeroual et al., 2003).

Klebsiella pneumoniae is a Gram -ve bacillus that possesses a thick polysaccharide capsule of glucose, mannose, galactose, glucuronic and galacturonic acids and lacks phosphoryl residues (Fresno et al., 2006; Kobayashi et al., 2002). While, *Pseudomonas aeruginosa* is also a Gram -ve bacillus that possesses a slime polysaccharide layer of rhamnose, glucose, mannose, glucosamine, galactosamine, glucuronic acid and acetyl groups (Arsenis and Dimitracopoulos, 1986; Bartell et al., 1970). *K. pneumoniae* was successively used for the biosorption of lead (Al-Garni, 2005) and *P. aeruginosa* was studied for the biosorption of mercury (Hassen et al., 1998). The objectives of the present work are: first, is to characterize some physicochemical parameters of mercury biosorption by local isolates of *K. pneumoniae* ssp. *pneumonia* and *P. aeruginosa*, and second, to evaluate the efficiency of capsule and slime layer forming Gram -ve bacilli for mercury biosorption from polluted liquids.

MATERIALS AND METHODS

Microorganisms and maintenance

K. pneumoniae ssp. *pneumonia* and *P. aeruginosa*, local isolates, were kindly provided by Microbiology Laboratory, King Abdul-Aziz Medical City, Jeddah, Saudi Arabia, and their identification was routinely assessed using PHONIX 100BD and MICRO SCAN walk away 9651 apparatus.

Growth and preparation of the dried powdered dead cells

The tested bacteria were maintained on nutrient agar slants. The stock cultures were transferred weekly and stored at 10°C. Biomass of the tested bacteria was developed by growing in MacConky broth medium (Nentech, LTD, U.K.), as a selective medium for the tested genera, at pH 7.0, incubated for 48 h at 37 ± 1°C, under shaken conditions (120 rpm). Cells were harvested by centrifugation at 8000 rpm for 10 min (Sorvall Ultra Pro 80, Surespin 630, Kendro Laboratory, USA). Harvested cells (biomass) were washed twice with deionized distilled water and dried in an oven at 80°C for 48 h (Puranik and Pakniker, 1999). To assess complete death of the dried cells, samples of the dried cells were inoculated to Petri-dishes containing MacConky agar medium, absence of any growth indicated positive results (complete death of the bacteria). The dried cells were then grinded in a blinder and sieved to obtain a fine powder (0.2 mm), and stored at 5°C until use.

Metal solution

A stock mercury ion solution (1000 mg/L) was prepared by dissolving mercuric chloride (HgCl₂) (Fisher Scientific Ltd) in deionized distilled water (Chen et al., 2005). Solutions were adjusted to the

desired pH values using 0.1 N NaOH and 0.1 N HCl (Svecova et al., 2006). The mercury concentration was determined using atomic absorption spectrophotometer (Unicam 929AA).

Metal absorption studies

A batch equilibrium method was used to determine sorption of mercury by the tested bacteria. All biosorption experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of the tested mercury solution. Powdered biomass (100 mg, unless otherwise stated) was exposed to metal solution for 60 min (otherwise stated) at 25 ± 2°C on a rotary shaker at 120 rpm. The biomass was separated by centrifugation at 10000 rpm for 10 min, and residual mercury concentration was measured in the supernatant. Metal adsorbed by the biomass (mg metal/g biomass) was calculated (Volesky and May-Phillips 1995) as:

$$Q = V (C_i - C_f) / 1000 \times M$$

Where, Q = mercury uptake (mg Hg (II)/mg biomass), V = volume of mercury solution (mL), C_i = initial Hg (II) concentration (mg/L), C_f = final Hg concentration (mg/L) and M = mass of powdered dried cells (mg).

Effect of pH

To test the effect of pH value on Hg (II) biosorption, the dried powdered cells of *K. pneumoniae* ssp. *pneumonia* and *P. aeruginosa* were suspended in mercury solution with pH values ranging from 2 - 7 for 60 min on a rotary shaker at 120 rpm. Thereby, the necessary analyses were carried out.

Effect of initial mercury concentration

Metal solutions (100 mL) of varying concentrations of mercury (25 - 400 mg/L) adjusted to the optimum pH of 5 for *K. pneumoniae* ssp. *pneumonia* and pH 5.8 for *P. aeruginosa*, were treated with 100 mg of the dried powdered cells of the test bacteria. Thereby, the biosorption was completed and necessary analyses were carried out.

Effect of dried powdered cells concentration

Dried powdered cells (100 - 1000 mg) were exposed to 100 mL of mercury solution (100 mg/L) at the optimum pH 5 for *K. pneumoniae* ssp. *pneumonia* and (150 mg/L) at pH 5.8 for *P. aeruginosa* for 60 min on a rotary shaker at 120 rpm. Thereby, the residual mercury in the supernatant after centrifugation at 10000 rpm for 10 min was measured using atomic absorption spectrophotometer.

Effect of contact time

To examine the mercury biosorption mechanism, 100 mg of dried powdered cells of the bacteria were contacted with 100 mL aliquots of mercury solutions (100 mg/L for *K. pneumoniae* ssp. *pneumonia* and 150 mg/L for *P. aeruginosa*) in 250 mL Erlenmeyer flasks. The flasks were incubated at 25 ± 2°C for different time intervals (5 - 180 min), thereafter analyzed for residual mercury content.

All experiments were carried out in triplicates and the recorded results are the arithmetic mean.

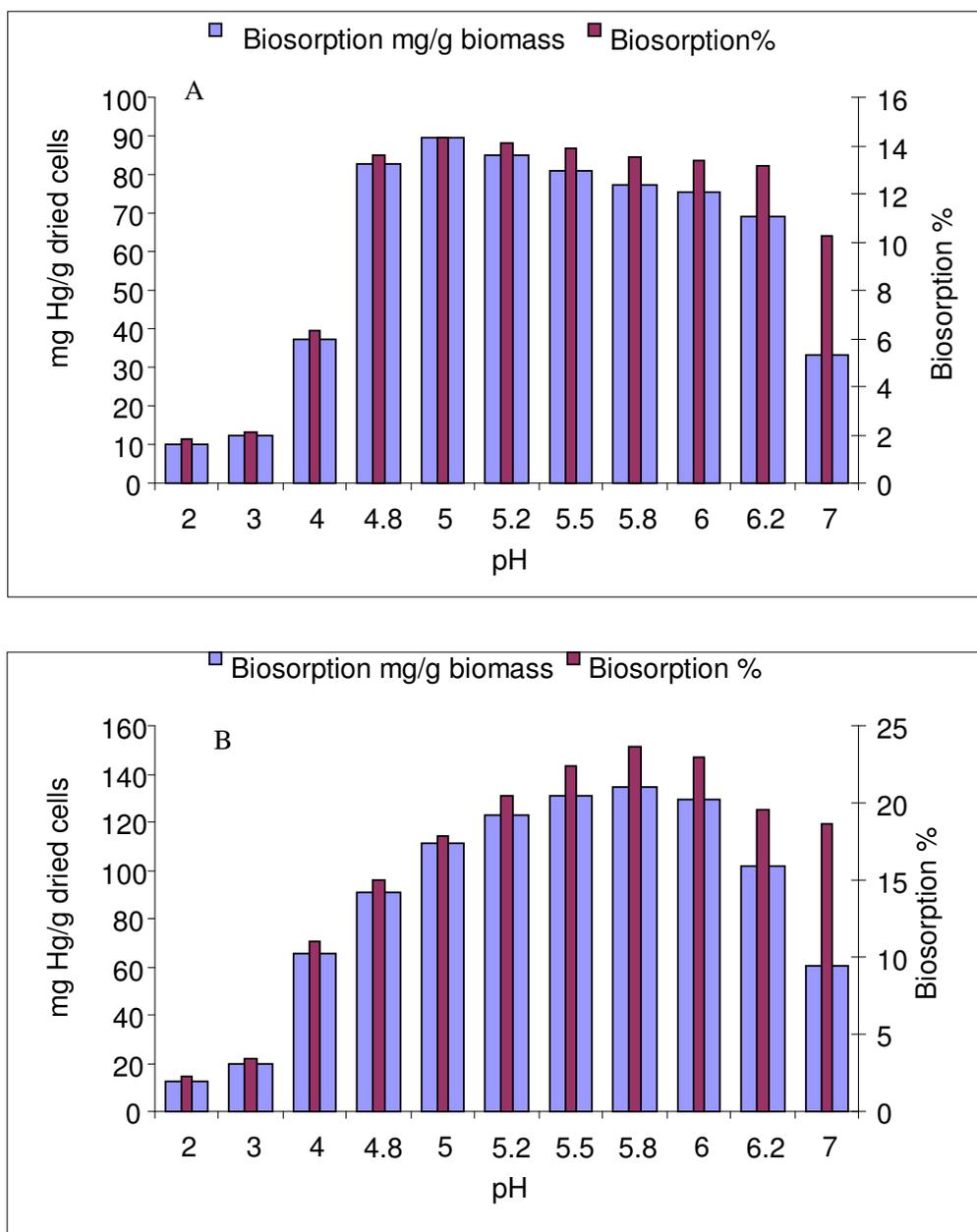


Figure 1. Mercury biosorption (mg Hg/g dry cells and as %) by dry biomass of *K. pneumoniae* (A) and *P. aeruginosa* (B) as influenced by the pH value.

RESULTS AND DISCUSSION

Effect of pH values on Hg (II) biosorption

The results (Figure 1) revealed that a pH range of 4.8 to 5.2, (especially pH 5) was optimum for biosorption of Hg by *K. pneumoniae*, where maximum Hg biosorption (14.36%) was recorded at pH 5. However, a pH range of 5.5 to 6 was optimum for Hg biosorption by *P. aeruginosa* and pH value of 5.8 proved to be the most suitable for maximum Hg sorption (23.61%) by the bacterium. The

results indicated that *P. aeruginosa* was more efficient to biosorb Hg, 64.4% more than *K. pneumoniae*. It was reported that pH value has an important role in metal ions biosorption (Al-Garni et al., 2009; Bae et al., 2002; Green-Ruiz, 2006), where the active biosorbing groups have the ability to accept or lose protons that depend mainly on the pH value (Yalcinkaya et al., 2002). Also, it affects metal ions solubility and ionization of the biosorbing groups in the biomass surfaces (Fourest and Volesky, 1996). It was also reported that high acidity makes the biosorbent surface accept protons (H^+) and

therefore reduce their ability to adsorb positive Hg ions and inversely, decrease acidity to its optimum value, which differ from one biomass to another and type of adsorbed metal ions. The adsorbing surface saturated with negative charges, resulted in increased efficiency to bind and adsorb metal ions of positive charges (Bayramoglu et al., 2003). The pH affects the network of negative charges on the surface of the biosorbing cells and the chemistry of the walls, as well as physicochemistry and hydrolysis of the metal (Collins and Stotzky, 1996; Lopez et al., 2000).

In accordance with our finding that Hg adsorption was low at low and high pH values by the tested bacteria, it was reported that at low pH values, adsorption of metals decreases because of competition for binding sites between cations (as Hg^{++}) and protons (Sahoo et al., 1992), while at pH higher than 7, hydroxo species of the metals can be formed and do not bind to the adsorption sites on the surface of the adsorbent (Kacar et al., 2000). The higher efficiency of *P. aeruginosa* to absorb Hg than *K. pneumoniae* at the different tested pHs may be due to the high content of reducing sugars, amino sugars, carboxylic acids, deoxysugars and acetyl groups of the slime layer of *P. aeruginosa* (Arsenis and Dimitracopoulos, 1986; Bartell et al., 1970) as compared to the capsule content of *K. pneumoniae* of non-polar lipopolysaccharides, carboxylic acids and amino sugars (Fresno et al., 2006), instead of the two bacteria which are Gram -ve bacilli. It may also be due to the presence of compact dense capsule of *K. pneumoniae* which may hide and/or block some adsorbing surfaces on the bacterial cell wall and make them unavailable to bind to the positive metal ions, as compared to the loose slime layer of *P. aeruginosa*.

Effect of initial mercury concentration on biosorption

The effect of initial concentrations of Hg (II) (25 – 400 mg/L) on its biosorption by nonviable cells of *K. pneumoniae* ssp. *pneumonia* and *P. aeruginosa* was evaluated. The results (Figure 2) indicated that in terms of percentage, the removal of mercury from the solutions appeared to be more efficient at 100 mg Hg/L for *K. pneumoniae* and at 150 mg Hg/L for *P. aeruginosa* (28.65 and 29.83%, respectively) than at lower or higher metal concentrations. The enhancement in metal sorption could be due to an increase in electrostatic interactions, involving sites of progressively lower affinity for metal ions (Al-Asheh and Duvnjak, 1995; Puranik and Pakniker, 1999). These data indicated that mercury uptake by the two tested Gram -ve bacteria (capsulated and slime forming) was chemically equilibrated and saturation was attained at an initial Hg(II) of 100 mg/L for *K. pneumoniae* and at 150 mg Hg/L for *P. aeruginosa*. So, there was no increase in metal uptake where the binding sites were saturated by the metals. The results indicated that the

adsorbing sites for mercury of *P. aeruginosa* are more than that of *K. pneumoniae* by more than 50%, whereas, the adsorbing sites of *K. pneumoniae* was saturated by 100 mg Hg(II)/L and with adsorbing percentage of 28.65%, while the adsorbing surfaces of *P. aeruginosa* was equilibrated by 150 mg Hg(II)/L (with 29.83% adsorption). At the same experimental conditions, this may due to the high content of negatively charged compounds of its slime layer, as compared to that of *K. pneumoniae* capsule with less negatively charged constituents.

It was reported by many workers that increasing initial mercury concentrations in liquids resulted in increasing its biosorbing efficiency until saturation of adsorbing sites, followed by lower adsorption efficiency (Green-Ruiz, 2006; Kacar et al., 2000; Saglam et al., 2002).

Effect of dried powdered cells level on biosorption

The results given in Figure 3 shows that the specific metal uptake (mg Hg/g cell mass) at different concentrations (1 to 10 g/L) of dried powdered cells of *K. pneumoniae* and *P. aeruginosa* were decreased with increasing dry mass concentrations. Thus, about 58.5 and 70.7% decreases were recorded for *K. pneumoniae* and *P. aeruginosa*, respectively, as the dry mass increased from 1 to 10 g/L. This could be attributed to interference between binding sites at higher mass levels (De Rome and Gadd, 1987). Reduction in metal uptake by the biosorbent with increasing biomass concentration was also attributed to an insufficiency of metal ions in solution with respect to available binding sites (Al-Asheh and Duvnjak, 1995; Fourest and Roux, 1992; Sampedro et al., 1995). Higher specific uptake at lower dry mass concentrations could be due to an increased metal-to-biosorbent ratio, which decreases upon an increase in dry mass concentration (Puranik and Pakniker, 1999). The obtained results indicated that the number of adsorbing negative charges on the biomasses' levels (1 g/L) of both bacteria was at equilibrium with metal ions, therefore increase in biomass level is not accompanied by increasing adsorption efficiency (Al-Garni, 2005; Al-Garni et al., 2009; Mashitah et al., 2008; Tawfik et al., 2005). However, as the biosorbed Hg was calculated on the basis of percentages, as expected, it was increased regularly with increasing dry mass of the cells. Thus, as the mass increased from 1 to 10 g/L about 4.2 and 2.9 fold increases in the percentage of biosorbed Hg (II) were recorded with *K. pneumoniae* and *P. aeruginosa*, respectively. However, in the basis of specific Hg uptake (mg Hg/g cell mass) about 2.4 and 3.4 fold decrease were recorded, respectively. This is in accordance with previous work in which it was reported that increased biomass concentration of the microbial cells was attained with increased metal sorption as g/L, and in the basis of absorbed metal as mg/g cell mass biosorption decreased regularly (Al-Garni, 2005; Gupta and Keegan, 1998;

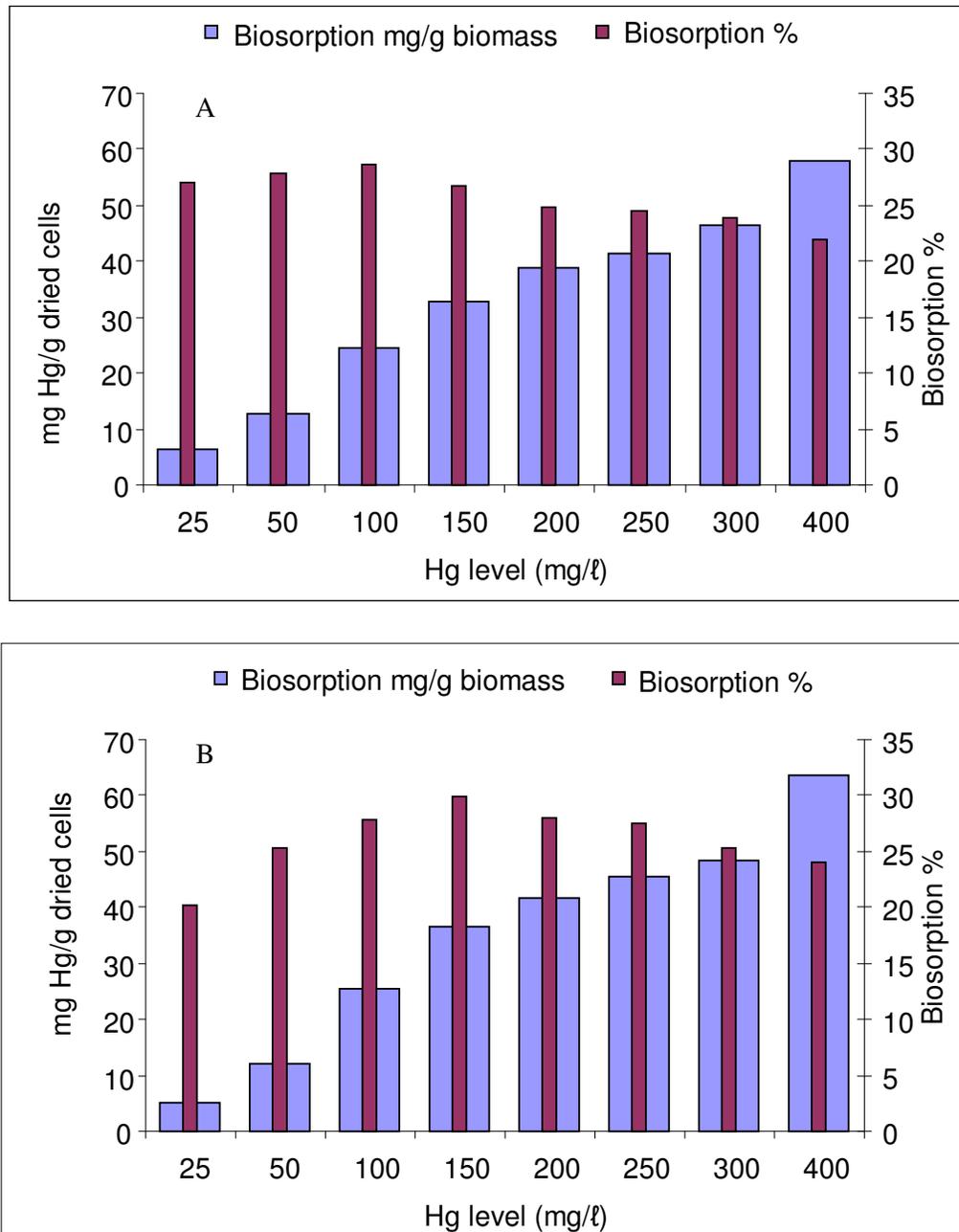


Figure 2. Effect of initial mercury level on biosorption of mercury (mg Hg/g dry cells and as %) by dry biomass of *K. pneumoniae* (A) at pH 5 and *P. aeruginosa* (B) at pH 5.8.

Selatnia et al., 2004).

Biosorption time

The data of mercury uptake (0.1 g Hg/L) at the optimum pH 5 (for *K. pneumoniae*) and pH 5.8 (for *P. aeruginosa*) with 1 g dried cells/L (Figure 4) showed that the rapid uptake of mercury occurred in the first 5 min, accounting for about 6.17 mg Hg/min for *K. pneumoniae* and about

8.87 mg Hg/min for *P. aeruginosa*. This probably is due to the availability of sorption sites at the beginning of the experiments, which is occupied suddenly by the mercuric ions from the solutions (Green-Ruiz, 2006). The results showed that time required for attaining equilibrium was less than 60 min, where biosorption of Hg mg/min dropped markedly (about 72 and 69% for *K. pneumoniae* and *P. aeruginosa*, respectively) as the contact time extended from 5 to 60 min. It is known that the rate of metal uptake is influenced by factors affecting mass

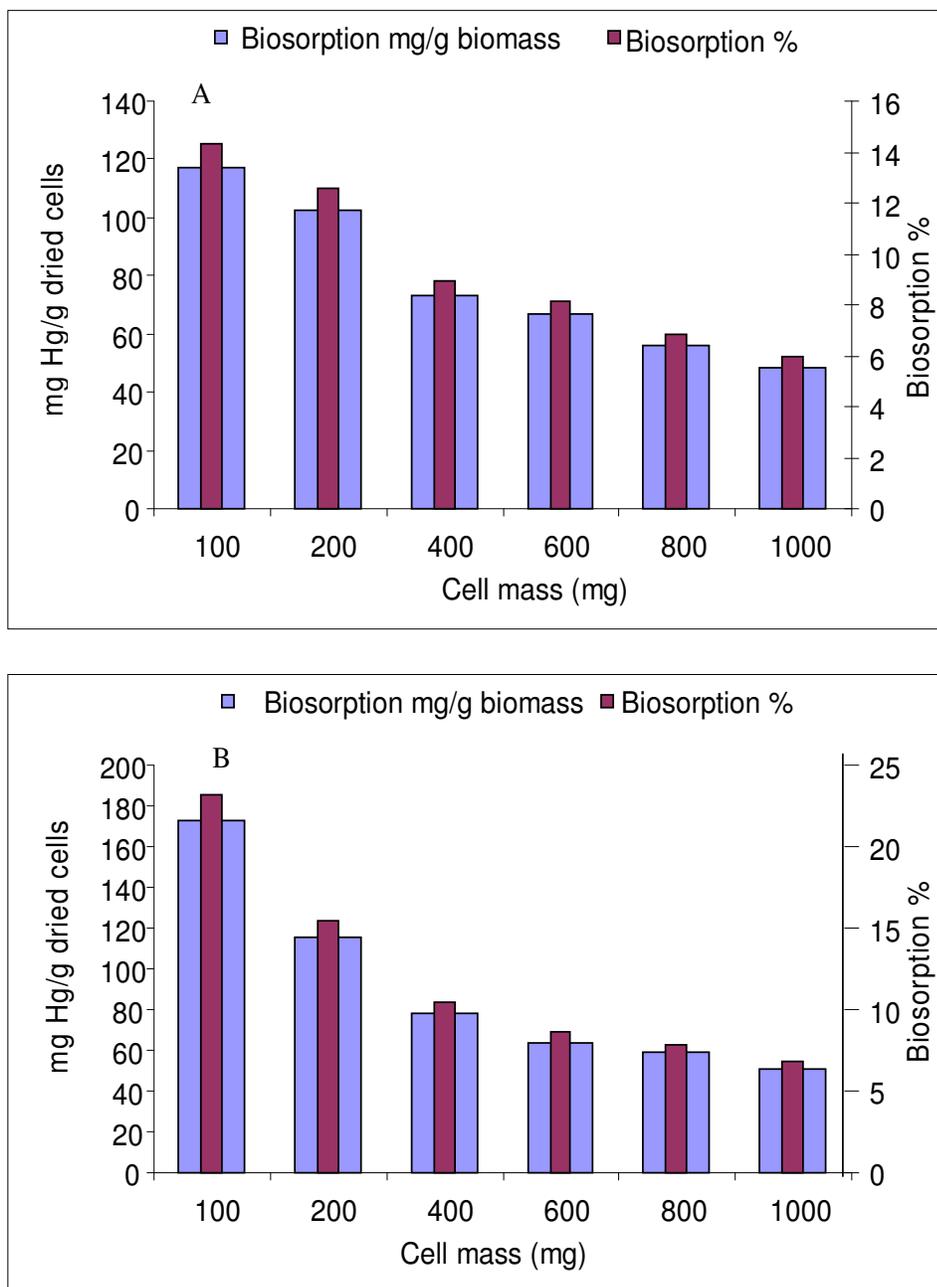


Figure 3. Effect of dried powdered cells level on biosorption of mercury (mg Hg/g dried cells and as %) by *K. pneumoniae* (A) at pH 5 and *P. aeruginosa* (B) at pH 5.8.

transfer from bulk solution to binding sites. It was indicated that various steps are involved in the transfer of metal from bulk solution to binding sites (Weber, 1995). First is the bulk transport of metal ions in solution phase, which is usually rapid because of mixing and adaptive flow (Gadd, 1988). Second, film transport involves diffusion of metal through a hydrodynamic boundary layer around the biosorbent surface, and third, actual adsorption of metal ions by active sites of the biomass is considered to be rapid and equivalent to an equilibrium

reaction (Weber, 1985). In the case of mercury biosorption by the tested bacteria, the experimental conditions allowed a normal mixing of solutes and biomass (dry cells) in the system that partially suppressed the kinetic limitations of the first and second steps and hence equilibrium was attained at less than 60 min. Therefore, the kinetics of the process was influenced by the three steps. Similarly, it was reported that biosorption of mercury by *Bacillus* sp. was rapid in the first 20 min and equilibrium was reached around 60 min (Green-Ruiz,

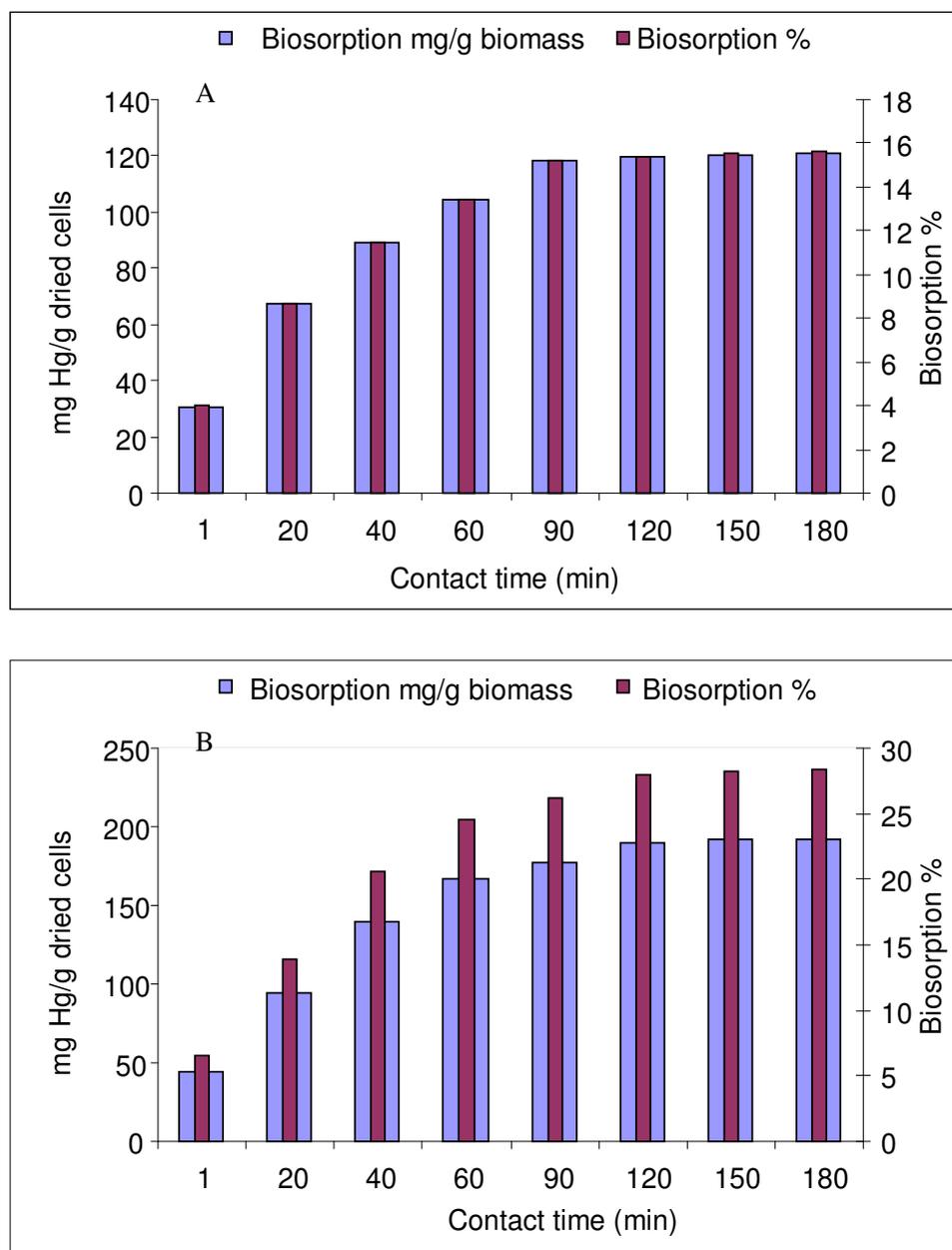


Figure 4. Effect of contact time on biosorption of mercury (mg Hg/g dried cells and as %) by *K. pneumoniae* (A) at pH 5 and *P. aeruginosa* (B) at pH 5.8.

2006). While, Al-Garni (2005) found that biosorption of lead by *Citrobacter freundii* and *K. pneumoniae* was rapid in the first 10 min and equilibrium was attained at less than 70 min. However, it is difficult to compare between those results and the results of this study because of the differences between the different parameters that play a role in the adsorption mechanisms, such as inherent characteristics of the adsorbent organisms (surface area, protein and carbohydrate composition, surface charge capacity), metal affinity and experimental conditions (pH, temperature and sampling periods) (Kacar et al., 2000).

The results also indicated that the average of Hg (II) absorption, as mg/min, at the different contact periods of *P. aeruginosa* has 1.5 fold increase as compared to *K. pneumoniae*. This finding correlates with the study's finding that the first bacterium had maximum biosorption of mercury at 150 mg/L, while *K. pneumoniae* attained maximum biosorption at only 100 mg Hg/L. Also, it correlates with the fact that *P. aeruginosa* slime forming Gram -ve bacillus is more efficient to biosorb mercury than capsulated Gram -ve bacillus (*K. pneumoniae*), due to the structural differences between the slime layer

components (contain high negative charge) and the constituents of *K. pneumonia* capsule with low negatively charged components.

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