

CHARACTERIZATION OF SORBENT PRODUCED THROUGH IMMOBILIZATION OF HUMIC ACID ON CHITOSAN USING GLUTARALDEHYDE AS CROSS-LINKING AGENT AND Pb(II) ION AS ACTIVE SITE PROTECTOR

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ABSTRACT

Sorbent produced through immobilization of humic acid (HA) on chitosan using glutaraldehyde as cross-linking agent and Pb(II) ions as active site protector has been characterized. Active sorption site of HA was protected by reacting HA with Pb(II) ion, and the protected-HA was then activated by glutaraldehyde, crosslinked onto chitosan, and deprotected by 0.1 M disodium ethylenediamine tetra-acetic acid (Na₂EDTA). The protected-crosslinking method enhanced the content of immobilized-HA and its chemical stability. Based on the FTIR spectra, crosslinking of HA on chitosan probably occurred through a chemical reaction. The sorption capacity of sorbent still remains unchanged after the second regeneration, but some of HA start to be soluble. The latter shows that cross-linking reaction between HA and chitosan is through formation an unstable product. The effectiveness of sorbent regeneration can also be identified by the XRD pattern.

Keywords: immobilization, cross-linking, humic acid, chitosan, sorption

INTRODUCTION

Heavy metals in soil are considered a major environmental problem facing many countries around the world. Heavy metal ions must be removed from wastewater streams of mineral operating industries, because the increasing level of heavy metals in the environment represents a serious threat to human health, living resources and ecological systems [1]. Various treatment technologies have been developed for the removal of these metals from wastewater. To remove of heavy metal ions, sorption by naturally occurring materials is one of the most effective and low cost methods [2]. Among them, humic substance deserves particular attention. Lately, adsorption of metal ions on humic substances of various origins was extensively studied. There were studies of adsorption of metal ions on humic acid extracted from brown coals [2], on peats and their humic acids [3-6], as well as on humic acids extracted from soil [7-8]. Humic acids are highly functionalized carbon-rich biopolymers with a great capacity to bind metal cations [9].

The total acidity values of humic materials are mainly attributed to the sum of the carboxyl and phenolic hydroxyl contents (–OH and –COOH), which are the most active sorption sites responsible for the formation of metal–humic substances complexes, and these groups indicate the cation-exchange and complexing capacities of humic matter. A high total acidity value is indicative of a high cation-exchange capacity and complexing power [10]. Infrared spectroscopy study has confirmed that carboxyl group plays its important role in complexing metal cations [4,11-16].

Humic substances are classified according to their solubility in water. Humic acid (HA) is the component that is insoluble at acidic pH (below pH 2), humin is the fraction that is insoluble at any pH, and finally, fulvic acid is substances that are soluble at any given pH [17]. The solubility of HA in aqueous media depends on the number of –COOH and –OH groups present in HA on a large scale and with increasing content of these groups the solubility increases. Because these groups are the most active sorption sites responsible for the

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formation of metal–HA complexes, so the high solubility of HA in aqueous media is a limiting problem for taking advantage of the interaction ability of HA (as a solid phase) with metal ions. The existence of HA as a solid material can only be maintained at the medium acidity equivalent to pH 2 or lower. Accordingly, it is not advisable to use untreated HA as a sorbent in aqueous media, so an appropriate treatment of HA is needed [18].

Considering the high binding ability of HA for metal cations generally exceeds that of metal oxides, bentonite, and active carbon, it is interesting to immobilize of humic acid to obtain an insoluble humic-like sorbent [19]. Several approaches for immobilization of HA have been suggested [20-26]. Unfortunately, these immobilization methods gave an implication on reducing the most active sorption site of HA because of reducing the number of carboxylic group as the most active sorption site during immobilization process.

Klavin et al. [27] have suggested that the most probable structures in HS that can be used for their immobilization, according to hypothetical structural models, are functional groups such as phenolic hydroxyl groups, carboxyl groups, and amino groups in the peripheral part of the humic macromolecules. Based on this structure and the contradiction solubility properties of HA compared to chitosan, it is interesting to immobilize HA on chitosan using glutaraldehyde as a crosslinker. Glutaraldehyde, as an aldehyde molecule, can react with amine groups of chitosan or HA, but cannot react with carboxylic groups of HA. In addition, Ge and Huang [28] have shown that that modification of amine groups of chitosan to carboxylic, through reaction between epichlorohydrin O-crosslinked chitosan and EDTA dianhydride, enhanced the sorption capacity of this polymer toward metal ions significantly. Wan Ngah and Fatinathan [29] have also shown that chitosan-alginate beads possessed sorption capacity toward Cu(II) higher than chitosan beads.

The previous study [30] has shown that HA can be immobilized on chitosan using glutaraldehyde as a crosslinker and Pb(II) as a active site protector. The sorbent produced through this protected-crosslinking method possessed of high sorption capacity toward Pb(II), Cd(II), and Cr(III), i.e. 416.7 mg/g, 332.3 mg/g, and 714.3 mg/g, respectively. The next result [31] has shown that sorption capacity of this sorbent toward Pb(II) was enhanced significantly by improving the deprotection degree of Pb(II) from the sorbent. Although optimization of sorbent to enhance the sorption capacity has been done [31], but characterization and the stability test has not been carried out yet. It is necessary to examine the stabilization of this sorbent because the stability of the sorbent will affect the sorbent regeneration and finally can affect the cost in wastewater treatment. This paper was aimed to characterization of

the sorbent using FTIR spectrophotometer and XRD crystallography. The examination of the stability of the sorbent was also reported.

EXPERIMENTAL SECTION

Sampling and Sample Preparation

Sample of peat soil as source of HA was taken in Gambut District of South Kalimantan, Indonesia. The sample was taken using a Teflon pipe dipped in weak and partly submerged of peat soil. Peat soil trapped inside the Teflon pipe was pouring out and collected in a black plastic bag. Peat soil was air-dried in room condition. Sample of crustacean shell waste as a source of chitosan was collected from traditional market in Banjarbaru, South Kalimantan, Indonesia. The crustacean shell was dried under sun light and then crushed and sieved to the size of 80 mesh.

Materials

All reagents in analytical grade i.e. Pb(II)NO₃, KOH, NaOH, KCl, and disodium ethylenediamine tetra-acetic acid (Na₂EDTA) salt, solutions of HCl, HF, and HNO₃ were obtained from Merck Co Inc. (Germany) and used without further purification.

Instrumentation

Instruments used in this research were glass equipments, electrical oven, hot plate with magnetic stirrer, analytical scale (Metler AE160), and pH Meter (Cyberscan). Spectrophotometer UV-Vis (Kruss-Optonic Germany UV-6500) was used for determination of humic acid concentration, whereas atomic absorption spectrophotometer (Avanta ver. 2.02) was used for measuring Pb(II), Cd(II), and Cr(III) concentrations. Characterization of humic acid, chitosan and the sorbents were conducted by using spectrophotometer FTIR (IR Shimadzu 8201-PC) and Diffractometer XRD (Shimadzu XRD-6000).

Procedure

Extraction of humic acid, chitin, and chitosan

HA was extracted from the peat soil sample according to the procedure of IHSS (International Humic Substances Society) with some modification [32]. Chitin was isolated through deproteination and followed by demineralization processes, and the obtained chitin was transformed to chitosan by common deacetylation method according to the method of No and Meyer [33].

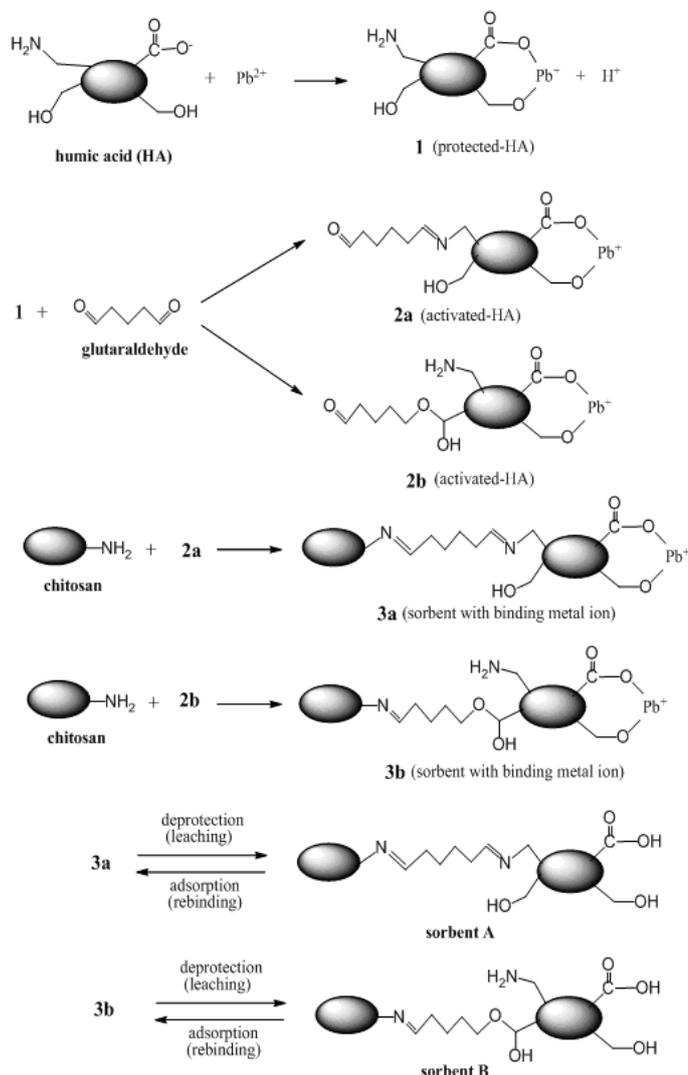


Fig 1. Schematic representation of sorbent preparation

Immobilization of humic acid on chitosan

Detail method of immobilization of HA on chitosan using glutaraldehyde as a crosslinking agent and Pb(II) as a protector ion has been described in our previous paper [30-31]. Hence, immobilization of HA using this method is described briefly. Active sites of HA was protected by reacting of HA with excess Pb(II) ion in aqueous solution before crosslinking of HA on chitosan. About 100 mL of HA solution 20 000 mg/L and 200 mL Pb(II) solution 10 000 mg/L were mixed and stirred for 3 h at room temperature. HA was then activated by addition with 10 mL glutaraldehyde 5% and stirred for 2 h at room temperature in order to obtain an aldehyde-HA derivate. Crosslinking reaction was performed by reacting an aldehyde derivate of protected-HA with 100 mL chitosan 2% (w/v). Reaction was carried out at room temperature for 24 h. The mixed suspension was filtered, and the bound-ion protector in the sorbent was

deprotected by 0.1 M Na_2EDTA solution at room temperature, stirred magnetically for 24 h. The mixture suspension was filtered and the Pb(II) concentration in the leaching filtrate was analyzed using atomic absorption spectrophotometer (AAS). The precipitated residue was washed with water until neutral. The obtained sorbent was dried in oven at 70 °C until constant weight. This deprotection process was repeated several times until the metal concentration in the leaching filtrate no longer change significantly. The obtained powder of the hybrid sorbent containing chitosan-HA was then characterized using FTIR spectroscopy.

Determination of total amount of humic acid contained in sorbent

Humic acid contained in 100 mg of each sorbent was dissolved on 50 mL of 0.1 M NaOH. Using standard addition method, the content of HA released from the sorbent to the solution was determined by ultraviolet-visible spectrophotometer at wavelength of 400 nm.

Solubility test of humic acid contained in sorbent

Five hundred mg of sorbent was added to 50 mL of distilled water and the acidity was adjusted to pH 2, 4, 6, 8, 10, and 12 using either HCl or NaOH solution. The mixture was stirred for 30 min and followed by standing for 24 h. After separating supernatant from the solid, the content of dissolved HA in supernatant was determined spectrophotometrically at 400 nm using standard addition method.

Sorption of Pb(II) onto sorbent

The sorption experiments were carried out by interacting the sorbent with solution of Pb(II) 1000 mg/L for 24 h. After filtering, the concentrations of metal ion in the supernatants were analyzed using AAS. The amount of ion metal sorbed was considered to be the difference between the initial amount and the amount remaining in the reacting solution. The sorption capacity was calculated using the following equation:

$$Q = \frac{(C_0 - C_e)V}{W}$$

where Q represents the sorption capacity (mg/g), C_0 and C_e represent the initial and final concentration of metal ion (mmol/L), V is the volume of metal ion solution (L), and W is the mass of the sorbent.

RESULT AND DISCUSSION

Immobilization of HA on chitosan was carried out by protected-crosslinking method with several steps

(Fig. 1). First step is protection of active sorption site of HA by reacting Pb(II) ion (as protector) with HA in aqueous solution. Second step is activation of protected-HA using glutaraldehyde. Here, it is possible that aldehyde group (from glutaraldehyde) can react either with amine group or alcohol group of HA. Thus, it is possible that there are two type of activated-HA can be formed (**2a** and **2b**). Third step is crosslinking of activated-HA with chitosan to produce **3a** and **3b**. The final step is deprotection of the sorbent by releasing of metal ion from the sorbent using 0.1 M Na₂EDTA.

The total amount and stability of humic acid contained in sorbent

The immobilization method performed in this study produced the content of HA on the sorbent as much as of 301.18 mg of HA/g chitosan for the sorbent obtained by entrapment method, and 997.21 mg of HA/g chitosan for the sorbent obtained by protected-crosslinking method (Table 1). These contents of HA were then used as a base to calculate the stability of immobilized HA on those support materials at various medium acidities. The stability of HA contained in certain sorbent was calculated by comparing the eluted amount of HA in each medium acidity relative to its total amount in that sorbent.

The immobilization of HA on chitosan by using protected-crosslinking method greatly improved the stability of HA toward dissolution (Table 2). Without protected-crosslinking method, HA was gradually dissolved from about 3% to 7% at pH 2 to 8, and then dissolved sharply from about 10% to 31% from pH 10 to 12. On the contrary, after protected-crosslinking, HA was stable at pH 2 to 12 at solubility value of lower than 0.5% (Table 2).

Characterization of sorbent

FTIR analysis was initially conducted for HA, chitosan, and sorbents (with and without active site protection) prior to sorption in order to characterize the available functional groups. Fig. 2(a) - a spectrum of pure chitosan shows the broad peak at 3448 cm⁻¹ is caused by amine N-H symmetrical vibration. Another broad peak at 1034 indicates the C-O stretching vibration in chitosan. Peak at 2800 cm⁻¹ is the typical C-H stretch vibrations. Strong absorption peaks at 1639

and 1323 cm⁻¹, which are characteristic of chitosan and have been reported as amine and amide III peaks, respectively [34-35]. The sharp peaks at 1381 and 1423 cm⁻¹ were assigned to the CH₃ symmetrical and unsymmetrical deformation mode, respectively [34]. Spectrum of the sample shows a pronounced band at 2345 cm⁻¹. This band has been assigned to CO₂. The presence of bands due to CO₂ at about 2350 cm⁻¹ is evident in spectrum of the heated HA examined by Piccolo and Stevenson [13].

Table 1. The content of immobilized-HA on the sorbent obtained by entrapment and protected crosslinking method

Type of sorbent preparation	% w/w	mg/g
Entrapment method	30.10	301.18
Protected-crosslinking method	99.72	997.21

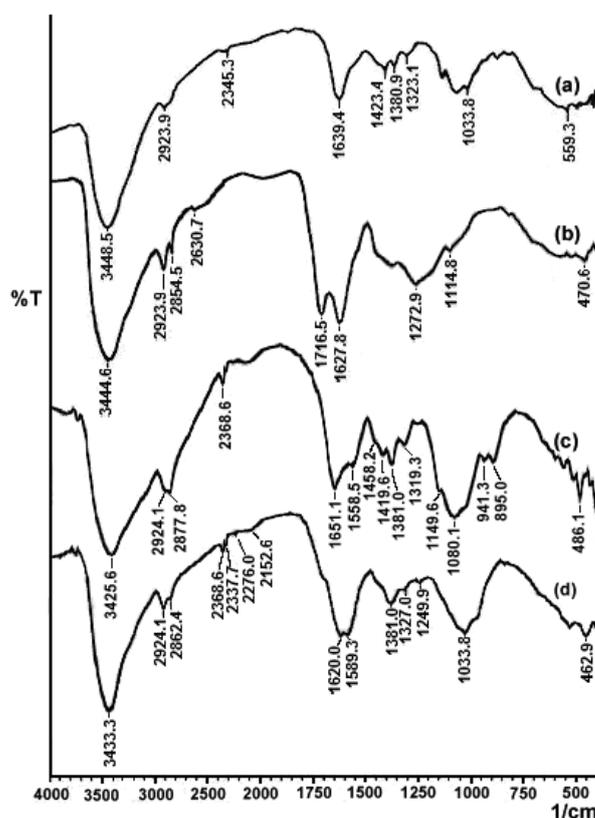


Fig 2. FTIR spectra of chitosan (a), humic acid (b), and sorbent without active site protection (c), and sorbent with active site protection (d)

Table 2. Solubility of HA on the each sorbent (% w/w) at various medium pH

Type of sorbent preparation	pH of medium					
	2	4	6	8	10	12
Entrapment method	3.47	4.01	4.86	6.88	10.19	31.39
Protected-crosslinking method	0.07	0.11	0.14	0.25	0.29	0.42

Infrared spectrum of the HA is shown in Fig. 2(b). Major absorption bands occur at 3445 cm^{-1} (primarily O-H stretching), 2631 cm^{-1} (strongly H-bonded OH of COOH), 1716 cm^{-1} (C=O stretching of COOH and ketones), 1628 cm^{-1} (aromatic C=C, strongly H-bonded C=O, C=C conjugated with C=O, or a combination of these), and 1273 cm^{-1} (C-O stretching and OH deformation of COOH). The shoulder at 2924 and 2854 cm^{-1} can be assigned to aliphatic C-H stretching; the one at 1115 cm^{-1} to OH deformation and C-O stretching of phenolic and alcoholic OH groups.

FTIR spectrum of the HA-chitosan sorbent hybrid obtained by crosslinking method without using Pb(II) as protector ion is shown in Fig. 2(c). The immobilization of HA on chitosan resulted small but remarkable modification on the FTIR spectrum of chitosan. A new peak at around 1420 cm^{-1} is attributed to the bending vibration of $-\text{NH}_3^+$ groups of chitosan [36,29]. The new peak appears at around 1558 cm^{-1} can be assigned to a symmetric deformation of NH_3^+ [34]. Another new peak appeared at 1651 cm^{-1} can be assigned to the characteristic $-\text{COO}^-$ symmetric stretching vibration of carboxylate anion [37-38]. It suggests that the amine groups of chitosan reacted with the carboxylic groups of HA electro-statically. It can be occurred because carboxylic groups of HA as an acid can release protons (H^+) and convert to COO^- anions, whereas amine groups of chitosan as a base can accept protons and convert to cations [39].

FTIR spectrum of the HA-chitosan sorbent hybrid obtained from protected-crosslinking method using Pb(II) as protector ions is shown in Fig. 2(d). The immobilization of HA on chitosan also resulted small but remarkable modification on the FTIR spectrum of chitosan. Before reacting with HA, the spectra of chitosan is lack of absorption bands at about $1250\text{-}1260\text{ cm}^{-1}$ (C-O stretching and OH deformation of COOH), but after reacting with HA, this absorption band appeared. In the case of FTIR spectrum of HA, it resulted the unappearance of absorption band at about 1115 cm^{-1} that was attributed to the OH deformation and C-O stretching of phenolic and alcoholic OH. The disappearance of the 1115 cm^{-1} band can be occurred probably because hydroxyl groups of HA was reacted with glutaraldehyde. Hence, crosslinking between HA and chitosan can be considered occur chemically.

Chemical reaction between HA and chitosan using glutaraldehyde as a crosslinker probably can occur in two ways. First, amine groups of chitosan react with glutaraldehyde by the Schiff base formation and hydroxyl groups of HA react with glutaraldehyde by the hemiacetal or acetal formation. Second, aldehyde groups of glutaraldehyde react with both amine groups of chitosan and amine groups of HA by the Schiff base formation. From these FTIR spectra (Fig. 2(d)), Schiff

base formation is verified by the 1589 and 1568 cm^{-1} bands associated with the imine (C=N) groups [37]. Acetal or hemiacetal bridge formation can be verified by the disappearance of the 1115 cm^{-1} band. In addition, the formation of the crosslinked structure was also confirmed by the absorption peak at 1381 cm^{-1} . This 1381 cm^{-1} absorption is caused by the CH_2 deformation vibration, because the number of the CH_2 groups was increased with proceeding the crosslinked reaction (with glutaraldehyde). Tanuma et al. [40] showed that the intensity at 1380 cm^{-1} , was due to CH_2 deformation vibration, increased with the content of diepoxyPEG in the PEG-crosslinked chitosan hydrogel films while those of the chitosan and the chitosan/PEG blend film did not increase. Thus, there are two type of sorbent can be formed in this experiment, i.e. sorbent A and sorbent B, as shown in Fig. 1.

Based on the disappearance of absorption band at 1716 cm^{-1} in Fig. 2(c), but the remaining of absorption band at about $1250\text{-}1260\text{ cm}^{-1}$, it can be concluded that some of carboxylic groups of HA have been converted to COO^- . According to Piccolo and Stevenson [13], conversion of COOH to COO^- led to the expected loss of absorption band at about 1700 cm^{-1} (C=O of COOH) and 1200 cm^{-1} (C-O stretching and OH deformation of COOH). As addition, conversion of COOH to COO^- can also occur because of interaction between carboxylic groups and Pb^{2+} during protection process. Furthermore, the another result [41] showed that, on the sample of HA adsorbed physically on crosslinked-chitosan epichlorohydrin beads, about 50.3% HA could be desorbed by 0.1 M HCl at contact time 180 min. However, sorbent resulted in this research shows no loss of HA during deprotection sorbent by 0.1 M HCl at a long period of contact time 24 h. It indicates that in sorbent resulted in this research, most HA was bound chemically on chitosan.

Sorbent regeneration

One of the important characteristics of a sorbent is its ability to be regenerated. A cycle of sorption and desorption study will help to elucidate the nature of sorption process and to reuse the sorbent. For examining stability of sorbent, it was also necessary to evaluate the sorption capacity of sorbent after regenerating several times. Sorption and desorption performances of this sorbent before and after regenerating are shown in Fig. 3.

Fig. 3 shows that after regenerating, sorption capacity toward Pb(II) tend to decrease slowly. It occurs probably because not all of adsorbed Pb(II) ions can be fully desorbed by Na_2EDTA treatment. Furthermore, some immobilized HA start to be soluble at the second regeneration. Solubilization of HA was

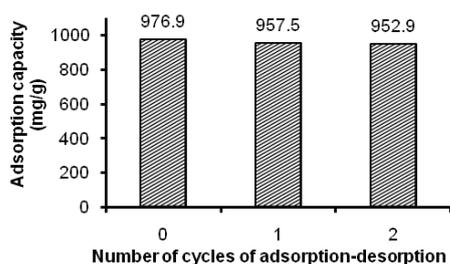


Fig 3. Sorption capacity of sorbent before and after regeneration

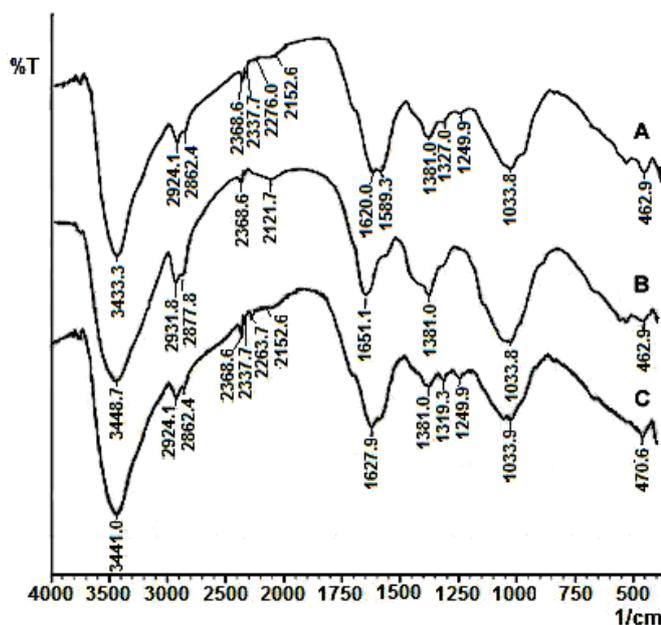
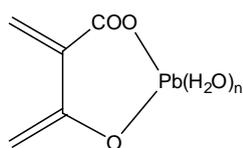


Fig 4. FTIR spectra of sorbent: A. Before adsorbing of Pb(II), B. After adsorbing of Pb(II), and C. After regenerating by using Na₂EDTA



Scheme 1

observed by color of the filtrate to be yellowish after Na₂EDTA treatment at the second regeneration. It indicated that some extend of crosslinking reaction was formed through Pb(II) ions as a crosslinker.

To examine the functional groups of sorbent involving in sorption of Pb(II), the FTIR spectra of sorbent before and after sorption, and after regeneration were also analysis as comparison (Fig. 4). Fig. 4A and 4B show FTIR spectrum of sorbent before and after absorbing Pb²⁺, respectively. Absorption band at 1250 cm⁻¹ in Fig. 4B disappears because carboxylic groups was used for Pb(II) sorption. A increasing band at 1381 cm⁻¹ and disappearance band at 1589 cm⁻¹ are

probably due to imine groups was involved in Pb(II) sorption. Monteiro Jr. and Airoldi [42] have shown that although the primary amine is transformed in an imine, but the final form is also sensible to the sorption of cations. In addition, Piccolo and Stevenson [13] have shown that complex of HA-Pb²⁺ also have sorption band at 1385 cm⁻¹.

The ability of humic acids to form stable complexes with metal ions has been known for a long time and has been attributed to their high content of O-containing functional groups, including carboxylic COOH; phenolic, alcoholic and enolic OH; and ketonic C=O structures of various types. Carboxylate groups are by far the most abundant. But despite the high concentration of COOH groups, it was showed that metal ions are predominantly complexed to humic substances by phenolic OH and carboxylic COOH groups located in ortho position, i.e., by salicylate-type functional groups. Chelation of metals by COOH (benzoate-type complex), two adjacent COOH (phthalate type complex), or OH (ca phthalate type techol-type) groups are also possible but seem to be of a lesser quantitative importance as compared to salicylate [43]. However, although Pb(II) ions have been bonded by HA through carboxylic and hydroxyl groups of HA (as shown in Fig. 1), absorption band at around 3449 cm⁻¹ (O-H stretching) still appear in Fig. 4D. This suggests that part of the absorption at 3449 cm⁻¹ was due to hydration water of the metal in the complex, such as the following (Scheme 1).

The effect of hygroscopic water on absorption band near 3420 cm⁻¹ was investigated by Stevenson and Goh [13] by obtaining spectra on pellets heated at 100 °C for 24 h. Absorption at 3420 cm⁻¹, although somewhat reduced, was still pronounced, thereby confirming the formation of aquocomplexes between the metal and fulvic acid.

Xia et al. [44] have investigated the binding environments of Cu²⁺ and Pb²⁺ complexed by soil humic substances using synchrotron-based X-ray absorption spectroscopy with the assistance of bond network analysis, analysis of X-ray absorption near edge structure (XANES) and radial structure functions derived from extended X-ray absorption fine structure (EXAFS). Their EXAFS study indicates that most Pb²⁺ binding sites in the soil humic substances are 4-coordinate with two O-containing functional ligands. The average distances of Pb-O decrease from 2.46 Å to 2.32 Å when the pH of humic substances increased from 4 to 6, while average Pb...C distances are 3.26 Å in this pH range. They used 4-coordinate Pb[H₂O]₂[RCOO]₂ as bond network model for Pb²⁺ humic complex. Because there was a good match between the FEFF-simulated and the experimental RSF curves, so the models of the binding sites represent

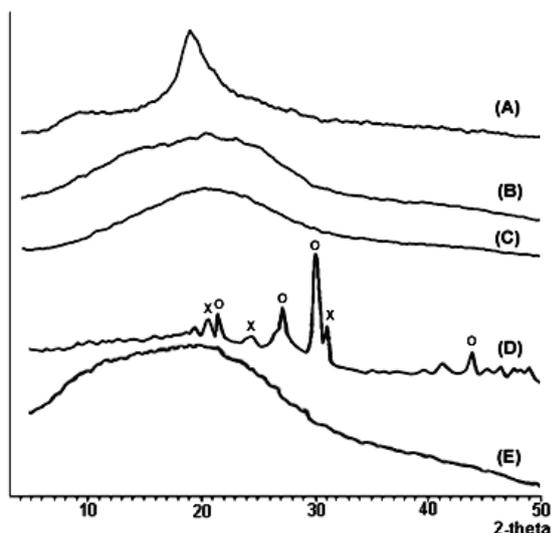


Fig 5. XRD pattern of chitosan (A), humic acid (B), sorbent (C), sorbent-Pb complex (D), sorbent-Pb complex after regeneration with Na_2EDTA (E). $x = \text{Pb}(\text{CH}_3\text{COO})_2 \cdot \text{PbO} \cdot \text{H}_2\text{O}$ (tentative) and $o = \text{Pb}(\text{OH})_2$ (tentative, no indexing available)

the actual metal sites. Their results also predict that although the sample was dried by freeze-drying, Pb in the humic materials they studied are still coordinated by two or more water molecules. This is not surprising in light of the high moisture-absorbing capacity of humic substances.

Fig. 4C shows that after regeneration of the sorbent, releasing Pb(II) from the sorbent cause absorption band at 1250 cm^{-1} appears again. It indicated that reconversion COO^- to COOH has been occurred. This spectrum also showed that absorption band at 1381 cm^{-1} also decreased because of releasing of Pb(II) from imine groups.

Furthermore, for ensuring the effect of Pb(II) ion on structure of sorbent, it is necessary to characterize the sorbent before and after crosslinking, and also before and after regeneration, using XRD crystallography. The result of this characterization is shown in Fig. 5.

Fig. 5(A) shows that chitosan is a polymorphic form, whereas Fig. 5(B) is amorphous form. This result is in accordance with Zhang et al. [45] that chitosan is polymorphic forms occurring as the α -form in shrimp shell wastes. Vissep and Mendel [46] also have shown that HA is amorphous. The diffraction pattern of sorbent obtained by crosslinking between chitosan and HA, Fig. 5(C) also shows an amorphous form. The pattern for sorbent treated with Pb(II) reveal new sharp bands, Fig. 5(D), indicating a new highly crystalline structure. Because no previous data exist for Pb(II)-humic complex, it is necessary to compare this result with known XRD pattern of appropriate lead compounds. The

powder XRD patterns were complicated by the presence of amorphous HA and polymorph of chitosan, but showed a progression of peaks consistent with lead hydroxide, $\text{Pb}(\text{OH})_2$, [47-48] and alkaline lead acetate, $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot \text{PbO} \cdot \text{H}_2\text{O}$ [49]. Lead ions occupy and fixed positions in the sorbent and a chemical bond is established mainly between the sorbent carboxyl groups and the lead ions. Of course there is also a contribution by water molecule (based on FTIR characterization in Fig. 4D). After regeneration, the diffraction pattern of sorbent shows amorphous form, Fig. 5(E) indicates that much of the Pb ions can be released from the sorbent. These results show that XRD pattern can be used to characterization of effectiveness of sorbent regeneration.

CONCLUSION

The present work demonstrated that immobilization of humic acid (HA) on chitosan using glutaraldehyde as cross-linking agent and Pb(II) ions as active site protector can enhance the total amount and stability of HA contained in sorbent. Based on the FTIR spectrum, it can be identified that the crosslinking of HA on chitosan through a chemically reaction. FTIR spectrum also confirmed that sorption of Pb(II) on sorbent occurred through formation of aquocomplexes between the metal and HA. The sorbent obtained by this method is effective enough in order to be used as sorbent for the removal of aqueous Pb(II), Cd(II) and Cr(III) ions. However, after two times of regeneration, HA start to be soluble. The later indicated that the cross-linking reaction between HA and chitosan occurred through forming unstable product like acetal or hemiacetal. The effectiveness of sorbent regeneration can also be identified by the XRD pattern.

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REFERENCES

1. Bailey, S.E., Olin, T.J., Ricka, B.R.M., and Adrian, D.D., 1999, *Water Res.*, 33, 11, 2469–2479.

2. Martyniuk, H., and Wieckowska, J., 2003, *Fuel Process. Technol.*, 84, 1, 23–36.
3. Al-Faqih, L., Johnson, P.D., and Allen, S.J., 2008, *Bioresour. Technol.*, 99, 5, 1394–1402.
4. Fukushima, M., Nakayasu, K., Tanaka, S., and Nakamura, H., 1995, *Anal. Chim. Acta.*, 317, 1-3, 195–206.
5. Jin, X., Bailey, W., Yu, Y., and Lynch, A., 1996, *Soil Sci.*, 161, 8, 509–520.
6. Prado, A.G.S., and Airoidi, C., 2003, *Thermochim. Acta*, 405, 2, 287–292.
7. Manunza, B., Deiana, S., Maddau, V., Gessa, C., and Seeber, R., 1995, *Soil Sci. Soc. Am. J.*, 59, 1570–1574.
8. Buhani, and Suharso, 2006, *Indo. J. Chem.*, 6, 1, 43-46.
9. Cezikova, J., Kozler, J., Madronova, L., Novak, J., and Janos, P., 2001, *React. Funct. Polym.*, 47, 111–118.
10. Tan, K.H., 1994, *Environmental Soil Science*, Dekker, New York, 159–177.
11. Alimin, 2000, *Fractionation of Humic Acid and Its effect on the Solubility of Zn(II), Cd(II), Mg(II), and Ca(II)*, Master Thesis, Universitas Gadjah Mada, Indonesia, 83–85.
12. Fadiawati, N., 2000, *Sorption-Desorption Characteristics of Magnesium(II), Calcium(II), Zinc(II), and Cadmium(II) on Humic Acid and Bentonite as a Model of Peat Soil System*, Master Thesis, Universitas Gadjah Mada, Indonesia, 46–47.
13. Piccolo, G.A., and Stevenson, F.J., 1982, *Geoderma*, 27, 3, 195–208.
14. Sari, L.P., 2000, *Sorption-Desorption Studies of Chromium(III), Cobalt(II), Nickel(II), and Copper(II) on Humic Acid and Bentonite as a Model of Soil System*, Master Thesis, Universitas Gadjah Mada, Indonesia, 51–53.
15. Sudiono, S., 2001, *Acid-Base Properties of Humic Acid and Their Interaction with Chromium(III), Copper(II), Cobalt(II), and Nickel(II)*, Master Thesis, Universitas Gadjah Mada, Indonesia, 66–68.
16. Terkhi, M.C., Taleb, F., Gossart, P., Semmoud, A., and Addou, A., 2008, *J. Photochem. Photobiol., A*, 198, 205–214.
17. Aiken, G.R., McKnight, D.M., Wershaw, R.L., and McCarthy, P., 1985, An introduction to humic substances in soils, sediments and waters, in: G.R. Aiken, D.M. McKnight, R.L. Wershaw, P. McCarthy (Eds.), *Humic Substances in Soil, Sediment, and Water: Geochemistry, Isolation and Characterization*, John Wiley and Sons, New York, 1-9.
18. Gezici, O., Kara, H., Ersöz, M., and Abali, Y., 2005, *J. Colloid Interface Sci.*, 292, 2, 381–391.
19. Santosa, S.J., Tanaka, S., Siswanta, D., Kunarti, E.S., Sudiono, S., and Rahmanto, W.H., 2007, *Indonesian Peat Soil Derived Humic Acid: Its Characterization, Immobilization, and Performance as Metal Sorbent*, Proceeding of International Conference on Chemical Sciences (ICCS-2007), Yogyakarta, Indonesia, 1–6.
20. Seki, H., and Suzuki, A., 1995, *J. Colloid Interface Sci.*, 171, 2, 490–494.
21. Koopal, L.K., Yang, Y., Minnard, A.J., Theunissen, P.L.M., and Van Riemsdijk, W.H., 1988, *Colloids Surf., A*, 14, 385–395.
22. Carey, 2000, *Organic Chemistry*, 4th ed., McGrawHill, Boston, 740–742, 802, 864–868.
23. Yang, Y.H., and Koopal, L.K., 1999, *Colloids Surf., A*, 151, 1-2, 201–212.
24. Santosa, S.J., Siswanta, D., Sudiono, S., and Sehol, M., 2007, *Surf. Sci.*, 601, 2, 5148–5154.
25. Santosa, S.J., Siswanta, D., Sudiono, S., and Utarianingrum, R., 2008, *Appl. Surf. Sci.*, 254, 23, 7846–7850.
26. Irawati, U., Santosa, S.J., and Sudiono, S., 2005, *Imobilization of Humic Acid on Chitosan and Its Application as Sorbent of Cd(II)*, Proceeding of International Seminar on Environmental Chemistry and Toxicology (2nd InSECT), Yogyakarta, Indonesia, 129–134.
27. Klavins, M., and Eglite, L., 2002, *Colloids Surf., A*, 203, 47–54.
28. Ge, H., and Huang, S., 2010, *J. Appl. Polym. Sci.*, 115, 1, 514–519.
29. Wan Ngah, W.S., and Fatinathan, S., 2008, *Chemical Engineering Journal*, 143, 1-3, 62–72.
30. Santoso, U.T., Irawati, U., Umaningrum, D., and Nurmasari, R., 2008, *Indo. J. Chem.*, 8, 2, 177–183.
31. Santoso, U.T., Irawati, U., Umaningrum, D., Nurmasari, R., Santosa, S.J., Siswanta, D., Rusdiarso, B., 2009, *Improvement of Degree of Active Sites Deprotection for Enhancing the Ability of Crosslinked Humic Acid-Chitosan as Sorbent for Pb(II)*, Proceeding of International Seminar on Sains and Technology (ISBN : 978–979–19201–0–0), Yogyakarta, Indonesia, 706–712.
32. Santoso, U.T., and Herdiansyah, 2004, *Indo. J. Chem.*, 4, 1, 12–25.
33. No, H.K., and Meyer, S.P., 1989, *J. Agric. Food Chem.*, 37, 3, 580–583.
34. Costa-Júnior, E.S., Barbosa-Stancioli, E.F., Mansur, A.A.P., Vasconcelos, W.L., and Mansur, H.S., 2009, *Carbohydr. Polym.*, 76, 3, 472–481.
35. Ma, G., Yang, D., Zhou, Y., Xiao, M., Kennedy, J.F., and Nie, J., 2008, *Carbohydr. Polym.*, 74, 1, 121–126.

36. Shiu, J.C., Ho, M.H., Yu, S.H., Chao, A.C., Su, Y.R., Chen, W.J., Chiang, Z.C., and Yang, W.P., 2009, *Carbohydr. Polym.*, 79, 3, 724–730.
37. Silverstein, R.M., Bassler, G.C., and Morrill, T.C., 1991, *Spectrometric identification of organic compound*, John Wiley & Sons Inc., 118, 127.
38. El-Tahlawy, K.F., El-Rafie, S.M., and Aly, A.S., 2006, *Carbohydr. Polym.*, 66, 2, 176–183.
39. Wan Ngah, W.S., and Musa, A., 1998, *J. Appl. Polym. Sci.*, 69, 12, 2305–2310.
40. Tanuma, H., Saito, T., Nishikawa, K., Dong, T., Yazawa, K., and Inoue, Y., 2010, *Carbohydr. Polym.*, 80, 1, 260–265.
41. Wan Ngah, W.S., Hanafiah, M.A.K.M., and Yong, S.S., 2008, *Colloids Surf., B*, 65, 18–24.
42. Monteiro Jr., O.A.C., and Airoidi, C., 1999, *Int. J. Biol. Macromol.*, 26, 2-3, 119–128.
43. Manceau, A., Boisset, M.C, Sarret, G., Hazemann, J.L, Mench, M., Cambier, P., and Prost, R., 1996, *Environ. Sci. Technol.*, 30, 5, 1540–1552.
44. Xia, K., Bleam, W., and Helmke, P.A., 1997, *Geochim. Cosmochim. Acta*, 61, 11, 2211–2221.
45. Zhang, Y., Xue, C., Xue, Y., Gao, R., and Zhang, X., 2005, *Carbohydr. Res.*, 340, 11, 1914–1917.
46. Vissep, S.A., and Mendel, H., 1971, *Soil Biol. Biochem.*, 3, 3, 259–262.
47. Sajadi, S.A.A., Alamolhoda, A.A., and Hashemian, S.J., 2007, *Scientia Iranica*, 14, 2, 169–173.
48. Ponder, S.M., Darab, J.G., and Mallouk, T.E., 2000, *Environ. Sci. Technol.*, 34, 12, 2564–2569.
49. Sajadi, S.A.A., Alamolhoda, A.A., and Hashemian, S.J., 2008, *Scientia Iranica*, 15, 4, 435–439.