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Biosorption of Cr^{6+}, Pb^{2+} and Cu^{2+} ions in industrial waste water on *Bacillus* sp.

M. Nurbaş Nourbakhsh [a,*], S. Kiliçarslan [a], S. Ilhan [c], H. Ozdag [b]

[a] *Department of Chemical Engineering, Osmangazi University, Eskisehir, Turkey*
[b] *Department of Mining Engineering, Osmangazi University, Eskisehir, Turkey*
[c] *Department of Biology, Osmangazi University, Eskisehir, Turkey*

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Abstract

Biosorption of each of the ions Cr^{6+}, Pb^{2+} and Cu^{2+} on *Bacillus* sp. in a batch stirred system was investigated and optimum conditions were determined. Then, the multi-metal ions, containing different concentrations of Cr^{6+}, Pb^{2+} and Cu^{2+} ions together were prepared and partial competitive adsorptions of these mixtures were investigated at 27 °C and for the pH values of 4.0 and 7.0 that are the most frequently seen conditions in industrial waste waters. Parameters such as co-adsorption of metals to microorganism, combination, concentration and adsorption sequence affect the partial competitive adsorption. In conclusion, in the metal ions mixture, lead biosorption increases widely while chromium and copper biosorptions decrease in comparison with the biosorption of only one kind of metal ion.

Keywords: Heavy metal contamination; Heavy metal biosorption; *Bacillus* sp.; Partial competitive biosorption; Waste water; Biosorption; Heavy metals

1. Introduction

Heavy metal ions are used in various industries due to their technological importance. Waste waters from these industries include metal ions having permanent toxic effect [1]. Algae, fungi, yeast and bacteria remove heavy metals from waste waters through functional groups such as ketones, aldehydes, carboxyls on their cell walls. Feasible and useful treatment methods have been developed to purify industrial waste waters [2,3]. Removal and recovery of heavy metals are very important with respect to environmental and economical considerations. Investigations on the biosorption mechanism of heavy metals show that the metal ions are deposited by adsorption to the functional groups present on the cell wall. Dead as well as living cells are used in the removal of metal ions [4–6]. Most of the studies involve the removal of only one kind of metal ion by microorganisms from aqueous solutions. However, the presence of only one kind of heavy metal ion is a rare situation either in nature or in waste waters.

Simultaneous removal of metal ions by microorganisms and immobilized microorganisms (biomass) from solutions including two or more metal ions has gained importance recently [7,8]. When two or more metal ions are present together, they may increase or decrease or may not change the metal ion adsorption capacity of the microorganism [9]. Parameters such as binding of metals to the cells together, combinations, concentrations and binding sequence are effective in the partial competitive adsorption [10,11]. The pH values and metal ion concentrations also affect biosorption rates and equilibrium uptake [12]. In this study, biosorption of each of the Cr^{6+}, Pb^{2+} and Cu^{2+} ions by *Bacillus* sp. was investigated and optimum conditions and partial competitive adsorptions of multi-metal ions were investigated.

2. Materials and methods

2.1. Isolation and identification of microorganisms

The strain used in the study was isolated from the soil. Enrichment was carried out in shaken culture in the medium with the following content: D[+] glycose monohydrate, $4.0\,g\,l^{-1}$; KH_2PO_4, $0.8\,g\,l^{-1}$; $[NH_4]_2SO_4$, $4.0\,g\,l^{-1}$; NaH_2PO_4, $0.1\,g\,l^{-1}$; $MgSO_4{\cdot}7H_2O$, $0.2\,g\,l^{-1}$;

* Corresponding author. Tel.: +90-222-239-10-74x3278;
fax: +90-222-239-36-13.
E-mail address: mnurbas@ogu.edu.tr (M. Nurbaş Nourbakhsh).

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yeast extract, 4.0 g l^{-1}; $CaSO_4 \cdot H_2O$, 0.1 g l^{-1}; pepton, 4.0 g l^{-1}; nutrient broth (oxoid), 4.0 g l^{-1}; $FeCl_3 \cdot 6H_2O$, 0.1 g l^{-1}; $Na_2MoO_4 \cdot 2H_2O$, 0.1 g l^{-1}. Pure culture was obtained by inoculation to nutrient plates from an enrichment culture. Three different bacteria obtained from soil were named as OGUB 001, OGUB 002, and OGUB 003. Isolates were investigated with respect to their removal capacity of lead, copper and chromium from the solutions containing these ions. OGUB 001 was determined as the isolate having the best adsorption capacity [13]. Microscopic and biochemical tests were applied to OGUB 001 according to Bergey's Manual of Systematic Bacteriology. The genus to which this isolate belongs was detected [14,15].

2.2. Growth of microorganisms and biosorption

OGUB 001 was incubated at 27 °C and at 150 rpm for 40–48 h in the nutrient broth (oxoid). Upon incubation, cells were removed from medium by centrifuging at 5000 rpm and the free moisture was removed by retaining the pellet in an oven at 50 °C. Then, it was suspended in deionized water for adsorption studies. By using stock solutions including 1000 mg l^{-1} metal, 200 ml solutions containing 100 mg l^{-1} Cr^{6+},150 mg l^{-1} Pb^{2+}, and 100 mg l^{-1} Cu^{2+} ions were prepared. Then, 4.0 g wet cells were added to the medium (20 g wet cells l^{-1}) and adsorptions of metals were investigated for different pH values within the ranges specific to the best adsorption of each metal ion to cells. These ranges are between 1.1–2.5, 3.5–5.0 and 3.0–5.0 for chromium, lead and copper ions, respectively. The pH was adjusted by using HCl and NaOH. Microorganism-containing solutions were agitated in a shaker of 150 rpm at 27 °C during adsorption. In partial competitive biosorption, 200 ml solutions containing copper (100 mg l^{-1}), chromium (100 mg l^{-1}), and lead (150 mg l^{-1}) ions were prepared and experiments were carried out under the same conditions. Samples taken at intervals were prepared for supernatant analysis by centrifuging. Analyses for Cr^{6+}, Pb^{2+} and Cu^{2+} ions were carried out by atomic absorption spectrophotometer (Perkin-Elmer) after dilution of the samples.

3. Results and discussion

Three different bacteria were obtained from soil named OGUB 001, OGUB 002, and OGUB 003. Isolate OGUB 001 was used in partial competitive adsorption of heavy metal mixtures. Tests performed for identification and other properties are in Table 1 and OGUB 001 was determined as a member of the *Bacillus* genus.

While the parameters such as binding of metals to the cells together, combinations, concentrations and binding sequence affect the partial competitive biosorption, pH affects the distribution coefficient and the adsorption capability of the functional groups on the biosorbent [16]. For instance, a functional group on the cell, the carboxyl group, may give a cation exchange reaction as follows [17]:

$$Me^{n+} + nHR \leftrightarrow MeR_n + nH^+ \quad (1)$$

Optimum pH values in single adsorptions of Cr^{6+}, Pb^{2+} and Cu^{2+} ions are 2.0, 4.5, and 4.0, respectively (Table 2) [13]. In partial competitive adsorption, a significant part of total adsorption capacity was used by Pb^{2+} ion. At pH 4.0, 71.8% of the capacity was used by lead, while 16.3 and 11.9% were used by copper and chromium ions, respectively (Table 3); and, at pH 7.0, 75.42% of the capacity was used by lead while 0.9 and 23.68% by copper and by chromium ions, respectively (Table 4). Approximately, 2.5 times increase for lead and approximately, 2.5 times decrease for chromium and copper were observed in the partial competitive adsorption in comparison with single metal ion adsorption. It was observed by comparing the results obtained at pH 4.0 and 7.0 that lead adsorption did not change significantly and copper adsorption increased and chromium adsorption decreased with increasing pH.

Table 1
Tests for identification of OGUB 001 and other determined properties[a]

Properties	OGUB 001	Properties	OGUB 001
Cell shape	Rod	Citrate utilization	−
Width	1.0	Growth in pH 6.82	+
Length	3.0	Growth in pH 5.7	+
Gram reaction	+	Growth in 2%	+
Catalase	+	NaCl growth in ≥5%	−
Spore shape	Ellipse	NaCl growth at 5 °C	−
Anaerobic growth	−	Growth at 30 °C	+
Voges-Proskauer	+	Growth at 40 °C	+
Starch hydrolysis	+	Growth at ≥50 °C	−

[a] (+): 90% or more strain positive; (−): 90% or more strain negative.

Table 2
Effect of initial values of pH and concentration on biosorption of Cr^{6+}, Pb^{2+} and Cu^{2+} ions (T = 27 °C, 20 g wet cells l^{-1})[a]

Co = 96.83 mg Cr^{6+} l^{-1}		Co = 100 mg Pb^{2+} l^{-1}		Co = 70 mg Cu^{2+} l^{-1}		Co (mg Cr^{6+} l^{-1}) at pH = 2	Adsorption (mg Cr^{6+} l^{-1})	Co (mg Pb^{2+} l^{-1}) at pH = 4.5	Adsorption (mg Cr^{6+} l^{-1})	Co (mg Cu^{2+} l^{-1}) at pH = 4.5	Adsorption (mg Cr^{6+} l^{-1})
pH	Adsorption (mg Cr^{6+} l^{-1})	pH	Adsorption (mg Pb^{2+} l^{-1})	pH	Adsorption (mg Cu^{2+} l^{-1})						
1.1	38.83	3.5	21	3.0	49	48.41	38.41	50	15	35	25
1.5	51.83	4.0	25	3.5	64	96.83	56.83	100	35	70	52
2.0	56.83	4.5	35	4.0	52	145.24	98.24	150	61	105	80
2.5	28.83	5.0	33	4.5	70	193.66	123.66	200	88	140	108
				5.0	58						

[a] Co: Initial metal ion concentration.

Table 3
Partial competitive adsorption of Cr^{6+}, Pb^{2+} and Cu^{2+} ions onto *Bacillus* sp. (pH = 4.0, 20 g wet cells l^{-1})

Time (min)	Cr^{6+}		Pb^{2+}		Cu^{2+}	
	Adsorption (mg l^{-1})	Adsorption (%)	Adsorption (mg l^{-1})	Adsorption (%)	Adsorption (mg l^{-1})	Adsorption (%)
5	15.25	15.25	150	100	33.00	33.00
15	22.25	22.25	150	100	37.50	37.50
30	25.25	25.25	150	100	39.25	39.25
60	17.00	17.00	150	100	31.75	31.75
90	19.75	19.75	150	100	35.00	35.00
120	19.00	19.00	150	100	33.75	33.75
180	19.25	19.25	150	100	33.75	33.75
240	24.00	24.00	150	100	33.50	33.50
360	24.25	24.25	150	100	32.50	32.50
1440	24.75	24.75	150	100	34.00	34.00

Table 4
Partial competitive adsorption of Cr^{6+}, Pb^{2+} and Cu^{2+} ions onto *Bacillus* sp. (pH = 7.0, 20 g wet cells l^{-1})

Time (min)	Cr^{6+}		Pb^{2+}		Cu^{2+}	
	Adsorption (mg l^{-1})	Adsorption (%)	Adsorption (mg l^{-1})	Adsorption (%)	Adsorption (mg l^{-1})	Adsorption (%)
5	0.00	0.00	149.89	99.927	56.00	56.00
15	1.50	1.50	149.87	99.913	54.00	54.00
30	0.00	0.00	149.69	99.793	52.50	52.50
60	3.50	3.50	149.95	99.967	55.25	55.25
90	0.25	0.25	149.92	99.947	53.50	53.50
120	0.00	0.00	149.94	99.960	55.00	55.00
180	4.25	4.25	150.00	100.000	56.25	56.25
240	3.50	3.50	149.65	99.767	51.00	51.00
360	4.50	4.50	149.78	99.853	52.50	52.50
1440	1.75	1.75	149.66	99.773	47.00	47.00

4. Conclusions

Biosorption of various metal ions onto *Bacillus* sp. (OGUB 001) was investigated. At 27 °C, pH values of 2.0, 4.5 and 4.5 were determined for Cr^{6+}, Pb^{2+} and Cu^{2+} ions, respectively. Next, the multi-metal ions, consisting of these ions, were prepared. Partial competitive adsorptions of these mixtures were investigated at 27 °C for the pH values of 4.0 and 7.0. In this process, pH affects the distribution coefficient and the adsorption capability of the functional groups on the biosorbent [16]. In our study, it was found that the initial values of pH and the concentration of metal ions were effective on biosorption. It was also determined that 72% of the adsorption capacity was used by lead in competitive adsorptions.

The findings of our laboratory-scale studies are encouraging, and should be applicable to industrial waste waters biosorption.

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