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Original paper

CHELATING ABILITY OF CRAB SHELL PARTICLES AND EXTRACTED ACETAMIDO GROUPS (CHITIN AND CHITOSAN) FROM *Portunus* sp TO LEAD (Pb^{2+})

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ABSTRACT

Various toxic metal species, including lead, are produced rapidly by industrial activities and fossil fuel consumption. The use of biological component as sorbents for heavy metals might be an alternative method to reduce a heavy metal concentration from various aqueous systems. Here, we used crab shell particles and extracted acetamido component from Portunus sp (chitin and chitosan) to reduce the concentration of lead (II). The crab shell was powdered, sieved, and added with lead (II) in various pH values. The lead (II) solution added to extract of chitin and chitosan was separated, to determine the chelating ability of them. The result showed that the removal efficiency of lead with crab shell depend on pH value, but it was less sensitive than that of the control without crab shell. Biosorption of chitin and chitosan showed different phenomenon. It showed that both chitin and chitosan absorb with the best capacity at pH 4.0. Chitosan has higher sorption than chitin for all treatments.

Keywords: Lead (Pb^{2+}), Chitin, Chitosan, *Portunus* sp, Chelating ability

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INTRODUCTION

Since industrial revolution era, heavy metal waste has been increasing rapidly. Various toxic metal species are produced by industrial activities and fossil fuel consumption. These are accumulated through the food chain, leading to both ecological and health troubles. Various methods for removal of toxic metal species from contaminated environments have been studied based on ion exchange technologies and or precipitation of the cation in an inert form. Those are expensive and still use contaminating products for desorption of metals for cleaning up the organic matrix (Galli *et al*, 2003; Gomes *et al*, 1998; Hernadez *et al*, 1998).

Lead is one of the most dangerous, important, and potential toxic metal in marine ecosystem. The toxicity level may affect growth, and enzyme activity and even respiration of organism. The storage of metals by detoxifying mechanism makes them available for assimilation by the biota and bio-magnification along the aquatic food chains (Gerringa, 1995; Muhaemin, 2004).

Recent studies have focused on the materials improvement by increased affinity, capacity, and selectivity for metals

target (Jianlong *et al*, 2001; Schmuhl *et al*, 2001). The use of biological component as sorbents for heavy metals might be an inexpensive alternative method to reduce the heavy metal concentration from various aqueous systems (Gomes *et al*, 1998; Knorr, 1991).

Chitin and Chitosan are natural, biodegradable sorts of polysaccharide polymer which serve as major structural component of the exoskeleton of crustaceans and insects (Bittelli *et* al, 2001). Acetamido groups (such as chitin and chitosan) can be obtained from fungi, insect, lobsters, shrimp, and even krill; but the most important commercial sources are the exoskeletons of crab (Lee *et al*., 1997). These shells accumulate as waste by products of shellfish processing. Thus the raw product for chitosan is abundant. Chitin and Chitosan (de-acetylated derivative of chitin) are useful for various purposes. The industrial isolation of chitin and chitosan could reduce the problems both seasonal and limited supply and environmental pollution (Strandberg *et al*, 1981; Synowiecki and Al-Khatteb, 1997).

Chitin and chitosan extracted from *Cunninghamella elegans* showed heavy metal sorption significantly, though the metallic biosorption was dependent upon the concentration and pH of metal solutions (Franco *et al*., 2004). The use of another chitin and chitosan sources have been studied to determine the more/most effective biosorbent extracted materials (Bittelli *et al*, 2001; Gordon *et* al, 2000; Lee *et al*, 1997). In this research, we used chitin and chitosan extracted from *Portunus* sp. The research was aimed at determining biosorption of crab shell in different pH level and chelating ability of chitin and chitosan extracted from *Portunus* sp to lead (Pb^{2+}) in aqueous system.

MATERIALS AND METHODS

Sample Collection

The shell of *Portunus* sp, commonly captured on Lampung Bay, was obtained as a waste product of the seafood processing industry. The crab shells were powdered and sieved to pass through a 25 mesh but retained on a 100 mesh screen. To determine the effects of pH, crab shell (0.05 g) was added to each set of 200 ml flasks containing 100 ml of solution with 20 mg Pb l^{-1}. The pH in the flasks was adjusted quickly with $NaOH/HNO_3$ to cover the pH range from 2.0 – 11.0. Solution without crab shells was used as a control.

Extraction of Chitin and Chitosan

The extraction process involved de-proteinization with 2% sodium hydroxide solution (30:1 w/v, 90 ^{0}C, 2 h), separation of alkali-insoluble fraction (AIF) by centrifugation (4000 rpm, 15 min), extraction of chitosan from AIF under reflux (10% v/v acetic acid 40:1 w/v, 60 ^{0}C, 6 h), separation of crude chitin by centrifugation (4000xg, 60 ^{0}C, 6 h) and precipitation of chitosan from the extract at pH 9.0, adjusted with a 4 M NaOH solution. Crude chitin and chitosan were washed on a coarse sintered-glass funnel with distilled water, ethanol, and acetone and air-dried at 20 ^{0}C (Synowiecki and Al-Khatteb, 1997).

Metal Determination

Solution of lead (Pb^{2+}) at concentration 1, 2, 3, and 4 mM was prepared. The pH (3, 4, and 5) was adjusted using 1 N sodium hydroxide or 10% acetic acid. The polysaccharides (chitin and chitosan) were added into the flasks and submitted to orbital shaker at 200 rpm at 25 ^{0}C for 18 h. Chitin and chitosan were removed by filtration through a 0.45 Millipore membrane filter, and the metal concentrations in the filtrates were determined by using an atomic absorption spectrophotometry (AAS). All experiments were done in triplicate. The data were used

to calculate the number of moles of metal ion absorbed per gram of biomaterials. The metal recovery was calculated according to the equation $q = C_i - C_f/m$, where q is metal uptake; C_i is initial concentration; C_f is final concentration; and m is polysaccharides concentration (Volensky and Holan, 1995).

Removal efficiency was determined in the equilibrium experiment to assess the maximum uptake of samples (Lee *et al*., 1997). Removal efficiency of lead for initial pH range was given in percent unit. The maximum uptake of the sample described the highest steady stated value of lead removal (uptake) in the equilibrium condition of reaction.

RESULTS AND DISCUSSION

Effect of pH to Removal Efficiency of Lead

The solubility of calcium carbonate ($CaCO_3$) in the crab shell may vary with the pH solution. According to pH, the dominant carbonate species existed in three different forms H_2CO_3, HCO_3^-, and CO_3^{2-}. Among these, only HCO_3^- and CO_3^{2-} can form insoluble lead precipitates. The research was conducted to determine the optimum range of pH for maximum removal of lead by crab shell (**Fig. 1**). The pH of the solution with crab shell shifted rapidly up to 10 within 20-30 min before reaching a stable value. It is suggested that this phenomenon was a result of dissolution of $CaCO_3$ to CO_3^{2-}

$$\textbf{Crab shell } (CaCO_3\textbf{, chitin/chitosan}) + H_2O \rightarrow \textbf{Crab shell (chitin/chitosan)} + Ca^{2+} + CO_3^{2-}$$

$$CO_3^{2-} + H_2O \rightarrow HCO_3^- + OH^-$$

The removal efficiency of lead with crab shell was depended on pH, but it was less sensitive than that of the control without crab shell. At pH < 4.0 approximately the removal efficiency of lead was negligible in the control. The removal efficiency in the solution of 0.5 g crab shell l^{-1} was about 22 %. It indicated that the acetamido group (chitin and chitosan) acts as a non-specific chelating agent and establishes weak hydrogen bonds with lead in the solution. It has been suggested that the amine nitrogen on each chitin and chitosan monomer unit acts as the active site for metal ion coordination (Tsezos, 1983; Tsezos and Volensky, 1982).

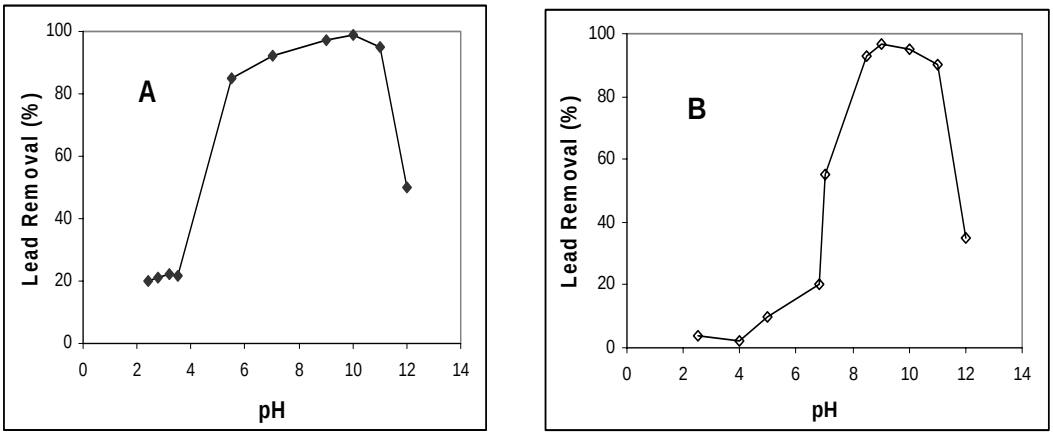

Fig. 1. Removal efficiency of lead for initial Pb concentration (20 mg Pb/l) at various pH values. (A) With 0.5 g crab shell/l; (B) without crab shell.

The removal efficiency of crab shell decreased rapidly in the solution with 0.5 g crab shell l^{-1} at pH 5.5 and below. It happened due to the change of HCO_3^- and

CO_3^{2-} to H_2CO_3. Theoretically, H_2CO_3 is a dominant species at pH < 6.3 and lead could not bind as complex molecule with HCO_3^- and CO_3^{2-}. Control showed that the removal efficiency decreased due to the deficiency of OH^- to form precipitates of $Pb(OH)_2$ at pH 8.0 and below. The optimum range of pH for maximum removal with crab shell was expanded 5.5 – 11.0, whereas the control was 8.5 – 11.0. Residual concentrations with 0.5 g crab shell l^{-1} were below 0.1 mg Pb l^{-1} at the optimum range of pH (compared with approximately 1 mg lead carbonate were better than those of lead hydroxide).

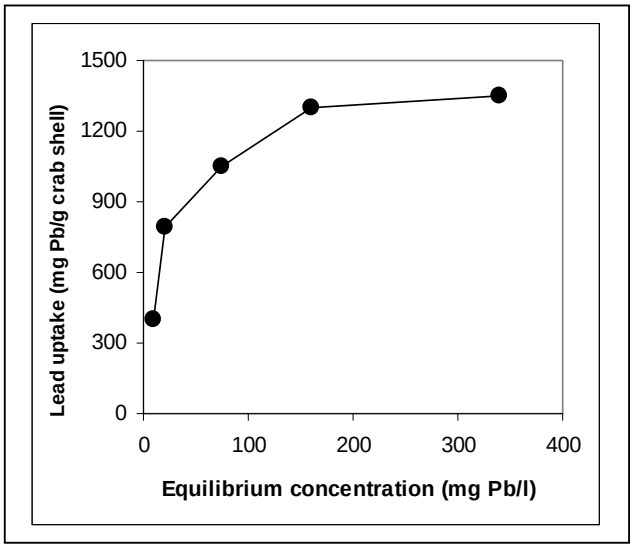

Fig. 2. Maximum uptake of lead by crab shell at ultimate pH 6.

The removal efficiency in the solution dramatically decreased. It may be due to the change from lead carbonate to lead hydroxide at the pH 11.0 and above. At this pH range given, lead hydroxides seemed to complex with hydroxide ions and then to form a soluble species because of the large amounts of hydroxide ions (Lee *et al*, 1987; Snoyink and Jenkins, 1980).

The lead ions would not form insoluble lead complexes suggested:

$$\mathbf{PbCO_{3(s)} + 2OH^- \rightarrow Pb(OH)_{2(s)} + CO_3^{2-}}$$

$$\mathbf{Pb(OH)_{2(s)} + OH^- \rightarrow Pb(OH)_3}$$

Control showed that removal efficiency also decreased rapidly due to the hydroxide ions (Lee *et al*, 1997; Snoyink and Jenkins, 1980). The optimum pH range for lead removal was expanded by using crab shell compared with the control. Isotherm experiments were carried out to confirm that the raw crab shell was a suitable biosorbent. The equilibrium isotherm showed that crab shell took up lead from aqueous solution up to 1300 mg Pb g^{-1} crab shell at ultimate pH 6.0, approximately (**Fig. 2**).

Biosorption of Chitin and Chitosan

The metal recovery of Pb^{2+} in aqueous solution by chitin and chitosan was observed. The metal concentration in aqueous solution (vary from 1, 2, 3, and 4 mM) and the effect of the pH (3, 4 and 5) were also investigated in order to determine the effect of the parameter on the metal ions removal by polysaccharides. Metallic removal was extremely dependent of pH, metal utilized and concentration of

solutions. The metal recovery values for chitin and chitosan, as well as the influence of the metal concentration and pH. **Table 1** showed that both chitin and chitosan absorb with the best capacity at pH 4.0. Chitosan has higher sorption than chitin for all treatments.

Table 1. Sorption of chitin and chitosan at various pH

Concentration (mM)	Chitin (%)			Chitosan (%)		
	3	4	5	3	4	5
1	38.2	43.1	37.5	47.2	50.3	35.0
2	38.2	45.3	35.2	42.2	52.6	46.0
3	27.5	32.1	28.9	38.3	48.1	27.2
4	16.1	27.3	18.7	29.7	32.4	30.1

Table 1 showed that the recovery by chitin and chitosan decreased with the increasing concentration of Pb^{2+} added in solution. The metal adsorption by chitin and chitosan in aqueous solution was directly influenced by the metal concentration. Metal removal increased with the increase of metal concentration up to 2 mM (Galli *et al*, 2003; Ozer *et al*, 1999). The pH of metal solutions affected the biosorption because it determines metal availability in soluble form for adsorption, and dictates the overall surface charge of the adsorbent (Tobin *et al*, 1994). The result showed clearly that the maximum metal ions adsorption occurred at pH 4 which affected the surface charge of biosorbents and the degree of ionization (Galli *et al*, 2003; Toei and Kohara, 1976).

CONCLUSION

The removal efficiency of lead with crab shell particles depended on pH. It showed that the acetamido group (chitin and chitosan) was non specific chelating agent and establishes weak hidrogen bond with lead. The best adsorption capacity of chitin and chitosan occurred at pH 4,0. Chitosan has higher sorption ability than chitin.

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REFERENCES

Bittelli, M., Flury, M., Campbell, G.S., and Nichols, E.J. 2001. Reduction of transpiration through foliar application of chitosan. *Agricul. Forest Meteorol*. 107: 167-175

Franco, L.O., Maia, R.C.C., Porto, A.L.F., Messias, A.S., Fukushima, K., and Campos-Takaki, G.M. 2004. Heavy metal biosorption by chitin and chitosan isolated from *Cunninghamella elegans* (IFM46109). *Braz. J. Microbiol*. 35: 243-247

Galli, E., Mario, F.Di., Rapana, P., Lorenzoni, P., and Angelini. 2003. Copper biosorption by *Auricularis polytrica*. *Let. Appl. Microbiol*. 37: 133-137

Gerringa, L.J.A., J.W. Rijstenbil, T.C.W. Poortvliet, Van Drie, M.C. Schot. 1995. Speciation of lead (II) and

response of the marine diatom *Ditylum brightwellii* upon inceasing lead (II) concentration. *Aquat. Toxicol*. 30: 77-90

Gomes, N.C.M., Mendonca-Hagler, L.C.S., and Savaidis, I. 1998. Metal bioremediation by micro-organisms. *Rev. Microbiol*. 29: 85-92

Gordon, A.S., Donat, J.R., Kango, R.A., Dyer, B.J., and Stuart, L.M. 2000. Dissolved copper complexing ligands in cultures of marine bacteria and estuarine water. *Mar. Chem.* 70: 149-160

Hernandez, A., Mellado, R.P., and Martinez, J.L. 1998. Metal accumulation and vanadium-induced multidrug resistence by environmental isolates of *Escherichia coli* and *Enterobacter cloacae*. *Appl. Environ. Microbiol*. 1: 4317-4320

Jianlong, W., Xinmin, Z., Decai, D., and Ding, D. 2001. Bioadsorption of lead (II) from aqueous solution by fungal biomass as *Aspergillus niger*. *J. Biotechnol*. 87: 273-277

Knorr, D. 1991. Recovery and utilization of chitin and chitosan in food processing waste management. *Food Technol*. 45: 114-122

Lee, M.Y., Park, J.M., and Yang, J.W. 1997. Micro precipitation of lead on the surface of crab shell particles. *J. Process Biochem.* 32(8): 671-677

Muhaemin, M. 2004. Toxicity and bioaccumulation of lead in *Chlorella* and *Dunaliella*. *J. Coast. Dev*. 8(1): 27-33

Ozer, A., Ozer, D., and Ekiz, H.I. 1999. Application of freudlich and langmuir models to multistage purification process to remove heavy metal ions using *Schizomeris leibleinni*. *Proc. Biochem.* 34: 919-927

Schmuhl, R., Krieg, H.M., and Keizer, K. 2001. Adosrption of Cu (II) and Cr (VI) ions by chitosan: kinetics and equilibrium studies. *Water SA*. 27(1): 1-8

Snoeyink, V.L., and Jenkins, D. 1980. *Water Chemistry*. New York: John Willey.

Stranberg, G.W., Shumate, S.E., and ParrotJr, J.R. 1981. Microbial cells as biosorbents for heavy metals: accumulation of uranium by *Saccharomyces cerevisae* and *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol*. 41: 237-245

Synowiecki, J., and Al-Khatteb, N.A.AQ. 2003. Production, properties, and some new applications of chitin and derivates. *Crit. Rev. Food. Sci. Nutr*. 43(2): 145-171

Tobin, J.W., White, C., and Gadd, G.M. 1994. Metal accumulation by fungi: applications in environmental biotechnology. *J. Ind. Microbiol.* 13: 126-130

Toei, K., and Kohara, T. 1976. *Analyt. Chem. Acta.* New York

Tsezos, M. 1993. The role of chitin in uranium adsorption by *Rhizopus.arrhius*. *Biotech Bioeng*. 25: 2025-2040

Tsezos, M., and Volesky, B. 1982. The mechanism of thorium biosorption by *Rhizopus arrhius*. *Biotech Bioeng*. 24: 955-969

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Volensky, B., and Holan, Z.R. 1995. Biosorption of heavy metals. *Am. Chem. Soc.* American Institute of Chemical Engineers. 235-251