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Biosorption lead(II) and nikel(II) from an aqueous solution by bacterial biomass

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The optimum conditions for biosorption of Pb(II) and Ni(II) from aqueous solution were investigated, by using living and nonliving *Pseudomonas fluorescens* and *Bacillus pumilus* isolated from wastewater treatment plant. It was found that the optimum pH for Pb(II) removal by living and nonliving cells was 6.0, while 7.0 for Ni(II) removal. At the optimal conditions, metal ion biosorption was increased as the initial metal concentration increased. The binding capacity by living cells is significantly higher than that of nonliving cells at tested conditions. The maximum biosorption capacities for lead and nickel by using *Ps. fluo-rescens* and *B. pumilus* were 77.6, 91.4 and 65.1, 73.9 mg/g, respectively. The results of bio-sorption time and desorption experiments suggested that Pb(II) and Ni(II) uptake by the living bacterial biomass might be enhanced by intracellular accumulation.

Keywords: biosorption, lead, nikel, *Pseudomonas fluorescens*, *Bacillus pumilus*.

INTRODUCTION

The presence of heavy metals in aquatic environments is known to cause severe dam-age to aquatic life, and the fact that these metals kill microorganisms during the biological treatment of wastewater causes a delay in the water purification process. Heavy metals are discharged from various industries such as electroplating, plastic manufacturing, textile, stor-age batteries, mining and metallurgical process. Most heavy metal salts are soluble in water from aqueous solutions and, as a consequence, cannot be separated by ordinary physical sepa-ration methods[1, 2]. Conventional methods for removing metals from aqueous solutions include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electro-chemical treatment, reverse osmosis, membrane technologies, and evaporation recovery. However, these conventional processes are not only expensive but are also ineffectual when the metal concentration in the water is less than 100 mg/l[3]. Compared with these processes, the biosorption process can offer the advantages of a low operating cost, minimization of the volume of sludge, high efficiency in detoxifying very dilute effluents, and no nutrients requirements[4, 5]. In general, the biosorption process includes the removal of metal or metalloid compounds from a solution by microorganisms in three ways: (1) the rapid binding of metal ions to a cellular surface or adsorption to the outer cell envelop (the surface adsorption mechanism); (2) gradual intracellular accumulation in which metals cross the cell wall during metabolism; and (3) complexation to excreted metabolic products in the surrounding envi-ronment[3]. The metal uptake process, however, is complex and dependent on the chemistry of the metal ions, specific surface properties of the organisms, cell physiology and the physico-chemical influence of the environment like pH, temperature and metal concentration. The same metal ions appear to be accumulated by different mechanisms in different microorgan-isms[6].

The objectives of the study are to compare the living and nonliving cells of *Pseudo-monas fluorescens* and *Bacillus pumilus* (isolated from wastewater treatment plant) in their removal capacity of Pb(II) and Ni(II). For these purposes, the removal capacity, desorption efficiency of living and nonliving cells and various factors affecting the adsorption, such as react time, initial pH of the solution and metal concentration.

EXPERIMENTAL

Microorganisms

The microorganisms used in study was isolated from wastewater treatment plant located in Głubczyce (Poland). Samples were diluted 10 – 10.000 fold in sterile distilled water and plated on Nutrient Agar (Merck) containing 50 mg/l of Pb(II) and Ni(II). These plates were incu-bated for 48 h at 30°C and colonies were randomly picked, isolated, and purified on the same medium. In this preliminary screening, colonies showing resistance to Pb(II) and Ni(II) were selected for further study. For preliminary tests bacterial strains were investigated for theirs biosorption capacities for Pb(II) or Ni(II) in a batch system. All the batch biosorption experi-ments were performed at 30°C on a rotary shaker (200rpm) using 100 ml beakers containing 100 mg/l of Pb(II) or 100 mg/l of Ni(II). The pH of the solutions was adjusted to 6.0. After 2 h the biosorption mixture was centrifugated at 4000rpm for 20min and the residual Pb(II) or Ni(II) concentrations in the solutions were analyzed. The most effective bacterial strains for the biosorption of Pb(II) and Ni(II) was identified according to Bergey's Manual of Systematic Bacteriology[7].

Preparation of biomass

The bacterial isolate was cultivated aerobically at 30°C in Nutrient Broth (Merck) by con-stantly agitating at 150rpm in glass flasks. The cells were harvested by centrifugation (4000rpm, 20min) from culture at early-stationary phase and washed twice with distilled wa-ter. Biomass concentration in cell suspensions was determined by dry weight at 105°C. The remaining harvested cells which were freeze dried, autoclaved at 115°C for 15 min, crushed in a blender and resuspended with distilled water, were defined as nonliving cells.

Preparation of metal solutions

Stock solution (1.0 g/l) of Pb(II) and Ni(II) were prepared by dissolving analytical grade $Pb(NO_3)_2$ and $NiSO_4$ in distilled water. Before mixing with the biosorbents, the stock solu-tions were diluted to required concentration. The initial pH of the solutions was adjusted to the required values by adding 0.1N HNO_3 or 0.1N NaOH solutions for the biosorption ex-periments. All glassware washed with 0.1N HCl before and after each experiment to avoid binding of the metal to it.

Heavy metal assay

The concentration of unadsorbed Pb(II) in the biosorption medium was determined spectrophotometrically at 520 nm in a spectrophotometer Photolab Spectral (WTW, Germany) using 4-(2'-pyridylazo)resorcinol (PAR) as the complexing agent. The concentration of free Ni(II) in the biosorption medium was estimated using dimethylglyoxime (DMG) at 366 nm[8]. In case of Pb(II) the applicable concentration limits is 0.1 – 5 mg/l using PAR method. Estimation of Ni(II) using DMG is a very sensitive method and it can estimate as low as 0.02 mg/l. The ini-tial and the final concentration of heavy metals used in batch mode studies were calculated by estimating the concentration of metals spectrophotometrically. From the difference in concen-tration the removal efficiencies of the microorganism has been calculated.

Procedures of biosorption experiments

Effect of pH. To check the effect of pH on biosorption, the biomass with the final concentration of 2 g/l were inoculated into a series of 250 ml conical flasks containing either 100 mg/l of Pb(II) or Ni(II). The pH value of metal/cell suspension was adjusted to 3 – 8 by 0.1N HNO_3 or 0.1N NaOH. The cultures were shaken in a rotary shaker (150rpm). After 1 h the biosorp-tion mixture was centrifugated at 4000rpm for 20min and the residual Pb(II) or Ni(II) concentrations in the solutions were analyzed.

Effect of contact time. The biomass with the final concentration of 2 g/l were added into a 250 ml flask containing either 100 mg/l of Pb(II) or Ni(II). This metal solution were mixed in a rotary shaker (150rpm) at optimal pH and temperature 30°C for 1 h. During the incubation period, heavy metal concentration was monitored for ten minutes interval until heavy metal removal attains a saturation level.

Effect of heavy metal concentration. The biosorption of heavy metal at the concentrations of 50, 100, 150, 200, 250 and 300 mg/l using the living and nonliving suspension of the selected strain at the optimum pH were compared. The adsorption test was conducted in an incubator shaker (150rpm) at 30°C for 1 h. After the incubation period, heavy metal concentration was analyzed as mentioned above.

Desorption of Pb(II) and Ni(II). Desorption of Pb(II) and Ni(II) was studied by using 0.1 M HCl as eluent. For this purpose, 0.01 g biomass was added to 10ml of eluent in a 50ml flask. After 24 h of shaking (150rpm), supernatants of centrifuged (4000rpm for 20min) samples were analyzed for the Pb(II) and Ni(II) concentrations.

Data evaluations. Metal adsorbed by bacterial biomass (Q mg metal/g dry biomass) was calculated as:

$$Q = \frac{(C_o - C_e)V}{m} \quad (1)$$

Where Q is the specific metal uptake (mg metal/g biosorbent), V is the volume of the metal solution (l), C_o is the initial concentration of metal in the solution (mg metal/l), C_e is the final concentration of metal in the solution (mg/l), and m is the dry weight of the biomass (g).The metal sorption ability of the biomass was determined by the above-mentioned procedure, in all the following experiments unless stated otherwise.

RESULTS AND DISCUSSION

Bacterial biosorbent

The bacterial cell used in biosorption studies was *Bacillus pumilus*; it was identified according to Bergey's Manual of Systematic Bacteriology[7] as *Pseudomonas fluorescens*; the biochemical testes for identification of the isolate are shown in Table 1.

Effect of pH

It has been shown that the affinity of cationic species towards the functional groups present in the cellular surface is strongly dependent on the pH[9]. Fig. 1 and 2 summarizes the results of the adsorption of Pb(II) and Ni(II) ions by living and nonliving bacterial biomass as a function of pH. In all cases, metal uptake by the biomass increases with increasing pH till it reaches a maximum after which the metal uptake decreases. Living cells demonstrated the maximum biosorption of 69.1 mg/g Pb(II) for Ps. *fluorescens* and 74.1 mg/g for *B. pumilus* at pH 6.0, where as that was 40.4 and 51.1 mg/g for nonliving cells,

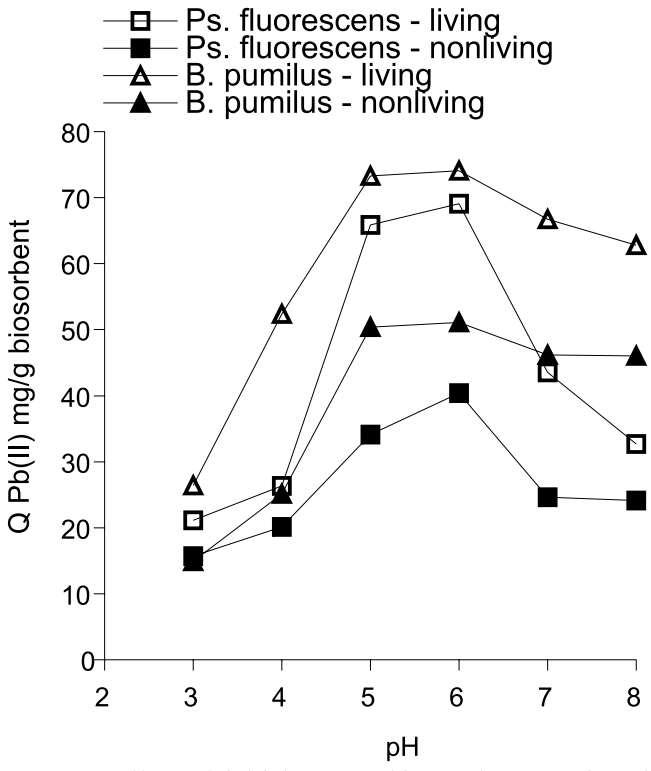

Figure 1. Effect of initial pH on biosorption capacity of Pb(II) by living and nonliving cells. Metal concentration 100 mg/l, temperature 30°C, incubation time 1 h

Table 1. Biochemical characterization of the isolate strains

Properties	Result	Properties	Result
Gram reaction	+	Gram reaction	-
Cell shape	Rod	Cell shape	Short rod
Cell diameter >1.0 μm	-	Cell diameter >1.0 μm	-
Spores round	-	Spores round	-
Sporangium swollen	-	Fluorescent pigment	+
Catalase	+	Catalase	+
Oxygen requirements	Aerobic	Oxygen requirements	Aerobic
Voges-Proskauer test	+	Voges-Proskauer test	ND
Glucose	+	Glucose	+
Xylose	+	Maltose	-
Mannitol	+	Mannitol	-
Hydrolysis of casein	+	Ossidase	+
Hydrolysis of gelatin	+	Hydrolysis of gelatin	-
Hydrolysis of starch	-	Hydrolysis of starch	-
Utilization of citrate	+	Utilization of citrate	+
Nitrate reduced to nitrite	-	Nitrate reduced	+
Formation of indole	-	Formation of indole	-
Growth at pH 6.8, nutrient broth	+	Growth at pH 6.8, nutrient broth	+
Growth at pH 5.7, nutrient broth	+	Growth at pH 5.7, nutrient broth	+
Growth in NaCl 2%	+	Growth in NaCl 2%	+
Growth in NaCl 5%	+	Growth in NaCl 5%	+
Growth in NaCl 7%	+	Growth in NaCl 7%	-
Growth in NaCl 10%	-	Growth in NaCl 10%	-
Growth at 5^0C	-	Growth at 5^0C	+
Growth at 10^0C	+	Growth at 10^0C	+
Growth at 30^0C	+	Growth at 30^0C	+
Growth at 40^0C	+	Growth at 37^0C	+
Growth at 50^0C	+	Growth at 41^0C	-
Growth at 55^0C	-	Growth at 50^0C	-
Growth at 65^0C	-	Growth at 65^0C	-
Type strain	*Bacillus pumilus*	Type strain	*Pseudomonas fluorescens*

+: 90% or more strain positive, -: 90% or more strain negative, ND: not detected

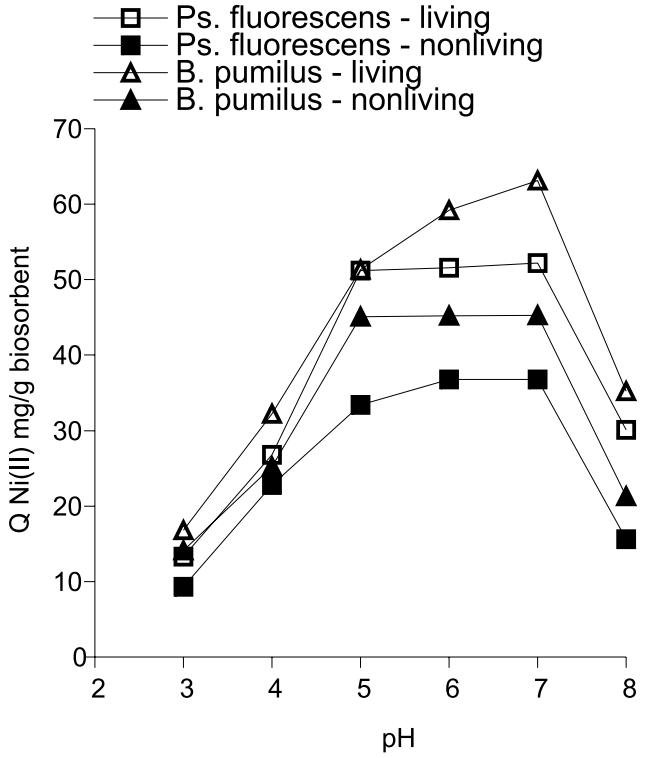

Figure 2. Effect of initial pH on biosorption capacity of Ni(II) by living and nonliving cells. Metal concentration 100 mg/l, temperature 30°C, incubation time 1 h

respectively. The effect of pH on the biosorption capacity of Ni(II) with bacterial biomass is shown in Fig. 2. It indicated the same trends as in the biosorption of Pb(II), for both type of cells achieved its maximum at pH 7.0. The maximum biosorption of Ni(II) by living cells *Ps. fluorescens* and *B. pumilus* was 52.2 and 63.1 mg/g, while that was 36.8 and 45.3 mg/g for nonliving cells, respectively. This dependence of biosorption on pH is caused by the presence of functional groups in the cell wall and the chemical configuration of the metal. The cell wall contains amines, amides and carboxylic acid functional groups that are either protonated or deprotonated, depending on the pH of the aqueous medium. Increasing the pH increases the negative charge at the surface of the cells until all relevant functional groups are deprotonated, a situation that favours electrochemical attraction and sorption of cations. Furthermore, the increase in metal uptake on increasing the pH may be the result of more effcient competition between cations and H^+ for the binding sites on bacteria[10]. The observed maximum lead biosorption at pH 5 – 7 by ex-amined species biomass is in accordance with the result given by Gabr et al[11] for *Pseudomonas areuginosa* ASU 6a with maximum biosorption founded at pH 6.0. Other investigators like Cabuk et al.[2] , Lopez et al.[9] and Fowle and Fein[13], reported that the maximum pH by *Bacillus* sp. ATS-2, *Pseudomonas fluorescens* 4F39, and *Bacillus subtilis* were 4.0, 7.0 and 8.0, respectively. Maximum pH for nickel biosorption in case of the present biomass is 7.0. This is in agreement with that obtained by Congeevaram et al.[8] and Gabr et al.[11] also reported the maximum pH for Ni(II) biosorption by *Bacillus subtilis* at 7.0. At the same time Liu et al.[14] and Hasan et al.[15] reported a maximum biosorption for nickel at pH 5 – 6 and low Ni(II) adsorption at lower pH values. This was due to the effect of pH on metal binding sites of the biomass surface and on metal speciation in aqueous solutions. The increased number of pro-tons on the sites of

biomass surface restricted the approach of metal cations as a result of repulsive forces. In contrast, El-Sersy and El-Sharouny[16], Kaewchai and Prasertsan[17], Lopez et al.[18] found that maximum pH for nickel by *Bacillus subtilis* N10, *B. subtilis* WD90 and *Pseudomonas fluorescens* 4F39 are 7.6, 8.0 and 9.0, respectively. In many instances, biosorption experiments conducted at high alkaline pH values have been reported to complicate evaluation of the biosorbent potential as a result of metal precipitation. Metal ions in solution undergo hydrolysis as the pH increases. The extent of which differs at different pH values and with each metal, but the usual sequence of hydrolysis is the formation of hydroxylated monomeric species, followed by the formation of polymeric species, and then the formation of crystalline oxide precipitates after aging[4]. Lopez et. al.[9] in the case of nickel solution indicated that within the pH range from 1 to 7, nickel existed in solution as Ni^{2+} ions (90%), whereas at pH 9, Ni^{2+} (68%), $Ni_4OH_4^{4+}$ (10%) and $Ni(OH)^+$ (8.6%) coexisted.

Effect of time on biosorption

Fig. 3 and 4 shows the effect of reaction time on the biosorption of Pb(II) and Ni(II) by biosorbent from aqueous solutions. In all cases, the rate of metal uptake increases rapidly in the first part within 10 min of contact. After that the rate decreases till we reach a constant value of metal concentration after 1 h. These changes in metal uptake may be due to the fact that, initially, all adsorbent sites were vacant and the solute concentration was high. After that period, only a very low increase in the metal uptake was observed because there are few sur-face active sites on the cell wall of bacteria. Therefore, one can conclude that the appropriate equilibrium time for measurements was taken at 1h. The quick equilibrium time is due to the particle size. The effective surface area is high for

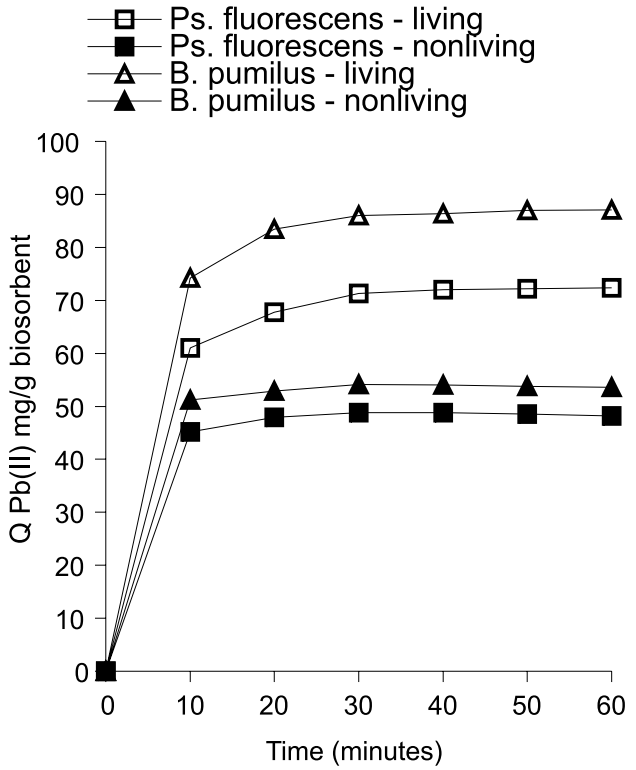

Figure 3. Effect of reaction time on biosorption capacity of Pb(II) by living and nonliving cells. Metal concentration 100 mg/l, temperature 30°C, pH 6.0

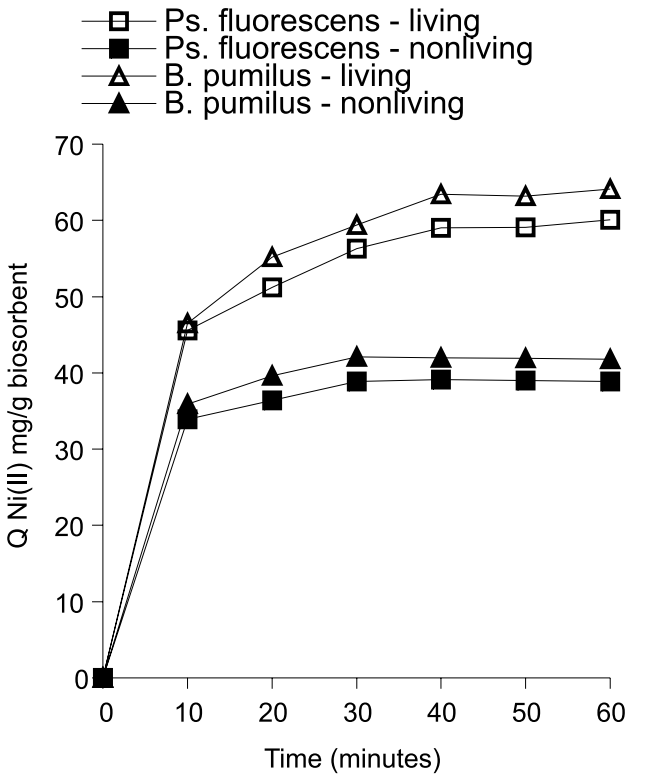

Figure 4. Effect of reaction time on biosorption capacity of Ni(II) by living and nonliving cells. Metal concentration 100 mg/l, temperature 30°C, pH 7.0

small particles. This short time required for biosorption is in accordance with the result given by many authors[11, 14, 16, 19].

The rate of lead biosorption by the nonliving cells *Ps. fluorescens* and *B. pumilus* was very rapid, reaching almost 92.6% and 94.6% of the maximum adsorption capacity within 10 min of contact time. However, it took a longer biosorption time for nickel, which reached 93.1% and 94.3% of the maximum biosorption capacity within 20 min. Microbial metal uptake by nonliving cells, which is metabolism-independent passive binding process to cell walls (adsorption), and to other external surfaces, and is generally considered as a rapid process, taking place within a few minutes[19].

It also can be seen that metals biosorption by living cells consisted of two phases: a primary rapid phase (within 10 – 30 min) and a second slow phase. It indicates that living cells may be not only having surface sorption but also slower and metabolism dependent active uptake of metals. In all cases, the metal adsorption capacity for living cells is apparently higher than that of nonliving cells (Fig. 3, 4). This result may be attributed to the intracellular accumulation of metal ions occurring in living cells, resulting in the enhancement in metal uptake capacity. The other possibility is that the autoclave-sterilization step, which may destroy or lose some of metal binding sites, resulting in the decrease in metal uptake capacity of the nonliving cells[19]. The maximum biosorbtion of Pb(II) by living cells *Ps. fluorescens* and *B. pumilus* were 72.4 and 87.1 mg/g dry cell. Similar result was obtained by Gabr et al.[11] and Chang et al.[20]. Studies on the Ni(II) biosorption showed the same trends as in the biosorption of Pb(II). The maximum biosorption of Ni(II) by living cells *Ps. fluorescens* and *B. pumilus* were 60.1 and 64.1 mg/g dry cell respectively (Fig. 4). For compari-

son, the maximum biosorption values (Ni(II) mg/g biomass) of some metal binging microbial biosorbents are 244 mg/g by *Bacillus* sp.[21], 145 mg/g by *Ps. fluorescens* 4F39[18] and 70 mg/g by Ps. aeruginosa ASU 6a[11].

The selectivity order for metal ion towards the studied biomass *Ps. fluorescens* and *B. pumilus* is Pb>Ni for a given initial metal ion concentration. This preferential type of adsorp-tion belonging to two different ions may be ascribed to the following: 1) The difference in their ionic radii. The ionic radius of Pb(II) is 1.20 A°, while that of Ni(II) is 0.9 A°. The smaller the ionic radius, the greater its tendency to be hydrolyzed, leading to reduce biosorption. 2) This may be explained on the basis of electronegativity of the metal ions[11]. Pb(II) (2.33 Pauling) has greater value that Ni(II) (1.91 Pauling). For these reasons the bacterial biomass has greater affinity for lead than nickel.

Effect of initial concentration

The effect of lead and nickel concentration was investigated at the concentration range of 50 – 300 mg/l and the optimum pH 6.0 or 7.0. Biosorption capacity of Pb(II) and Ni(II) by living cells rapidly increased when the initial metal concentration increased up to 100 mg/l and a slight increase thereafter as shown in Fig. 5 and 6. Nonliving cells indicated a gradual increase of biosorption of Pb(II) and Ni(II) up to the concentration of 150 mg/l and followed by a slight increase. When the initial Pb(II) concentration was increased from 50 to 300 mg/l, the biosorption capacity of living cells *Ps. fluorescens* and *B. pumilus* increased from 43.2 to 77.6 mg/g and 48.9 to 91.4 mg/g, but only from 22.0 to 48.0 mg/g and 31.0 to 53.0 mg/g for nonliving cells, respectively. The biosorption of Ni(II) showed to the same trends as indicated for Pb(II). When the initial Ni(II) concentration was 300 mg/l, the maximum biosorption by living cells *Ps. fluorescens* and

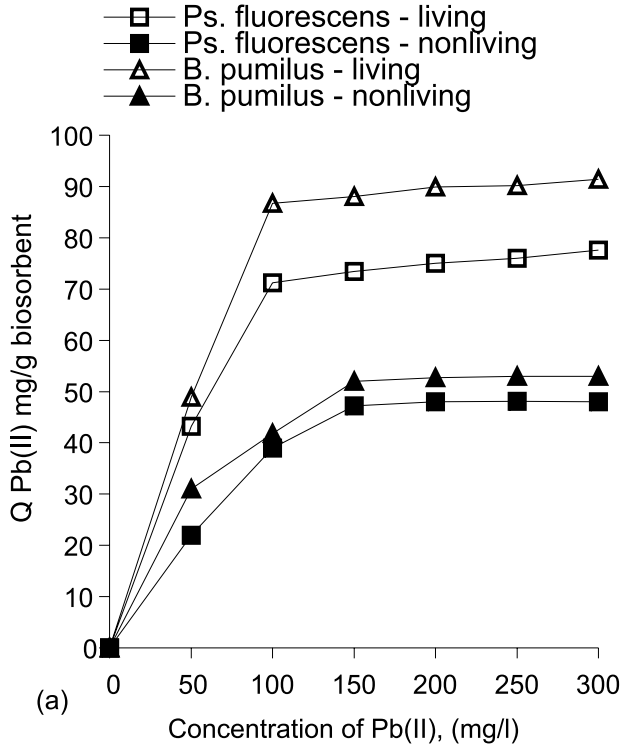

Figure 5. Effect of initial concentration of Pb(II) on their biosorption by living and nonliving cells. Incubation time 1h, temperature 30°C, pH 6.0

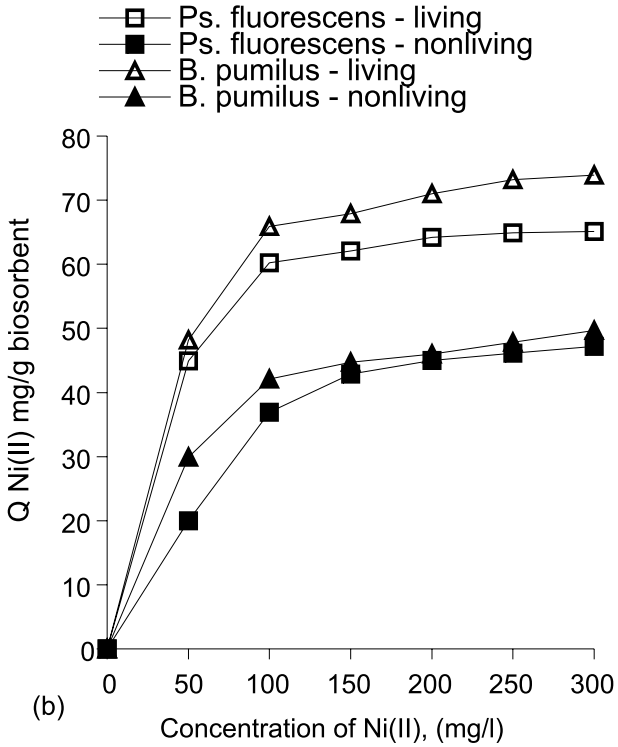

Figure 6. Effect of initial concentration of Ni(II) on their biosorption by living and nonliving cells. Incubation time 1h, temperature 30°C, pH 7.0

B. pumilus was 65.1 and 73.9 mg/g, while that was 47.2 and 49.7 mg/g for nonliving cells, respectively. It was also found that the biosorption capacities of living cells to Pb(II) and Ni(II) were significantly higher than that of the nonliving cells at all metal concentrations. The increase of biosorption capacity of biomass with the increase of metal concentration could be attributed to higher probability of interaction between metal ions and biosorbents[19]. Moreover, higher initial metal concentration provides an increased driving force to overcome all mass transfer resistance of metals between aqueous and solid phases and accelerate the probable collision between metal ion and sorbents which results in higher metal uptake.

Desorption of Pb(II) and Ni(II)

Biosorption is a process of treating pollutant-bearing solution to make it contaminant free. However, it is also necessary to be able to regenerate the biosorbent. This is possible only with the aid of appropriate elutants, which usually results in a concentrated pollutant solution. Therefore, a successful desorption process requires the proper selection of elutants, which strongly depends on the type of biosorbent and the mechanism of biosorption[4]. The experiment results of lead and nickel biosorption and desorption are presented in Table 2. Desorption efficiency of Ni(II) and Pb(II) by living cells *Ps. fluorescens* was 67.1 and 75.8% under 0.1 M HCl and it was 88.0 and 92.4% by nonliving cells, respectively. Nearly 88.8-91.9% of bound nickel and lead by biomass *B. pumilus* could be desorbed. However, only 61.7 – 69.6% of bound Ni(II) and Pb(II) by living cells could be desorbed. Chang et al.[20] reported desorption of lead ions from biomass *Pseudomonas aeruginosa* with 0.1 M HCl is 98%. It indicates that 0.1 M HCl can effectively desorb the bound Ni(II) and Pb(II) form bacterial biomass. And the desorption of Pb(II) is more effective than that of Ni(II). It also may be

Table 2. Desorption of Pb(II) and Ni(II) from living and nonliving cells

Metals	Biosorbent	Sorption (mg/g)	Desorption (mg/g)	Desorption (%)
Pb(II)	*Ps. fluorescens* – living	72.4	54.9	75.8
	Ps. fluorescens – nonliving	48.8	45.1	92.4
	B. pumilus - living	87.1	69.3	69.6
	B. pumilus – nonliving	54.1	48.1	91.9
Ni(II)	*Ps. fluorescens* – living	60.0	40.3	67.1
	Ps. fluorescens – nonliving	39.1	34.4	88.0
	B. pumilus - living	64.0	44.6	61.7
	B. pumilus – nonliving	42.1	37.4	88.8

due to the living cells uptake a part of Pb(II) and Ni(II) through intracellular accumulation, since it is possible to remove metals from cell surfaces after biosorption but not bioaccumulation[19].

CONCLUSION

In this study lead- and nickel-resistant microorganism was isolated from wastewater treatment plant, and the applicability of their heavy metal removal from aqueous solution was evaluated at a laboratory scale. Isolated strains were identified as *Ps. fluorescens* and *B. pumilus*. The optimum conditions for both the growth and heavy metal removal were determined for each isolate. It was found that the optimum initial pH for Pb(II) and Ni(II) removal by living and nonliving cells was 6.0 and 7.0, respectively. At the optimal conditions, metal ion biosorption was increased as the initial metal concentration. Results of this study demonstrat-ed that the binding capacity of living cells is significantly higher than that of nonliving cells at tested conditions. The maximum removal capacities of Pb(II) by living biomass *Ps. fluorescens* and *B. pumilus* were 77.6 and 91.4 mg/g, respectively. Regarding the case of Ni(II) removal using the same bacterial biomass, the relevant values were 65.1 and 73.9 mg/g. One can take assumption that the bacterial biomass has greater affinity for lead than nickel due to the difference in ionic radii and electronegativity. It was also found that 0.1M HCl can effectively desorb the bound Pb(II) and Ni(II) from nonliving cells, but not very effective for living cells. The results of biosorption time and desorption experiments suggested that Pb(II) and Ni(II) uptake by the living bacterial biomass might be enhanced by intracellular accumulation. The results suggest that *Ps. fluorescens* and *B. pumilus* is a potential adsorbing media for metals in the treatment of wastewater containing Pb(II) and Ni(II). However, many aspects of metalmicrobe interactions remain unexplored and further researches are necessary.

ACKNOWLEDGEMENT

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