

## Heavy Metal Removal from Aqueous Solution Using Biosurfactants Produced by *Pseudomonas aeruginosa* with Corn Oil as Substrate

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### ABSTRACT

The batch removal of Cu(II), Cd(II) and Pb(II) from individual heavy metal ion aqueous synthetic solution using biosurfactants produced by *Pseudomonas aeruginosa* with corn oil as substrate was investigated. The metal ion removal process of crude preparation biosurfactants (CPB) was established to be dependent on the initial pH and contact time. The optimum metal removal was observed at pH 6.0 of the initial metal solution and 10 min of contact time. The affinity sequence for metal ion removal was Pb(II)>Cd(II)>Cu(II). The removal capacity value of biosurfactant for Cu(II), Cd(II) and Pb(II) from single metal ions solution were 0.169, 0.276 and 0.323 mg/g, respectively. The removal capacity value of biosurfactant for Cu(II), Cd(II) and Pb(II) from multi metal ions solution were 0.064, 0.215 and 0.275 mg/g, respectively. The removal capacity of individual metal ion was diminished by the presence of other metal ions in multi metal ions from synthetic aqueous solution. The removal capacity value of biosurfactant for Cu(II), Cd(II) and Pb(II) from silver industry wastewater were 0.027, 0.055 and 0.291 mg/g, respectively. The results indicated that biosurfactants have potential to be used in the remediation of heavy metals in industrial wastewater.

**Keywords:** removal capacity; biosurfactants; corn oil; heavy metal; *Pseudomonas aeruginosa*

### ABSTRAK

Pengambilan ion logam Cu(II), Cd(II) dan Pb(II) dengan metode batch dari larutan menggunakan biosurfaktan yang diproduksi oleh *Pseudomonas aeruginosa* menggunakan minyak jagung sebagai substrat telah dipelajari. Proses pengambilan ion logam oleh biosurfaktan yang belum dimurnikan tergantung oleh pH awal dan waktu kontak. Pengambilan ion logam berat dalam larutan mempunyai kondisi optimum pada pH awal larutan 6,0 dan waktu kontak 10 menit. Urutan kekuatan pengambilan ion logam berat oleh CPB adalah Pb(II)>Cd(II)>Cu(II). Biosurfaktan mempunyai kapasitas pengambilan untuk logam Cu(II), Cd(II) dan Pb(II) dari larutan logam tunggal adalah berturut-turut 0,169; 0,276; and 0,323 mg/g. Biosurfaktan mempunyai kapasitas pengambilan untuk logam Cu(II), Cd(II) dan Pb(II) dari larutan logam bersaing adalah berturut-turut 0,064; 0,215; and 0.275 mg/g. Kapasitas pengambilan ion logam berat berkurang karena adanya ion logam lain dalam larutan. Pada pengambilan ion logam berat dalam limbah cair pencucian perak diperoleh kapasitas pengambilan untuk Cu(II), Cd(II) dan Pb(II) berturut-turut adalah 0,027; 0,055; dan 0,291 mg/g. Hasil penelitian menunjukkan bahwa biosurfaktan mempunyai potensi untuk digunakan dalam remediasi logam berat dalam limbah cair industri.

**Kata Kunci:** kapasitas penyerapan; biosurfaktan; minyak jagung; logam berat; *Pseudomonas aeruginosa*

### INTRODUCTION

Biosurfactants are amphiphile compounds which consist of hydrophobic and hydrophilic moieties. In comparison to synthetic surfactants, biosurfactants are environmentally friendly since they are less toxic, non-hazardous and biodegradable. Biosurfactants have been

applied for agrochemical solubilization, emulsification, and foaming in food processing and phase dispersion for cosmetic and textiles. Biosurfactants also have potential environmental applications for bioremediation, oil spills control, crude oil transferring and oil recovery enhancement [1-3].

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Research has shown an increasing interest in the application of biosurfactants in remediation technologies to clean up of metals [1,4-5]. The ability of biosurfactants to form complexes with metals is a key factor in their effectiveness in the reduction of heavy metal contaminated. Biosurfactants of anionic nature can also seize the metal ions using electrostatic interactions [6-9]. Juwarkar et al. have investigated that the removal of heavy metals from the soil by di-rhamnolipid biosurfactants produced by *P. aeruginosa* BS2 was in the sequence of  $Cd > Cr > Pb > Cu > Ni$  [10]. Das et al. have studied that biosurfactant produced by marine bacterium could be employed in the treatment of heavy metal containing wastewater because it can chelate heavy metals and develop insoluble precipitation [11].

We have reported that rhamnolipids biosurfactants could be produced by *P. fluorescens* using cassava flour wastewater as media. The biosurfactants exhibited critical micelle concentration (CMC) value of 715 mg/L and lowered the surface tension of the water from 80 mN/m to 59 mN/m [12]. We also have found that soybean oil could be used as a carbon source for rhamnolipids biosurfactants by *P. aeruginosa*. The biosurfactants had the CMC of 860 mg/L and decreased the surface tension of the water from 72 mN/m to 52 mN/m [13]. As an extension of this work, we are interested in the production of biosurfactants by *P. aeruginosa* using corn oil as substrate and the use of these biosurfactants in the removal of heavy metals from contaminated aqueous solution. The objective of this research is finding the alternative way for the reduction of heavy metals using non-hazardous and biodegradable materials to minimize human and environmental exposure of toxic heavy metals. Herein, we report the removal of heavy metal ions from synthetic aqueous solution and silver industrial wastewater using biosurfactants produced by *P. aeruginosa* with corn oil as substrate.

## EXPERIMENTAL SECTION

### Materials

An analytical grade of chemicals from E-Merck were used in this research, whereas corn oil from Sunbeam and palm oil from Bimoli. The strain used throughout this work, *P. aeruginosa* FNCC 0063, was purchased from Pusat Antar Universitas, Universitas Gadjah Mada, Indonesia.

### Instrumentation

Infrared spectra were obtained by a Shimadzu FT-IR-8201 PC Spectrometer. Metal ion concentrations

were measured by Atomic Absorption Spectroscopy (AAS), Shimadzu AA 6650.

### Procedure

#### Media used and growth condition

Nutrient agar media was used to maintain the cultures of bacteria. Biosurfactant production was performed using media composed of nutrient broth (8 g/L), NaCl (5.0 g/L) and 10% (v/v) corn oil. The cultures were incubated at room temperature on a reciprocal rotary shaker (150 rpm) for 4 days.

#### Biosurfactants recovery

Two forms of the biosurfactants were recovered from the culture liquid. Culture liquid of *P. aeruginosa* was centrifuged at 12,000 g for 20 min to obtain culture supernatant, namely crude preparation of biosurfactants (CPB). The extraction technique was applied to the culture supernatant with *n*-hexane and subsequently with chloroform. The extract was evaporated to get biosurfactants free of the solvent. This product was named partially purified of biosurfactants (PPB).

#### Emulsification index (E24) of biosurfactants

E24 of PPB was established by adding 2 mL of oil and the same amount of PPB, mixed by a vortex for 2 min, then left to stand for 24 h. The E24 is presented as a percentage of the height of emulsified layer (mm) divided by the total height of the liquid column (mm).

#### Surface tensions, interfacial tensions and CMC of biosurfactants

The surface tension and interfacial tensions of the PPB were quantified by the capillary rise method at room temperature. The PPB was dissolved in distilled water, and the surface tension of the water was evaluated in different concentrations of the PPB. The CMC value is the concentration at which micelles began to form. This value was obtained when the rapid change in the surface tension was noticed. The CMC was calculated by plotting the surface tension as a function of PPB concentration.

#### Emulsion type of biosurfactants

Conductivity test was used to identify the emulsion type of PPB. A small amount of an electrolyte was added to the emulsions. The emulsion is oil-water type when the conductance increases. Conversely, the emulsion is water-oil type when there are no significant changes in conductance. In this case, 1% (w/w) of sodium chloride was added to the emulsion, and the conductivity was measured.

### Emulsification properties of biosurfactants

The interfacial surface tension and E24 of the PPB using different hydrocarbons were tested. In a screw-capped tube containing 1 mL of distilled water and 1 mL of hydrocarbon was added 0.1 mg of PPB. The interfacial tensions of the emulsions with and without the addition of PPB were measured by the capillary rise method at room temperature. The E24 of the formed emulsions was monitored for 10 days.

### Preparation of metal solutions

The protocol of samples was made by dissolving specified weight of metal nitric salts (copper ( $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ ), cadmium ( $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ) or lead ( $\text{Pb}(\text{NO}_3)_2$ ) in 1 L of 0.1 M  $\text{HNO}_3$  to produce a single metal solution (1000 ppm). A mixture metal solution of concentration of 1000 ppm was formulated by dissolving appropriate amount of  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ ,  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  and  $\text{Pb}(\text{NO}_3)_2$  in 1 L of 0.1 M  $\text{HNO}_3$ . The desired pH metal solutions were modified using 0.1 M NaOH or 0.1 M HCl before the biosurfactant was added.

### Experimental design

Batch technique was performed in these experiments. Eight milliliters of 2.5 ppm heavy metal solution or silver industrial wastewater was mixed with CPB (10 ppm) or PPB (10 ppm) in a flask and stirred with pH adjustment using 0.1 M NaOH or 0.1 M HCl. The samples were kept at room temperature in a shaker at 150 rpm for definite periods of time. The solution was then filtered through Whatman filter paper No. 42. Residual metal ion concentrations were measured by Atomic Absorption Spectroscopy (AAS) in the filtered samples. The average value of the triplicate experiments was exhibited as a percentage of metal removed.

### Calculation

The adsorption capacity ( $q_e$ ) was quantified using eq. 1.

$$q_e = \frac{(C_i - C_f) \times V}{m} \quad (1)$$

where  $q_e$  is the amount of adsorbed ions per mass unit of sorbents (mg/g),  $C_i$  is the metal ions initial concentration (mg/L) and  $C_f$  is the metal ions final concentration (after treatment with PPB or CPB) (mg/L),  $V$  is the aqueous phase volume (L) and  $m$  is the amount of the CPB or PPB used (g).

### Analysis

The results were analyzed statistically, and one-way analysis of variance (ANOVA) was used to examine the differences between groups. Fisher's pairwise comparisons were used to carry out for post-test via statistical package Minitab TM 13 windows. Statistically significant differences were appointed at  $p < 0.05$ .

## RESULT AND DISCUSSION

### Production and Characterization of Biosurfactants

Biosurfactant production was performed using media containing 10% (v/v) of corn oil which was incubated for 4 days. The obtained peaks of PPB are similar to those commonly found in the FT-IR spectra of the rhamnolipids biosurfactants. The amphiphilic surface-active glycolipids usually are produced by *Pseudomonas* spp [14]. Fig. 1 presents the FT-IR spectra of PPB. The strong and broad band at  $3417 \text{ cm}^{-1}$  might be related to the O-H stretching vibration. The -CH stretch was confirmed by the band at  $2854 \text{ cm}^{-1}$ , and the asymmetric stretching vibration of C=O was identified by the band at  $1643 \text{ cm}^{-1}$ . The appearance of O-H stretching absorption at  $2923 \text{ cm}^{-1}$  and C=O stretching at  $1724 \text{ cm}^{-1}$ , confirming the presence of carboxylic acid groups. The weak band at  $1458$  and  $1377 \text{ cm}^{-1}$  are in the absorption range of  $1370\text{--}1470 \text{ cm}^{-1}$  resulting from deformation and bending

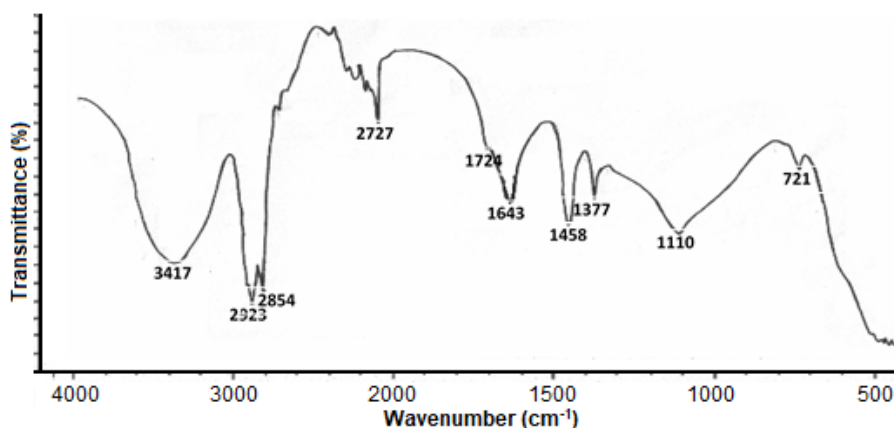


Fig 1. FT-IR spectrum of PPB

vibrations of aliphatic chains ( $-\text{CH}_3-$  and  $\text{CH}_2-$ ). These peaks reflect the presence of alkyl chains in the compounds. The stretching of C-O group was found by the presence of the intense and strong band at  $1110\text{ cm}^{-1}$ . FT-IR analysis showed that biosurfactants possess  $-\text{OH}$  and  $-\text{COOH}$  functional groups which could contribute to binding sites in removing toxic pollutants especially metals from the environment. These functional groups can attract and bind metal ion coordinately, thus form a stable complex resulting sorption of the metals.

These biosurfactants exhibited the CMC value of  $985\text{ mg/L}$  and the surface tension value of  $70\text{ mN/m}$ . The emulsion type of the biosurfactants is oil in water (o/w). The biosurfactant was able to decrease the interfacial tension by about 30% when palm oil and kerosene were used as water-immiscible compounds. The biosurfactant formed stable emulsion until 4 days when toluene, benzene, and kerosene were used as water-immiscible compounds and 8 days when palm oil was used as a water-immiscible compound.

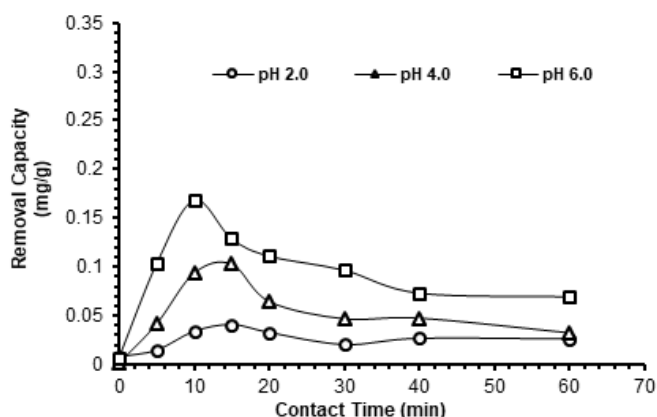
### Heavy Metal Removal from Single Metal Synthetic Solutions

Initially, a study was performed to investigate the removal ability of two forms of the biosurfactants. A crude preparation of biosurfactant (CPB) (10 ppm) and partially purified of biosurfactant (PPB) (10 ppm) was applied for removing of Pb(II) from aqueous solutions. It was observed that the Pb(II) absorption ability of CPB and PPB at pH 6 and contact time 10 min were  $0.316$  and  $0.044\text{ mg/g}$ , respectively. The results demonstrated that the CPB significantly has a much better absorption ability of Pb(II) removals than that of the PPB. This may be as a result of a decrease in the number of biosurfactants functional groups which are responsible for binding of the Pb(II) due to purification. The functional

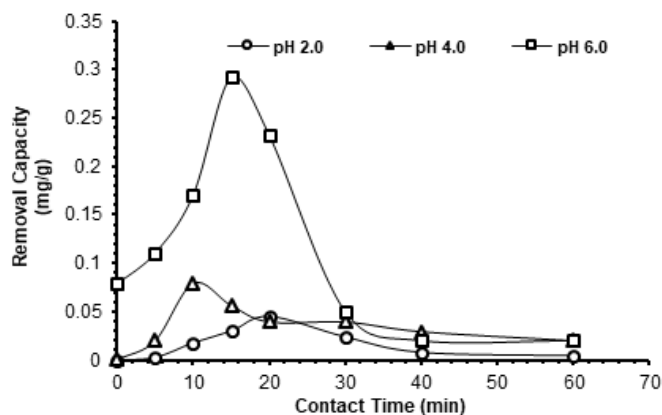
groups (amido, hydroxyl,  $\text{C}=\text{O}$ , and  $\text{C}-\text{O}$ ) have been reported to be involved in the biosorption of Pb(II) on the surface of *A. spherical* [15]. As the binding site decreased, the amount of Pb(II) removed by biosurfactant decreased, following the decrease in the removal capacity. Therefore, all subsequent metal ions removal experiments were examined for the CPB.

A set of experiments was conducted to assess the effects of pH and time of contact on the removal of Cu(II), Cd(II) and Pb(II) ions from the single metal aqueous solutions by CPB. The pH solution can cause changes in the ionic state of the metals and the surface charge of biosurfactants. As a consequent, the pH effects the removal of metal ions from aqueous solutions [16-18]. Since problems with precipitation of tested heavy metals were found to occur at pH above 6.0, a series of removal experiments were carried out at initial pH of 2.0, 4.0, and 6.0 to determine the influence of pH on removal. At pH below 2.0, the high proton concentration reduces the metal sorption, whereas, at pH above 6.0, the metal hydrate may be developed instead of complexation with CPB. Hence the removal process is difficult to accomplish.

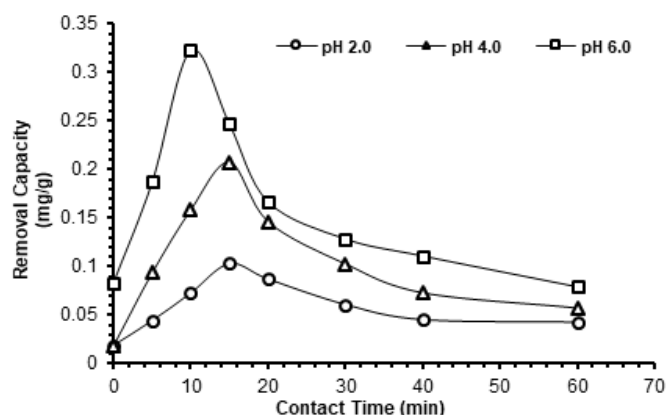
Fig. 2-4 illustrated the effect of initial pH on the removal of Cu(II), Cd(II) and Pb(II) ions by CPB. The number of metal ions adsorbed enhanced with an increase in pH. Significantly high removal capacity took place at the initial of pH 6.0, while low removal capacity took place at an initial pH of 2.0 and 4.0. Almost no removal was noticed at pH 2.0. These findings demonstrated that the presence of excess hydrogen ions at lower pH competed with the metal ions in the solutions and filled the existing binding sites in CPB. Moreover, at low pH, the metal binding sites became positively charged with repelling the Cu(II), Cd(II) and Pb(II) cations. The number of hydrogen ions decreases as the pH of the solution rises, hence causing the removal process more favorable [19-20]. Furthermore,



**Fig 2.** Effect of initial pH and contact time in the removal capacity of CPB for the removal of Cu(II) ion



**Fig 3.** Effect of initial pH and contact time in the removal capacity of CPB for the removal of Cd(II) ion



**Fig 4.** Effect of initial pH and contact time in the removal capacity of CPB for the removal of Pb(II) ion

**Table 1.** Competition among metal ions for time contact of 10 and 15 min

Contact time (min)	Removal capacity (mg/g)		
	Cu(II)	Cd(II)	Pb(II)
10	0.064	0.215	0.275
15	0.061	0.165	0.221

**Table 2.** Removal capacity comparison at pH 6.0 with time contact of 10 min for single metal, mixed metal ions solution and silver industrial wastewater

Solution	Removal capacity (mg/g)		
	Cu(II)	Cd(II)	Pb(II)
Single metal	0.169	0.276	0.323
Mixed metal ions	0.064	0.215	0.275
Silver industrial wastewater	0.027	0.055	0.291

the negative charge density of the biosurfactants increases with an increase in pH, due to the deprotonation of the metal binding sites, which leads increasing to heavy metal ion removal. For all subsequent experiments in this study, the pH of 6.0 was elected since optimum removal was noted and metal precipitation related to higher pH values could be prevented.

The removal capacities of metal ion were calculated as a function of time to determine an applicable contact time between the CPB and heavy metal ions. Removal experiments were examined with a varying contact time of 0, 5, 10, 15, 20, 30, 40, and 60 min. As shown in Fig. 2-4, the removal of metal ions by CPB was rapidly increased in first 10 min for Cu(II) and Pb(II) ions, and 15 min for Cd(II) ion. Conversely, further increase of contact time resulted in a decrease in the removal of heavy metal ions. The initial rapid stage is possibly because of the availability of more binding sites of CPB at the beginning stage of the removal [21-22]. This fast removal at initial phase was possibly due to the initial metal concentration in solution ascent with the number of binding sites available in biosurfactants. The

decrease in removal capacities with further increase in contact time may be as a result of the saturation of the binding sites of CPB with metal ions succeeded by the following removal and desorption processes, and in this case, the desorption process is more predominant over the removal [21,23]. Batch experiments for the competitive metal ions were then carried out at pH 6.0 for two different contact times, e.g., 10 and 15 min.

### Competition among Metal Ions

As metals studied are frequently discovered with some other metals in wastewaters, a series of experiment was performed to examine the binding of each metal ion in the presence of other metal ions. In the mixture metal ions solution, as presented in Table 1, the removal capacities of the metal ions with 10 min of contact time CPB were 0.064 mg/g for Cu(II), 0.0215 mg/g for Cd(II) and 0.275 mg/g for Pb(II). The removal capacities of Cu(II), Cd(II) and Pb(II) from a mixture of the metals ions with 15 min of time contact were found to be 0.061, 0.165 and 0.221 mg/g, respectively. The results exhibited that the removal capacity with 10 min of contact time was significantly higher than that of with 15 min of contact time. Thus, the removal of metal ions for the silver industrial wastewater was conducted at pH 6.0 with 10 min of contact time.

As presented in Table 2, it was noted that the removal capacities of multi metal ions solution are considerably lower than those of the single metal ion solution. Less availability of binding sites may be the reason why the removal capacity of the multi metal ions solution is lower than that of the single metal ion solution [24-25]. Binding sites present in CBP are competitively distributed among the different metal ions present in the mixed metal ions solution.

At silver industrial wastewater, the removal capacities of Cu(II), Cd(II) and Pb(II) with 10 min of time contact were noted to be 0.027, 0.055 and 0.291 mg/g, respectively. It is noticed that the removal capacity of silver industrial wastewater is considerably lower than those of the mixed metal ions solution possibly because of the presence of other metal ions of interest in silver industrial wastewater. Metal ions usually compete with one another for the binding sites, excluding the binding of Pb(II) which are relatively unchanged by other metals [26-27].

It was demonstrated that the metal ion removal capacities in three kinds of solutions are the sequence of Pb(II)>Cd(II)>Cu(II). There are some aspects which are affected the sorbent sorption capacity for metal ions from aqueous solution, e.g., the differences in ionic size, the affinity of metal ions for sorbent active group, electronegativity, nature of metal ion salt and atomic weight [28]. Commonly, the metal ion with larger ionic radius will compete slower for active groups than

those of smaller ionic radius [29]. However, the binding site present in CPB (adsorbents) has a bad affinity for metals with smaller ionic radii. In this study, the typical binding affinities of the CPB for the metals studied are  $\text{Cu(II)} < \text{Cd(II)} < \text{Pb(II)}$ , while the ionic radii of  $\text{Pb(II)}$ ,  $\text{Cd(II)}$  and  $\text{Cu(II)}$  are 1.22, 0.97, and 0.72 Å, respectively. The electronegativity designates the tendency of an atom to attract electrons, and the electronegativity sequence is  $\text{Pb} (2.33) > \text{Cu} (1.90) > \text{Cd} (1.69)$ . Overall, the affinity sequences for the three metals tested did not correlate with the ionic radii and electronegativity of the metals. This trend in the greater ionic size, the greater its affinity was also observed by Babatunde et al. for the removal of  $\text{Cu(II)}$  and  $\text{Zn(II)}$  (ionic radius of  $\text{Zn(II)}$  is 0.74 Å) from aqueous solutions by bamboo root biomass, wherein the sequence of metal ion uptake was  $\text{Zn}^{2+} > \text{Cu}^{2+}$  ions [28]. Similarly, Yasemin and Zeki reported that the sequence of the sawdust of walnut is  $\text{Pb(II)} \cong \text{Cd(II)} > \text{Ni(II)}$  (ionic radius of  $\text{Ni(II)}$  is 0.69 Å) [27].

## CONCLUSION

The rhamnolipids type biosurfactant produced by *P. aeruginosa* with corn oil as the substrate can be utilized for the removal of  $\text{Cu(II)}$ ,  $\text{Cd(II)}$  and  $\text{Pb(II)}$  from the aqueous solution by batch removal method. The removal process was obtained to be dependent on the initial pH and contact time. The affinity sequence of the adsorbent was  $\text{Pb(II)} > \text{Cd(II)} > \text{Cu(II)}$ .

In single metal ions solutions, optimum removal of  $\text{Cu(II)}$  and  $\text{Pb(II)}$  ions by CPB was achieved at pH of 6.0 and contact time of 10 min with removal capacities of 0.169 mg/g and 0.323 mg/g, respectively. Optimum removal of  $\text{Cd(II)}$  ion was obtained at a pH of 6.0 and contact time of 15 min with removal capacity of 0.276 mg/g. For mixture metal ions solutions, the removal capacities of the metal ions at pH 6.0 with 10 min of contact time on CPB were found to be 0.064, 0.0215 and 0.275 mg/g for  $\text{Cu(II)}$ ,  $\text{Cd(II)}$  and  $\text{Pb(II)}$ , respectively. The presence of other metal ions in the aqueous solutions decreased the removal capacity for  $\text{Cu(II)}$ ,  $\text{Cd(II)}$  or  $\text{Pb(II)}$ . In silver industrial wastewater, the removal capacity of  $\text{Cu(II)}$ ,  $\text{Cd(II)}$  and  $\text{Pb(II)}$  on CPB with 10 min of time contacts were established to be 0.027, 0.055 and 0.291 mg/g, respectively. The removal capacity of 0.027, 0.055 and 0.291 mg/g for  $\text{Cu(II)}$ ,  $\text{Cd(II)}$  and  $\text{Pb(II)}$ , respectively, were established for silver industrial wastewater with 10 min of time contact.

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