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Immobilization of *Nannochloropsis* sp Biomass by Sol-Gel Technique as Adsorbent of Metal Ion Cu(II) from Aqueous Solutions

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This work is related to algae biomass immobilization with sol gel technique using TEOS precursor as silica matrix to adsorb Cu(II) in aqueous solution. The data obtained showed that adsorption processes of Cu(II) are optimum at pH of 5-6, with an interaction time of 1 h. Adsorption capacity of adsorbent from *Nannochloropsis* sp biomass immobilized on silica through sol gel technique is 6-7 times higher than on silica gel. Adsorption capacities of Cu(II) are found to be in the range of 11.38-14.48 kJ/mol contributing as physical interaction.

Key Words: Immobilization, Sol gel, Adsorption, *Nannochloropsis* sp biomass.

INTRODUCTION

An abundance of cleaning, uncontaminated water is a fundamental requirement for human life and environmental protection. As a result of various technological activities in the past including mining, nuclear energy and the burning of fossil fuels, metals ion such as Cu(II), Pb(II) and Cd(II) have been released into the environment at unprecedented levels. Conventional methods applied to remove heavy metals in high concentration from aqueous solutions, include precipitation, ion exchange, evaporation, electroplating and membrane processes. However, these methods have been found to be inefficient or expensive when heavy metals are present in low concentrations and may generate secondary wastes which are difficult to treat[1]. Therefore, it is required to find new technologies or materials for removing heavy metal ions from wastewater.

Biosorption utilizes the ability of certain materials to accumulate heavy metal ions from aqueous solutions by either metabolically mediated or physicochemical path way of uptake[2]. It is an economical and effective method to remove heavy metals from waste water. Marine algae with large available quantities in many regions are a kind of promising biological resources. Many studies showed that the algae possess high metal binding capacities[3,4], due to the presence of polysaccharides, proteins or lipid on the cell wall surface containing functional groups such as amine, hydroxyl, carboxyl and sulfate, which can act as binding sites for metals. The cell wall matrix of green algae contains complex heteropolysaccharides that can provide amine, carboxyl and sulfate groups[5]. Protein can constitute10-70 % of the green algae cell wall[6].

Nannochlropsis sp, a kind of green filamentous microalgae is widely distributed in eutrophic waste water, lagoon and intertidal zones in many parts of the world. Therefore, utilization of the sea affluent resources to treat waste water is of much interest. In this work, it was carried out increasing of adsorption capacity of algae biomass *Nannochlropsis* sp using silica matrix by sol gel technique.

The sol gel technique is very potential because silica matrix can form a cage for molecules, resulting stronger environment for biomolecules[7,8]. Beside that, this method usually used to immobilize protein to endure stability of spectroscopic properties and biology activities[9]. Other researches also showed that sol-gel technique with silica matrix on fungi cells based polysaccharides has surface area bigger than cross-linked technique with epichlorohydrin[10,11].

Immobilization of algae biomass by sol-gel technique uses silica matrix hoped can kept activity of functional groups on biomass to adsorb effectively metal ions, especially heavy metal ions. In addition, the influence of several parameters such as initial pH, time and initial Cu(II) concentration will also be investigated. The adsorption data were obtained from batch experiments. The biosorbent was characterized by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM). For surface area of adsorbent identified by NOVA 1000 version 2.00 use nitrogen adsorption technique. The adsorption capacities were evaluated from equilibrium isotherms adsorption. The results indicated on comparing its adsorption capacity with some of the other adsorbents, that it is the suitable material for the development of high capacity biosorbent for Cu(II) removal.

EXPERIMENTAL

The tetraethoxysilane (TEOS) and ethanol used are commercial products of Merck, Germany. Copper(II) solutions and standards were prepared by using analytical grade copper chloride salt ($CuCl_2.2H_2O$), Merck, Germany. NaOH and HCl (37 %) were purchased from Alba Chemical.

Measurements of pH were made using a pH meter (Orion 4 Star). The copper solution was analyzed using an atomic absorption spectrophotometer (AAS) model Perkin-Elmer 3110. Jeol-T330A was used for a scanning electron microscopy (SEM). BET surface area was measured using a gas sorption analyzer NOVA 1000 version 2.00. Infrared spectra of the adsorbent were recorded on an IR Prestige-21 (Zhimadzu).

Biosorbent: Algae biomass was collected from Balai Budidaya Laut Lampung, Indonesia. It was washed with distilled water to remove dirt and was kept on a filter paper to reduce the water content. The biomass was dried for 3 days followed by drying in an oven at 60 ºC for 12 h and then ground on an agate stone pistol mortar. The biomass was then sieved to select the particles between 100 and 200 mesh sizes for use.

Immobilization of biomass algae with sol gel technique: The composition of the precursor solution listed in Table-1. The biosorbents A, B, C and D were prepared by mixing components with biomass algae. After 0.5 h, a gel pellet obtained which was filtered off, washed with ethanol and water and dried in an oven at 60 ºC for 6 h. The dried green pellet of biosorbents B, C, D and the white silica monolith of A were crushed and sieved. Particles of size 100-200 mesh were collected and used in sorption experiments.

TABLE-1
COMPOSITION OF PRECURSOR SOLUTIONS

Absorbent	Composition of precursor solution (weight ratio)					
	TEOS	Ethanol	H_2O	HCl (0.1 M)	NH_4OH (0.1 M)	Algae (g)
A	2	1	1	0.1	0.1	0.00
B	2	1	1	0.1	0.1	0.16
C	2	0	4	0.1	0.1	0.16
D	2	2	0	0.2	0.2	0.16

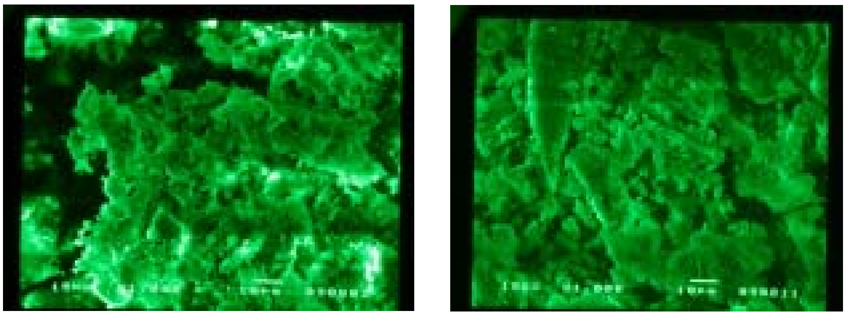
Batch adsorption studies: The adsorption features of the biosorbent were investigated as a function of initial pH, initial heavy metal concentration and contact time. The equilibrium adsorption was obtained from batch experiments, using 250 mL Erlenmeyer flasks containing 100 mL aqueous solution of copper and 0.1 g biosorbent kept at room temperature. The pH value was adjusted to the required value with 0.1 M HCl or 0.1 M NaOH hourly throughout the experiment. A magnetic stirrer was used to agitate the solution continuously. At the end of adsorption, sample was collected and centrifuged at 1500 rpm for 10 min on a centrifuge. The remaining concentration of copper in residual solution was analyzed by taking absorbance on the atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

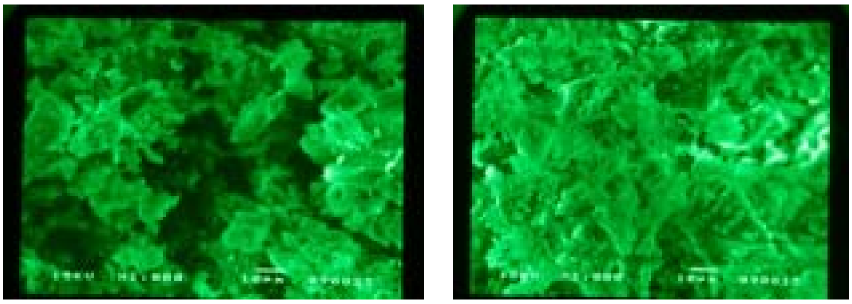
Characterization of adsorbent: The scanning electron micrograph clearly revealed the surface texture and morphology of the adsorbent (Fig. 1). Microscopy showed that silica biosorbent B, C and D are heterogeneous materials by a microporous silica matrix with inclusions of *Nannochloropsis* sp biomass.

The determination of the specific surface area (SBET) based on the adsorption on gaseous nitrogen at several pressures and at 77.40 K. The precursor silica (adsorbent A) gave values of 199, 800 $m^2 g^{-1}$, while the *Nannochloropsis* sp biomass immobilized on silica gel using sol gel technique (B, C and D), 170.577, 121.192 and 51.610 $m^2 g^{-1}$, respectively. The decreasing of the surface area after immobilization is due to the presence of pendant groups, which block the access of molecules of gaseous nitrogen into the structure of the silica.

The infrared spectroscopy (Fig. 2) presents a useful informatons to detect the success of immobilization process. This proposal can be clarified by comparing the precursor and modified surface. The broad symmetric stretching mode assigned to Si-O-Si appeared at 1087 cm^{-1}. The spectrum relating to the precursor silica shows a band at 964 cm^{-1} identified as terminal Si-OH deformation groups on silica surface



Adsorbent A Adsorbent B



Adsorbent C Adsorbent D

Fig. 1. SEM of the silica adsorbent from TEOS precursor (A) and the result of immobilization between *Nannochloropsis* sp biomass and silica using sol gel technique (B, C and D)

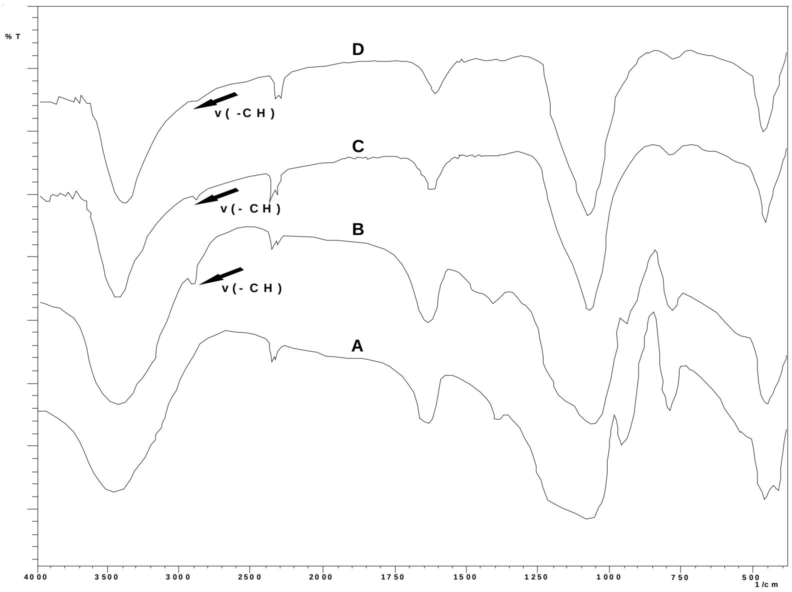


Fig. 2. Infrared spectrum of silica adsorbent from TEOS precursor (A) and the result of immobilization between *Nannochloropsis* sp biomass and silica using sol gel technique (B, C and D)

and another band associated with Si-O is located at 470 cm^{-1}. For three adsorbent (B, C and D) immobilized biomass algae with precursor TEOS, the spectra can be characterized for a partial loss of Si-OH deformation mode originally presented at 964 cm^{-1} and also for the appearance of new band at 2931 ascribed to stretching vibration modes of CH groups.

Effect of pH solution: The effect of pH solution in the adsorption process of metal ion Cu(II) on A, B, C and D adsorbent as shown in Fig. 3, generally seen that the higher pH is the higher metal ion adsorption and it is optimum at pH 5-6 and further it decreased above pH 6.

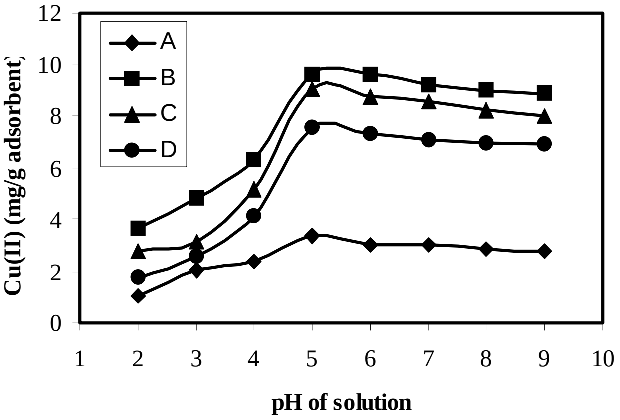


Fig. 3. Effect of pH solutions on adsorption of Cu(II) ion on silica gel (A) adsorbent and *Nannochloropsis* sp biomass immobilized on silica gel using sol gel technique (B, C and D)

At low pH, the adsorption of metal ion Cu(II) is relatively small. This can be explained that in acidic condition, functional group on the adsorbent is protonated and then occurring binding of hydrogen ion (H^+) and hydronium ion (H_3O^+). While, metal ions in the solution before adsorbed on adsorbent, first it is hydrolyzed resulting proton as equation below:

$$M^{2+} + nH_2O \rightleftharpoons [M(OH)_n^{2-n}]^+ + nH^+$$

Hydroxo complexes $[M(OH)_n^{2-n}]^+$ resulted from reaction above will be more adsorbed[12] than free metal cation (M^{2+}). In acidic condition, the equation above will move to left and then amount of metal hydroxo complexes formed is lower than amount of free metal cation. In the acidic condition also, an adsorbent surface has positive charge, therefore it will occur repulsion between adsorbent surface and metal ion resulting low adsorption.

At pH 5-6, the adsorption is relatively high, this case can be happened because metal hydroxo complexes (MOH^+) formed in solution are much and also adsorbent surface will be negative charge with releasing proton, so that the electrostatic force will attract each other causing adsorption increasing.

At pH = 7, the adsorption starts to decrease, this happens because at this pH, metal ion Cu(II) starts to be hydrolyzed to form a precipitating metal hydroxide species[13,14]. Beside that, at the high pH, adsorbent surface has negative charge, this causes repulsion between adsorbent surface and metal ion species and then adsorption will be decrease.

Interaction time: From the Fig. 4, it can be seen that interaction between Cu(II) ion on silica adsorbent and *Nannochloropsis* sp biomass immobilized on silica *via* sol gel technique (A, B, C and D) shows adsorption process increasing with adding interaction time. Interaction between metal ion and adsorbent is optimum at around 1 h and with increasing interaction time up to 2 h, the adsorption capacity does not increase sharply for each adsorbent.

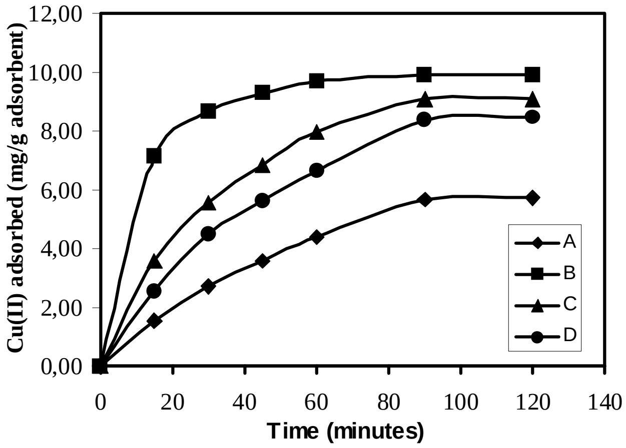


Fig. 4. Effect of interaction time on adsorption of Cu(II) ion on silica adsorbent (A) and *Nannochloropsis* sp biomass immobilized on silica gel *via* sol gel technique (B, C and D)

Adsorption rate and rate constants of metal ion on biomass can be obtain from non-linear kinetic adsorption explained by Langmuir-Hinshelwood[15] determining a zero and first-orders rate constants with assumption that the adsorption runs quickly and followed by slow reaction from an adsorbate. The equations of these are:

$$A + S \longrightarrow (A \times S) \quad (1)$$

$$-rA = \frac{\delta CA}{\delta t} = \frac{k_1 CA}{1 + KCA} \quad (2)$$

where K = Langmuir constants (M^{-1}); k_1 = first-order reaction rate constant at LH (min^{-1}) model; rA = rate constants (min^{-1}); CA = metal concentration in solution (M); t = time (min); A = metal ion; S = Adsorbent; A×S = metal ion concentration adsorbed on adsorbent.

With separating variable and integrating equation above, it can be obtained equation:

$$-\ln\frac{(\mathrm{Co}/\mathrm{CA})}{\mathrm{Co}-\mathrm{CA}}+\mathrm{K}=\frac{\mathrm{k}_1\mathrm{t}}{\mathrm{Co}-\mathrm{CA}} \qquad (3)$$

By plotting [ln (Co/CA/(Co-CA) *vs.* t/(Co-CA)], it will be obtained a straight line, where slope and intercept for each is k_1 (rate constants) tan K (Langmuir constants) as listed in Table-2.

TABLE-2
PLOT RESULT OF LH KINETICS ON THE ADSORPTION PROCESS OF Cu(II) ON *Nannochloropsis* sp BIOMASS IMMOBILIZED ON SILICA GEL *via* SOL GEL TECHNIQUE

Adsorbent	Cu(II)		
	R^2	K (mol/L)	k_1 (min^{-1})
A	0.988	32.800	9.493
B	0.980	239.800	51.399
C	0.995	270.000	25.963
D	0.989	49.000	19.288

Capacity and adsorption energy: Langmuir adsorption isotherm model is usually used to describe relationship between adsorbate activity and amount of sample adsorbed at constants temperature. From the study based on this adsorption isotherm model, it is hoped that it can result positive K value for all adsorbents, so that the energy and adsorption capacity can be determined.

Fig. 5 shows adsorption of Cu(II) ion on adsorbent A, B, C and D with various initial concentration of metal ion. Fig. 5 generally shows that adsorption process occurs on layer (monolayer). This also shows that adsorption model follows Langmuir adsorption isotherm which has assumption; maximum adsorption occurs at all active cites of adsorbents filled by adsorbate forming monolayer. Therefore, the data obtained in Fig. 5 can be applied into the Langmuir equation to determine capacity and adsorption energy.

Capacity and adsorption energy of Cu(II) ion was determined on the basis of Langmuir adsorption isotherm model[16] using the equation:

$$C/n = 1/n_mK + C/n_m \qquad (4)$$

where n = the amount of metal adsorbed per gram adsorbent at C concentration (mol/g); K = the equilibrium constant (adsorption affinity) $(mol/L)^{-1}$; C = free metal ion concentration in equilibrium (mol/L); n_m = the amount of metal adsorbed at supersaturated (maximum adsorption capacity) (mol/g).

By plotting C/n *versus* C, a straight line is obtained K, *i.e.*, the maximum capacity adsorption and nm can be determined from intercept and slope. In addition, the value of adsorption energy was determined based on Gibbs free energy equation.

$$\text{Adsorption energy} = \Delta G^o \text{ ads} = - R\ T \ln K \qquad (5)$$

K is the adsorption equilibrium constants obtained from Langmuir equations and adsorption of total energy is equal to Gibb free energy change at standard conditions[17].

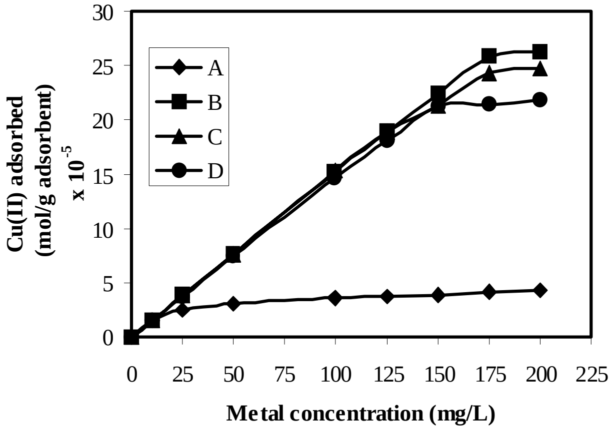


Fig. 5. Adsorption isotherm of Cu(II) ion on silica adsorbent (A) and *Nannochloropsis* sp biomass immobilized on silica gel using sol gel technique (B, C and D)

TABLE-3
LANGMUIR PARAMETRES OF ADSORPTION Cu(II) ION ON SILICA GEL AND *Nannochloropsis* sp BIOMASS IMMOBILIZED ON SILICA *via* SOL GEL TECHNIQUE

Absorbent	R^2	nm		K	E(kJ/mol)
		mg/g	mol/g × 10^{-5}		
A	0.991	2.708	4.261	96.660	-11.386
B	0.997	17.900	28.169	295.096	-14.185
C	0.984	16.378	25.773	331.908	-14.478
D	0.995	14.508	22.831	240.263	-13.672

Table-3 observed that generally adsorption capacity of adsorbent from *Nannochloropsis* sp biomass immobilized on silica *via* sol gel technique (adsorbent B, C and D) is greater around 6-7 times than silica gel. This means that Cu(II) adsorbed at each gram *Nannochloropsis* sp biomass adsorbent immobilized on silica *via* sol gel technique is higher than on silica adsorbent.

Silica gel obtained from TEOS precursor can adsorb divalent metal ion because there are functional groups of silanol (≡SiOH) and siloxane ((≡Si-O-Si≡). They are not effective because of low oxygen ability (silanol and siloxane) as electron-pair donor causing low bond of metal ion on silica surface. Low oxygen ability as (silanol and siloxane) as electron-pair donor is consequence of oxygen bounded directly on Si atom in silica structure (it is possible to occur a partial double bond between O and Si through dπ-pπ bond).

But, after immobilization application of *Nannochloropsis* sp biomass with silica *via* sol gel technique, adsorption capacity of Cu(II) as listed in Table-3, increases. This shows that there is immobilization of algae biomass adding type and amount of active cites participating at metal ion adsorption. Beside, silanol and siloxane group as on silica gel, there is also new active group derived from *Nannochloropsis* sp biomass as group: -COOH, -OH, C=O, NH and $-CONH_2$[14,18].

Increasing adsorption capacity of *Nannochloropsis* sp biomass occurs because sol gel technique is more simple and quick. This happens because bonding reaction of the active groups runs at the same process to form solid. In addition, the reaction can be done at room temperature so it can be used simple instrument. The advantage of sol gel technique is in producing mechanically stable material and porous material. In addition, based on other research results, this technique is potential to produce stronger environment for biomolecule because silica matrix can form cage for biomolecule. This method is also usually used to immobilize protein which has spectral properties and stable biology activity[9]. Other research also showed that sol gel technique with silica matrix on fungi cells derived from polysaccharide has bigger surface area than cross-linked technique with epichlorohidrine[10,11]. Therefore, the immobilization of algae biomass with the sol gel technique using silica matrix can keep activity of functional groups on biomass to be more effective and selective on adsorbing metal ion Cu(II). Furthermore, it is observed that adsorption energy of metal ion Cu(II) on silica gel and *Nannochloropsis* sp biomass immobilized on silica *via* sol gel technique has nearly the same value at a range of 11.38-14.48 kJ/mol. According to Stum and Morgan[19], chemical bond energy is usually higher than 40 kJ/mol such as energy for covalent bond and electrostatic interaction. While induction energy is lower than 10 kJ/mol. Adsorption energy indicated how strong bond between metal ions and adsorbent active cites. For four adsorbents (A, B, C and D) the adsorption energy to divalent ion Cu(II) is still grouped low. The value of this adsorption energy is too low to be identified as chemical bond energy, but it is too high if observed as physical induction.

Conclusion

Immobilization of algae biomass with sol gel technique using silica matrix can keep the activity of functional groups on biomass to be more effective on adsorbing heavy metal ions, especially heavy metal ion of Cu(II). Adsorption capacity of adsorbent from *Nannochloropsis* sp biomass immobilized on silica *via* sol gel technique (adsorbent B, C and D) was higher at around 6-7 times than an adsorption capacity of silica gel. Increasing of adsorption capacity of *Nannochloropsis* sp biomass occurs because sol gel technique is more simple and quick. This happens because bond reaction of active groups runs at the same time of solid forming process.

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