Biosorption of lead by Gram-ve capsulated and non-capsulated bacteria

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Abstract

The biosorption of lead by two Gram-ve bacteria, either non-capsulated (*Citrobacter freundii*) or capsulated (*Klebsiella pneumoniae*) was characterised. Lead biosorption was found to be influenced by the pH of the solution, initial metal concentration, and amount of the dried powdered cells and contact time. Thus, the optimum biosorption capacity, by the two tested bacteria, was attained at pH 4, initial lead concentration of about 481.2 mg/ℓ and contacted with 2 g dried cells/ℓ for 100 min. However, the dried powdered cells of both organisms can be safely stored for long periods (125 d) at room temperature (25 ± 2°C) without any loss of their biosorption efficiency, i.e. their binding sites not affected by storage. The results revealed that the presence of capsule (*K. pneumoniae*) increased the biosorption efficiency of the bacterium.

Keywords: biosorption, Gram-ve bacteria, capsulated bacteria, *Citrobacter freundii, Klebsiella pneumoniae*

Introduction

Mobilisation of heavy metals in the environment due to industrial activities is of serious concern due to the toxicity of these metals in humans and other forms of life. Removal of toxic heavy metals from industrial waste waters is essential from the standpoint of environmental pollution control (Puranik and Pakniker, 1999; Guangyu and Thiruvenkatachari, 2003). Among the toxic heavy metals, mercury, lead and cadmium, "called the big three" are in the limelight due to their major impact on the environment (Volesky, 1994). Many industries, especially plating and those manufacturing batteries, pigments and ammunition, release heavy metals such as lead, cadmium and zinc in wastewaters. Lead and cadmium are potent neurotoxic metals (Puranik and Pakniker, 1997).

Chemical oxidation, reduction, precipitation, adsorption, solidification, electrolytic recovery, and ion exchange are some of the physicochemical wastewater treatment processes which are being used for metal removal. Application of such methods, however, is sometimes restricted because of technical or economical constraints. (Bossrez et al.,1997; Yu and Kaewsarn, 1999). Biological metal removal (biosorption) has distinct advantages over conventional methods: it is non-polluting and it can be highly selective, more efficient, easy to operate, and hence cost-effective for treatment of large volumes of wastewaters containing low metal concentrations (Puranik and Pakniker, 1999).

Various biomass materials including microbial biomass have been identified and documented as effective metal-removing agents (Veglio and Beolchini, 1997; Volesky and Holan, 1995). The present work aimed to characterise lead biosorption by local Gram-ve bacteria, either non-capsulated (*Citrobacter freundii*) or capsulated (*Klebsiella pneumonia*).

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Materials and methods

Micro-organisms

Citrobacter freundii and *Klebsiella pneumoniae* were kindly provided by Microbiology Lab, King Abdulaziz Medical City, Jeddah, Saudi Arabia, and their identification was routinely assessed using Sin: Desca 5744 Vite apparatus and API 20E indicator for Gram-ve bacteria.

Growth and preparation of the powdered dried dead cells

The tested bacteria were maintained on nutrient agar slants. The stock cultures were transferred weekly and stored at 10°C in a refrigerator.

Biomass of the tested bacteria was developed by growing in MacConky broth medium (Nentech, LTD, UK), pH 7.0 at 37±1°C for 48 h, under shaken conditions (120 r/min). Cells were harvested by centrifugation at 8 000 r/min for 10 min (J-2/C plus centrifuge). Harvested cells (biomass) were washed twice with deionised distilled water and dried in an oven at 80°C for 48 h. 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 indicating positive results (complete death of the bacteria). The dried cells were then ground in a porcelain mortar to obtain a fine powder (0.2 mm), and stored at 5°C, until use.

Metal solution

A stock solution of lead (1 200 mg/ℓ) was prepared by dissolving 0.0113 M of lead nitrate in deionised distilled water, shaking it for 15 min and then leaving it to stand for 24 h to obtain complete dissolution. Stock solution was diluted with deionised distilled water to obtain the necessary concentrations. Solutions were adjusted to desired pH values with 0.1 M sodium hydroxide and 0.1 M nitric acid. The initial lead concentration was measured at the beginning of all experiments carried out using an atomic

absorption spectrophotometer (AAS) (Unicam 929 AA Spectrometer, UK) and not as calculated from the dilution.

Metal absorption studies

A batch equilibrium method was used to determine sorption of lead by *C. freundii* and *K. pneumoniae*. A set of 250 mℓ Erlenmeyer flasks containing 50 mℓ of the tested lead solution was used in the experiments. Powdered dried dead cells (100 mg, unless otherwise stated) were exposed to metal solution for 60 min (unless otherwise stated) at 25 ± 2°C on a rotary shaker at 150 r /min. The dried powdered cells were separated by centrifugation at 10 000 r /min for 10 min, and supernatants were analysed for residual lead concentration on an atomic absorption spectrophotometer. Metal adsorbed by the tested dried cells (mg metal/g dry cells) was calculated (Volesky and May-Phillips, 1995) as:

$$Q = V (Ci - Cf) / 1\,000\ M$$

where:

- Q = specific lead uptake (mg lead/g biosorbent)
- V = volume of lead solution (mℓ)
- Ci = initial concentration of lead in the solution (mg/ℓ)
- Cf = final concentration of lead in the solution (mg/ℓ)
- M = mass of the powdered dried cells (g)

The lead sorption ability of the dried cells was determined by the above procedure, in all of the following experiments, unless otherwise stated.

TABLE 1
Effect of different pH values on biosorption of lead by the dried powdered cells of *C. freundii* and *K. pneumoniae*

Bacteria	pH value	Initial lead conc. * (mg/ℓ)	Residual lead conc. (mg/ℓ)	Biosorbed lead** (%)	Specific lead uptake*** (mg/g dry wt.)
C. freundii	2	158.8±5.0	147.4± 2.5	7.2	5.7
	3	153.2±2.8	119.4±3.2	22.1	16.9
	4	147.2±2.6	94.5±2.5	35.8	26.4
	5	136.1±3.5	102.5±3.4	24.7	16.8
	6	125.7±4.8	103.4±3.5	17.8	11.2
	7	120.6±1.1	100.4±5.7	16.8	10.1
K. pneumaniae	2	158.8±5.0	146.1± 3.1	8.1	6.4
	3	153.2±2.8	102.1±3.6	33.4	25.6
	4	147.2±2.6	83.5±2.2	43.3	31.9
	5	136.1±3.5	101.8±3.4	25.2	17.2
	6	125.7±4.8	112.5±3.9	10.5	6.6
	7	120.6±1.1	108.4±5.6	10.1	6.2

* Determined at the beginning of the experiment using atomic absorption spectrophotometry.

** Biosorbed lead (%) = $\frac{\text{Initial lead conc.} - \text{Residual lead conc.}}{\text{Initial lead conc.}} \times 100$

*** Specific lead uptake (mg/g dried cells) = $\frac{\text{Biosorbed lead (mg/ℓ)} \times \text{volume of lead solution (ℓ)}}{\text{mass of dried cells (g)}}$ = $\frac{\text{Biosorbed lead (mg/ℓ)} \times 0.1}{1000 \times 0.1}$

Effect of pH

To test the effect of pH on biosorption, the dried powdered cells of *C. freundii* and *K. pneumoniae* were suspended in lead solutions with different pH values, ranging from 2 to 7, for 60 min on a rotary shaker at 150 r/min. Thereby, the necessary analysis was carried out.

Effect of initial lead concentration

Metal solutions (50 mℓ) of varying concentrations of lead (ranging from 98.2 to 579.3 mg/ℓ, as measured at the beginning of the experiment by AAS) adjusted to the optimum pH of 4, were treated with 100 mg of the dried powdered cells of *C. freundii* and *K. pneumoniae*. Thereby, the biosorption was completed and necessary analyses were carried out.

Effect of dried powdered cells concentration

Dried powdered cells (50 to 500 mg) were exposed to 50 mℓ of lead solution (481.2 mg /ℓ ≈ 24.1 mg/50ℓ) at the optimum pH 4 for 60 min on a rotary shaker at 150 r/min. Thereby, the residual lead in the supernatant after centrifugation at 10 000 r/min for 10 min was measured using AAS.

Effect of contact time

To examine the lead biosorption mechanism, 100 mg of dried powdered cells of the bacteria were contacted with 50 mℓ aliquots of lead solutions (481.2 mg/ℓ) in 250mℓ Erlenmeyer flasks. Flasks were incubated at 25 ± 2°C for different time intervals (10 to 130 min) and analysed for residual lead content.

Effect of storage of the dried powdered cells

To test the effect of storage on the efficiency of biosorbent material (its binding sites) to biosorb lead, the dried powdered cells of *C. freundii* and *K. pneumoniae* were stored at room temperature (25 ± 2°C) for 125 d. Thereafter, 100 mg of dried powdered cells (after different storage periods) were contacted with 50 mℓ aliquots of lead solutions (481.2 mg/ℓ) at the optimum pH 4 for 100 min. At the end of biosorption the residual lead content was measured.

Each treatment and assay were carried out in duplicate and the obtained results are the arithmetic mean.

Results and discussion

Effect of pH values

The results (Table 1) revealed that a pH range of 3 to 5, (especially pH 4) was optimum for biosorption of lead by both bacteria. *K. pneumoniae* was more efficient in the biosorption process than *C. freundii*. At pH 4 *K. pneumonia* sorbed about 20.7% and 20.8 % more lead, as percentage and mg/g dry cells (specific lead uptake) , respectively, than *C. freundii*. Previous authors have reported that the prevailing pH value is one of the main factors in biosorption efficiency by different organisms (Leung et al., 2000; Lopez et al., 2000; Senthilkumaar et al., 2000; Jalali

et al., 2002; Pagnanelli et al., 2003; Pardo 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 results, it has also been reported that pH 4.5 was optimum for biosorption of lead by *Citrobacter* strain MCM B-181 and pH < 3 or > 5 resulted in lower biosorption efficiency of lead (Puranik and Pakniker, 1999; Guangyu and Thiruvenkatachari, 2003).

It was also reported that at highly acidic pH (<3) lead ions compete with H on the binding sites of microbial cell. However, at higher pH (>5) solubility of lead was lowered (Change et al., 1997; Gadd, 1988). These findings were in accordance with our results.

The higher efficiency of *K.pneumoniae* to absorb lead than *C. freundii* at the different tested pHs may be attributed to the presence of capsules. In accordance, it has previously been reported that *K. pneumoniae* accumulate cadmium on the cell-wall surface and through the capsule (Scott and Palmer, 1990).

Effect of initial lead concentration

The results shown in Table 2 revealed that specific metal uptake increased with increasing initial lead concentration with both bacteria and *K. pneumoniae* was again more efficient in biosorption at the different lead concentrations due to the presence of the capsule. 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 lead uptake by the two tested Gram-ve bacteria was chemically equilibrated and saturation was attained at an initial lead concentrations of 481.2 mg/ℓ. Thus, there was no increase in metal uptake for as long as the binding sites were saturated by the metal. At an initial lead concentration of about 481 mg/ℓ *K. pneumoniae* showed a 14.4% increase in biosorption over that attained by *C. freundii* (42.8%). However, the percentage of biosorbed lead was regularly decreased with increasing initial lead concentration, as had been expected.

TABLE 2
Biosorption of different concentrations of lead by the dried powdered cells of *C. freundii* and *K. pneumoniae* at pH 4

Bacteria	Initial lead conc. (mg/ℓ)	Residual lead conc. (mg/ℓ)	Bio-sorbed lead (%)	Specific Lead up-take (mg/g dry wt.)
C. freundii	98.2±1.9	61.9±1.6	36.9	18.1
	147.2±2.6	94.5±2.5	35.8	26.4
	196.4±2.3	134.2±2.6	31.7	31.1
	285.4±5.7	211.1±4.7	26.1	37.2
	392.3±8.2	313.4±4.1	20.1	39.5
	481.2±4.6	395.6±4.3	17.8	42.8
	579.3±7.3	491.2±4.5	15.2	44.1
K. pneumoniae	98.2±1.9	54.4±1.2	44.6	21.9
	147.2±2.6	83.5±2.2	43.3	31.9
	196.4±2.3	114.5±2.9	41.7	41.0
	285.4±5.7	198.2±2.4	30.6	43.6
	392.3±8.2	302.2±5.3	23.0	45.0
	481.2±4.6	383.3±3.9	20.3	48.9
	579.3±7.3	480.0±4.4	17.2	49.7

TABLE 3
Effect of mass of the dried powdered cells of *C. freundii* and *K. pneumoniae* on the biosorption of lead (481.2 mg/ℓ) at pH 4

Bacteria	Weight of dried cells (g/ℓ)	Residual lead conc. (mg/ℓ)	Bio-sorbed lead (%)	Specific lead up-take (mg/g dry wt.)
C. freundii	1	431.2±2.8	10.4	50.0
	2 (basal)	395.6±4.9	17.8	42.8
	4	332.9±3.2	30.8	37.1
	6	310.4±2.8	35.5	28.5
	8	272.5±2.3	43.4	26.1
	10	252.0±2.3	47.6	22.9
K. pneumoniae	1	427.2±2.9	11.2	54.0
	2(basal)	383.3±3.9	20.3	48.9
	4	300.0±4.6	37.7	45.3
	6	260.9±2.5	45.8	36.7
	8	250.6±4.1	47.9	28.8
	10	241.0±2.2	49.9	24.0

Effect of dried powdered dead cells concentration

The results given in Table 3 show that the specific metal uptake values obtained at various concentrations (1 to 10 g/ℓ) of dried powdered cells of *C. freundii* and *K. pneumoniae* were decreased with increasing dry mass concentrations. Thus, about 54.2% and 55.2% decreases were recorded for *C. freundii* and *K. pneumoniae*, respectively, as the dry mass increased from 1 to 10 g/ℓ. This could be attributed to interference between binding sites at higher concentrations (De Rome and Gadd, 1987). Reduction in zinc uptake by *Rhizopus arrhizus* with increasing biomass concentration was attributed to an insufficiency of metal ions in solution with respect to available binding sites by Fourest and Roux (1992). Similar observations were repeated by other workers (Al-Asheh and Duvnjak, 1995; 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 percentage of biosorbed lead was increased regularly with increasing dry mass of the cells. Thus, as the mass increased from 1 to 10 g/ℓ a 4.5-fold increase in the percentage of sorbed lead was recorded with the two bacteria. This is in accordance with previous work in which it was reported that increased biomass concentration of the microbial cells was attained with metal sorption as g/ℓ (Gupta and Keegan, 1998; Selatnia et al., 2004).

Effect of contact time

The data of lead uptake at the optimum pH (4) and initial lead concentration of 481.2 mg/ℓ contacted with 2 g dried cells (Table 4) showed that rapid uptake occurred in the first 10 min, accounting for about 4.5 mg lead/min for *C. freundii* and about 6 mg lead/min for *K. pneumoniae*. Time required for

TABLE 4
Effect of different contact times of the dried powdered cells (2 g/ℓ) of *C. freundii* and *K. pneumoniae* on the biosorption of lead (481.2 mg/ℓ at pH 4

Bacteria	Contact time (min)	Residual lead conc. (mg/ℓ)	Bio-sorbed lead (%)	Specific lead uptake mg/g dry cell	Bio-sorbed lead /min
C. freundii	10	436.0±3.4	9.4	22.6	4.5
	40	410.6±6.7	14.7	35.3	1.8
	60 (basal)	395.6±4.9	17.8	42.8	1.4
	70	383.2±4.2	20.4	49.0	1.4
	100	364.1±4.2	24.3	58.5	1.2
	130	362.4±3.7	24.7	59.4	0.9
K. pneumoniae	10	421.2±3.8	12.5	30.0	6.0
	40	400.2±3.8	16.8	40.5	2.0
	60 (basal)	383.3±3.9	20.3	48.9	1.6
	70	377.0±6.5	21.7	52.1	1.5
	100	358.0±5.7	25.6	61.6	1.2
	130	356.1±3.4	26.0	62.5	1.0

TABLE 5
Effect of storage of the dried powdered cells (2 g/ℓ) of *C. freundii* and *K. pneumoniae* on the biosorption of lead (481.2 mg/ℓ) after 100 min of contact time at pH 4

Bacteria	Storage time (days)	Residual lead conc. (mg/ℓ)	Bio-sorbed lead (%)	Specific lead uptake (mg/g dry cells)
C. freundii	2 (basal)	364.1±4.2	24.3	58.5
	5	362.2±4.8	24.7	59.5
	25	362.1±3.9	24.7	59.5
	45	361.9±4.6	24.8	59.6
	85	363.7±6.0	24.4	58.8
	125	363.8±4.5	24.4	58.7
K. pneumoniae	2 (basal)	358.0±5.7	25.6	61.6
	5	356.8±5.0	25.8	62.2
	25	359.6±5.8	25.3	60.8
	45	356.0±5.6	26.0	62.6
	85	356.1±4.1	26.0	62.6
	125	356.5±4.1	25.9	62.4

attaining equilibrium was less than 70 min, under the tested conditions. It is known that the rate of metal uptake is influenced by factors affecting mass 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, 1985). First is the bulk transport of metal ions in solution phase, which is usually rapid because of mixing and advective 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, equivalent to an equilibrium reaction (Weber, 1985).

In the case of lead 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 70 min. Therefore, the kinetics of the process was influenced by the three steps. Similarly, it was reported that biosorption of lead by the fungus *Phanerochaete chrysosporium* was rapid in the first 15 min and equilibrium was attained after 3 h (Ceribasi and Yetis, 2001).

Effect of storage of dried powdered cells

The effect of storage of the biosorbent (dried powdered cells of the tested bacteria) for long periods (125 d) at room temperature 25±2°C (Table5) indicated that the binding sites of the biomass still had the same capacity to biosorb lead ions. The specific metal uptakes by both *C. freundii* and *K. pneumoniae* dried powdered cells, were almost the same and no detectable differences before and after storage were recorded. This finding can be considered as an advantage of applying biosorbents over the other conventional methods, as the biosorbents can be safely stored for long periods without any loss of metal uptake efficiency.

Conclusion

Different aspects of the biosorption of lead by the dried powdered dead cells of Gram-ve bacteria, one non-capsulated (*Citrobacter freundii*) and the other capsulated (*Klebsiella pneumoniae*) were characterised. Studies showed that lead biosorption capacity of the two organisms was influenced by the pH value of lead solution, initial lead concentration, amount of the sorbent and contact time. However, storage of the biosorbent cells for long periods had no detectable influence in their biosorption efficiency. The study showed that the capsule played an important role in biosorption efficiency, as *K. pneumoniae* showed higher efficiency to biosorb lead under the test conditions than the Gram–ve, non-capsulated *C. freundii*.

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