



BENEFICIAL EFFECTS OF CYSTEINE IN MODIFICATION OF CHITOSAN IN HANDLING MERCURY WASTE

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The present study put an emphasis on the handling of mercury waste by using modified chitosan which obtained from shrimp shell as waste of frozen shrimp. The main aim of the study was to find out the effectiveness of cysteine as modifier to chitosan and its application for adsorption of mercury metal at water bodies. The characterisation study of adsorbent, chitosan-cysteine was done using FT-IR and SEM-EDX, whereas maximum adsorption limit was determined by AAS. The effects of parameters like pH, contact time, maximum adsorption capacity was studied at isothermal condition, on the adsorption process under study. Mechanism of adsorption process was studied according to adsorption kinetic model based under pseudo-first order and pseudo-second order, whereas adsorption isothermal was determined based on Langmuir and Freundlich isotherms. The results of the study indicate that the increase of cysteine amount in the synthetic process has no significant effect on the acquisition of % yield. Adsorption of synthetic results were determined with FTIR, SEM-EDX. The optimum condition of metal ion Hg (II) adsorption by chitosan-cysteine at pH 5.5 with time of adsorption four hours. Kinetics model of adsorption follows pseudo-second order. Data on isothermal adsorption follows Langmuir isothermal model with maximum adsorption capacity to metal ion Hg (II) is 4.99 mmol/g, higher than that of earlier results that is 2.41 mmol g⁻¹.

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Introduction

Shrimp is a reliable commodity of Indonesia in fishing industry which is normally exported in frozen conditions. The shrimp production potential in Indonesia increasing year by year. The national shrimp production in 2011 was 412.000 tons, 1 out of which 288.000 tons (about 70%) are exported frozen shrimp and 12.950 tons (about 5%) exported to South Sulawesi. This gives the national shrimp export figures in last year.

During the process of shrimp freezing, about 25% of the shrimp go waste (skin and head) therefore it is assumed that last year waste produced was 70.000 tons nationally. Such amount of waste should be handled properly to avoid the negative effects on the environment. Since the use of shrimp shell waste is limited only to produce shrimp paste and cattle feed for ducks. Basically the waste has high economic value due to the presence of high percentage of chitin compound (about 20-30%) which also depends upon the types of species.² One of its uses are as biosorbent to mercury metal as pollutant, which is toxic in nature to the environment.

Several researchers have done modification to chitosan to enhance its mercury adsorption capacities. Among others are oxiran Crosslinked Cd- Complexed Chitosan (2.6 mmol/g),³ chitosan from lobster shell (2.14 mmol/g)⁴ and aminated chitosan bead (2.26 mmol/g).⁵ Modification to chitosan by adding cross binding agent is relatively expensive and in general it is toxic, therefore, it is the need of time to improve the ability of chitosan as adsorbent of heavy metal.

In this study L-Cysteine is used as modifier for improving the absorption capacity of Chitosan to mercury metal Hg (II), by making derivative of chitosan, as chitosan-Cysteine. The existence of amine (-NH₂) and thiol (-SH) of Cysteine put into chitosan derivative having higher adsorption capacity than chitosan alone. It can function as universal adsorbent to adsorb metal, both soft and hard according to Pearson classification.⁶

The aim of the study was to find out the effectiveness of Cysteine as modifier to chitosan and its application to adsorb mercury metal in water environment. The effects of parameters like pH, contact time, maximum adsorption capacity was studied at isothermal condition, on the adsorption process under study. The adsorption process mechanism was studied based on kinetic model of adsorption based on pseudo-first-order and pseudo-second order, whereas isothermal adsorption was determined based on Langmuir and Freundlich isotherms.

Materials and Methods

The materials used in this study were chitosan from Sigma (DD-80%), L-cystein, tetrahydrofuran, NaOH, HCl, HNO₃, sulfate acid, and acetic acid, KI, KIO₃, HgCl₂ and Na₂S₂O₃.

Preparation of cysteine-chitosan adsorbent

Five (5) grams of L-cysteine was dissolved in 50 mL of tetrahydrofuran and sulfuric acid as catalyst, then added to 5 grams of chitosan which was dissolved in acetate 1% with stirring. The amount chitosan and cysteine varied at a weight ratio of 1:1, 1:2, 1:3, and 1:4 respectively. Synthesis process was followed for 48 hours at a temperature of 50⁰C and 80⁰C with stirring by a magnetic stirrer. The resulting product is neutralized with aquabidest, filtered and washed until neutral. Then it washed with ethanol to eliminate the excess L-cysteine, and then filtered and dried in a freezing

drier. The resultant product obtained in the form of adsorbent stored in dry and dark containers avoid light and moisture for the further characterized by FTIR and SEM-EDS and for the study of Hg (II) adsorption.

Measurement of sulfhydryl group content

The content of the sulfhydryl group was determined by iodimetry. The procedure is as follows: 0.25 g cysteine-chitosan was taken in a 50-mL iodine flask and then add 15 mL distilled water, 2.5 mL acetic acid, and 0.5 g potassium iodide. When potassium iodide was completely dissolved, 10 mL potassium iodate (0.003 mol/L) was added. The flask was then put in ice water for 5 min. Then the I₂ produced was titrated with 0.03 mol/L sodium thiosulfate as a volumetric solution until the yellow color disappeared. The content of the sulfhydryl group was calculated by the following equation (1) and compared with elemental data analysis, EDX.

$$\text{SH}(\%) = \frac{6 \times 10 - \frac{M_2 V_2}{M_1} M_1 \times 33.07}{W \times 10} \quad \dots \quad (1)$$

where M_1 , M_2 are the concentration of KIO₃ and Na₂S₂O₃, respectively (mol L⁻¹); V_2 is the volume of Na₂S₂O₃ (mL); and W is the weight of cysteine-chitosan (g).

Adsorption Studies

Effect of pH

100 mL of metal ion solution Hg²⁺ at pH 4-8, 1.0 g cysteine-chitosan adsorbent each and stirred at room temperature for maximum 2 hours. Then keep it at rest for 1 hour, then filter with Whatman 42 filter paper. The concentration of metal ion before and after adsorption was determined by AAS. Each experiment was repeated twice. The control was done as earlier except without adsorbent and without stirring.

Effect of contact time

100 mL of metal ion Hg²⁺ plus cysteine-chitosan adsorbent and stirred at room temperature for 2-8 hours. Then keep it at rest for 1 hour, then filter it with Whatman 42 filter paper. The concentration of metal ion before and after adsorption was determined by AAS. Each experiment was repeated twice. The control was done as earlier except without adsorbent and without stirring.

Adsorption capacity

Solution of metal ion Hg²⁺ with concentration 50-1000 ppm was prepared at optimum pH. Put into each 100 ml of the solution 1 g cysteine-chitosan adsorbent and stirred at room temperature for an optimum time. Then let it left for 1 hour, then filter it with Whatman 42 filter paper. Concentration of metal ion before and after adsorption was determined by ASS. Each experiment was repeated twice.. The control was done as earlier except without adsorbent and without stirring. The adsorption capacity was calculated by the equation:

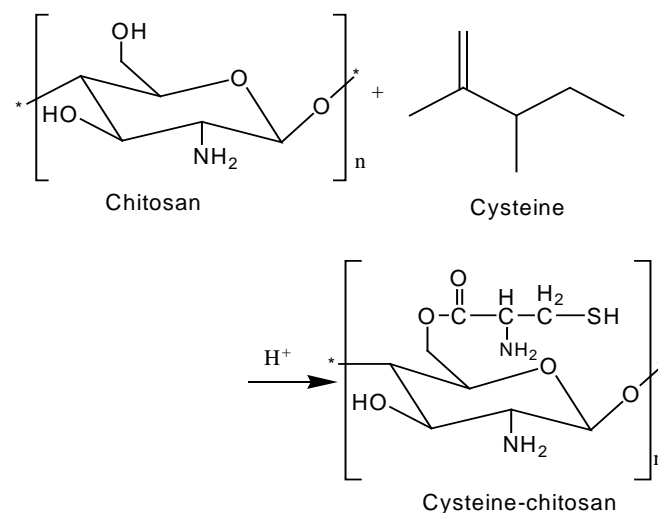
$$Q = \frac{V(C_0 - C_e)}{W} \quad (2)$$

in which V is volume of metal ion solution, W is weight of adsorbent (g), C_0 is concentration of metal ion before adsorption (mg/L), C_e is concentration of metal ion after adsorption (mg/L), Q is capacity of adsorption (mg/g).

Result and Discussion

Stage of Adsorbent Cystein-Chitosan Making

Adsorbent chitosan modified derivative, cysteine-chitosan was done by the reaction of chitosan and cysteine at the ratio 1: 2; 1: 3; 1: 4, whereas the effect of temperature was observed at 50°C and 80°C for 24 hours. The variation of comparison between chitosan and cysteine was meant to do a reaction of cysteine with chitosan with the amount of surplus cysteine so that to balance reaction to the formation of product, namely chitosan-cysteine (Scheme 1).



Scheme 1. The formation reaction of cysteine-chitosan

The percentage yield from cysteine-chitosan product of synthetic result are given in Fig.1, whereas sulphhydrylcontent in adsorbent cysteine-chitosan product was determined by iodometry titration method are in Fig.2. The increase of cysteine amount in synthesis process did not give significant result with the acquisition % yield (Fig.1). The result of the study indicate that the comparison 1:1 saturation reaction is achieved so that with similar condition process, the increase of cysteine amount in reaction given a significant effect in the acquisition of cysteine-chitosan adsorbent product. Therefore, the effect of temperature was also studied in this study, because in order to increase the reaction rate, greater energy is needed. However the result obtained shows that the increase of temperature up to 80°C, did not increase the acquisition of % yield even it tends to decrease (Fig. 2). This is due to temperature above 50°C can cause degradation at chitosan biopolymer, so that the % yield decreased.

The result of this study which is the result of analysis sulphhydrylcontent in chitosan-cysteine adsorbent of synthesis showing that the increase of temperature in the

process of cysteine-chitosan adsorbent tends to decrease the sulphhydryl content in the product (Fig.2).

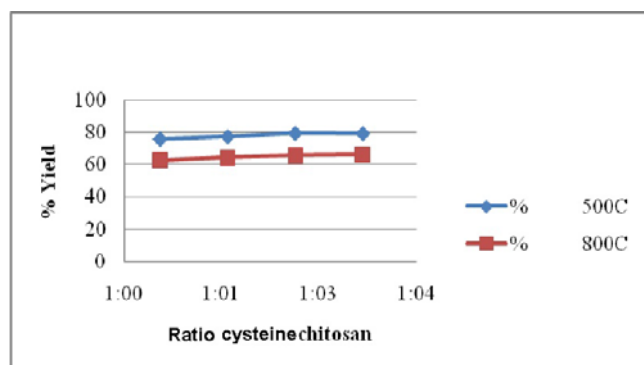


Figure 1. Effect of temperature reaction toward yield cysteine-chitosan

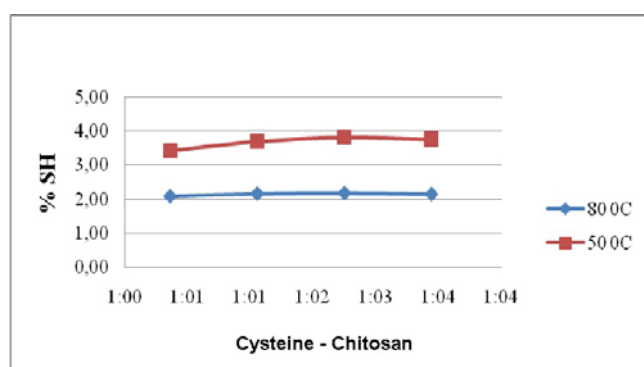


Figure 2. Content of sulfhydryl groups in cysteine-chitosan

Characterization of Chitosan-Cystain Adsorbent

Fourier Transformed infrared spectroscopy (FTIR)

Data on FTIR spectra can be used to identify functions in cysteine-chitosan adsorbent of synthetic result. The characteristic peaks for chitosan FTIR spectra is the peak at $3429\text{--}3442\text{ cm}^{-1}$ in the form of sharp band and width showing the existence of -OH stretching, weak adsorption at $2.881,65\text{ cm}^{-1}$ and $2.922,16\text{ cm}^{-1}$ indicating the existence of -CH stretching 1.649 cm^{-1} 1.597 cm^{-1} due to amida I and amida II at 1153 cm^{-1} indicating stretching of -CN ; 1080 cm^{-1} indicating stretching -C-O .

The FTIR spectra of cysteine-chitosan adsorbent of synthetic result where new band appears at 2.590 cm^{-1} indicating the existence of -SH , carbonyl ester (-CO) appears at the area 1622 cm^{-1} ; adsorption -CN appears at $1.296\text{--}1.408\text{ cm}^{-1}$; adsorption -CS appears at $1.114\text{--}1.134\text{ cm}^{-1}$.

Scanning Electron Microscope-Energy Dispersive Spectroscopy (SEM-EDX)

Samples of morphology and topography tested with SEM at Working Distance (WD) 7 mm with HV 20 kV shows property (Fig.3) as follows: chitosan-cysteine sample of synthetic result has homogenous, smooth and thin surface. At the magnification of 20.000 times several cylindrical pores were found with smaller size from $1\mu\text{m}$.

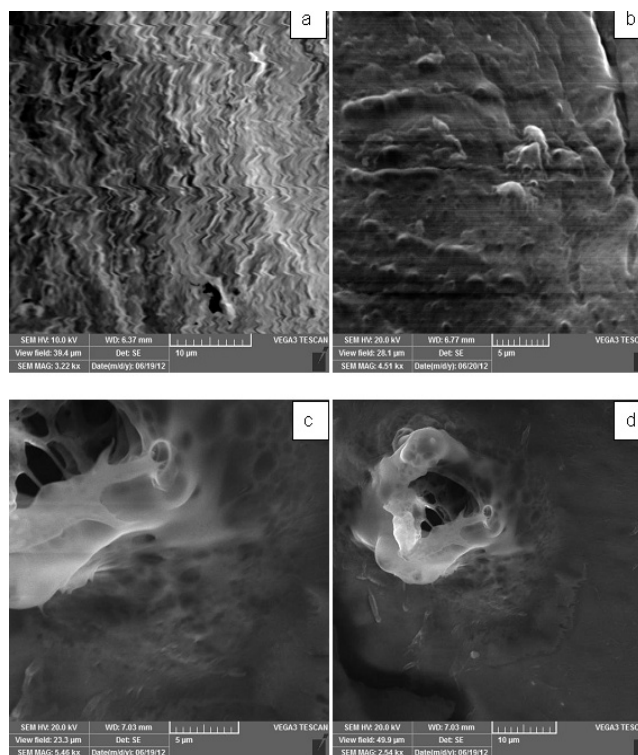


Figure 3. Micrograf SEM of chitosan (a, b) and cysteine-chitosan (c,d)

Chitosan sample has homogenous rough surface, curved and unfixed pores. The elemental analysis done with Energy Dispersive Spectroscopy (EDS) with excitation source of X-ray at HV 20kV with WD 15 mm. At chitosan-Cysteine adsorbent of synthetic result, C,O,N and S components were found, whereas at chitosan only C,O and N components were found. The EDS chitosan-Cysteine spectrum and chitosan is shown in Fig. 3 and in the supplementary material.

Study of Adsorption Optimum Condition and kinetics of Cysteine-Chitosan Adsorption toward Metal Ion Hg(II)

Effect of pH

The level of acidity (pH) of solution is an important parameter fully affecting the metal biosorption, surface charge of adsorbent, ionization level and speciation of adsorbate.

In this study the optimization of adsorption process (Hg(II)) was varied with pH metal solution at pH 4-8 with early concentration, contact time and constant temperature.

The increase pH increases the amount of metal Hg(II) adsorbed until it reached to an optimum condition at pH 5.5 for chitosan-cysteine adsorption and pH 6.0 for chitosan, then decreased above the optimum pH. This illustrates that the effectiveness of pure ion Hg(II) adsorption process by adsorbent was fully affected by pH of the solution. The denial of electrostatic property between adsorbent active sites of positive charge with metal ion Hg(II) adsorbate of positive charge at pH causing the ability of adsorption low below the optimum pH condition.

This is also caused by competition between metal ion Hg (II) and ion H⁺ in competing for active adsorption sites, so that its adsorption capacity becomes weaker and weaker. The increase of the pH decreases the protonation level of adsorbent functional groups and the partial positive charges, which increases the adsorption, however, above to this pH value, the adsorbed Hg(II) starts to settle as Hg(OH)₂ precipitated due to reaching the equilibrium hydroxide ion concentration determined by the K_{sp} value. This hampered the interaction of adsorbate and adsorbent causing the adsorption capacity decreased toward mercury metal.⁷

Effect of contact time

The result of the effect of contact time on the amount of metal ion Hg (II) adsorbed by chitosan and cysteine-chitosan is shown in Fig. 5.

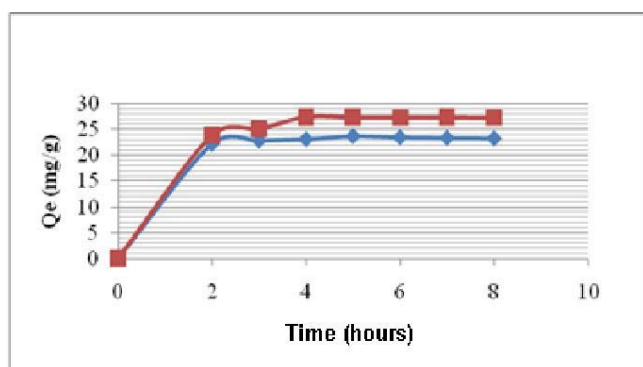


Figure 5. Effect of contact time at adsorption of ions Hg (II) onto cysteine-chitosan (CC) and chitosan

Fig. 5 shows that the increase of time, amount of ion adsorbed until the balance reached in which the amount of ion adsorbed tends to be constant due to the adsorbent surface has been saturated by metal ion. The results of the study indicate that optimum time of metal ion Hg (II) adsorption by chitosan is 5 hours and for cysteine-chitosan 4 hours. The balance adsorption of metal ion Hg(II) by chitosan-Cysteine adsorbent is quicker to be achieved compared with chitosan. The availability of greater functions at chitosan-Cysteine adsorbent needed for interaction with metal ion, to increase the adsorption capacity significantly and the adsorption process completes rapidly (Scheme 2). The time for adsorption balance is one of the important parameters for the economic method of waste water management.

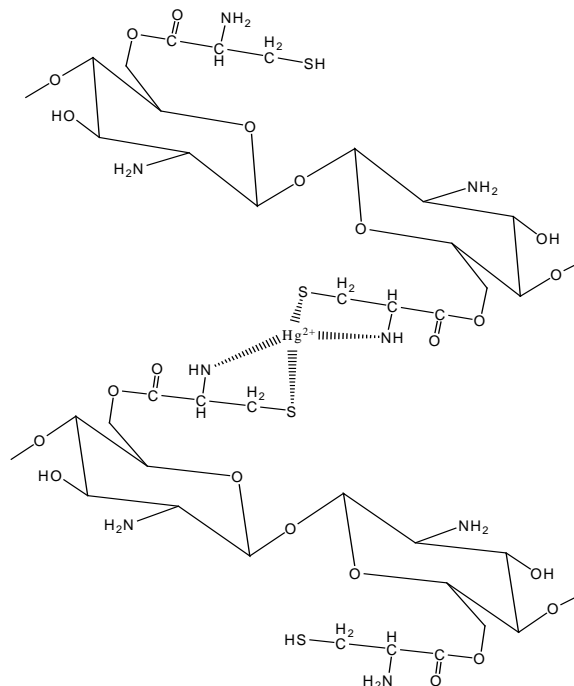
Kinetic model of adsorption based on as pseudo-first order (equation 3) and pseudo-second order (equation 4) is as follows:

$$\lg(q_e - q_t) = \lg \frac{k_1}{2.303} t q_e \quad (3)$$

$$\frac{t}{q_e} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad (4)$$

These equations are used to analyze mechanism of adsorption comprising movement of mass and chemical reaction process.

Information on kinetics of metal adsorption is needed to choose optimum condition for the process of metal ion adsorption of batch scale.



Scheme 2. The mechanism of binding of metal ions Hg (II) onto chitosan-cysteine..

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The linear form of the quasi first-order model for the adsorption of ion Hg (II) at chitosan and chitosan-cysteine has shown in (Fig. 6).

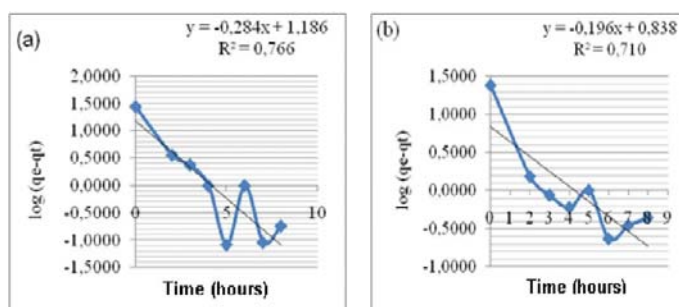


Figure 6. Pseudo-first order sorption kinetics of Hg(II) onto (a) Cysteine-Chitosan (b) Chitosan

The results of calculation based on equation of quasi order one indicate that coefficient correlation value is low for both chitosan and cysteine-chitosan. Besides that q_e value obtained by this method is different significantly with the value of experiment, so that the reaction cannot be classified as pseudo-first order. The parameter of kinetics adsorption based on similarity of pseudo-first order and pseudo-second order is shown in Table 1. The linear form of pseudo-second order is shown in Fig. 7.

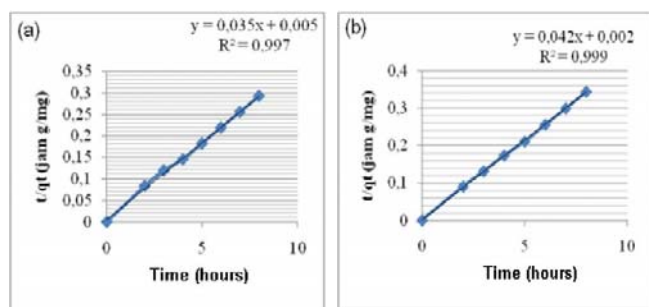


Figure 7. Pseudo-second order sorption kinetics of Hg(II) onto (a) cysteine-chitosan (b) chitosan

The results of the calculation of the data indicate the kinetic adsorption of Hg (II) follows pseudo-second order marked by the value of coefficient correlation (R^2) close to one for chitosan-Cysteine. Besides that the q_e value obtained by this method is not different significantly with the experimental value. This shows the occurrence of chemisorption involving the attracted power of metal atomic valence and functions through complexity of cysteine-chitosan with metal ion Hg (II). Several studies of kinetic adsorption of metal ion Hg (II) at various adsorbents have reported higher correlation for kinetics model of pseudo-second order.⁸

Isothermal Adsorption of Metal Ion Hg(II) by Cysteine-Chitosan

Study of isothermal adsorption is important to determine the effectiveness of an adsorption process. In this study adsorption models of Langmuir and Freundlich were used to determine adsorption model between metal ion Hg (II) and adsorbent. Isothermal constants for both models obtained by linear regression calculation based on equation (4) and (7) is shown in Fig. 8, Table 2 and Fig. 9.

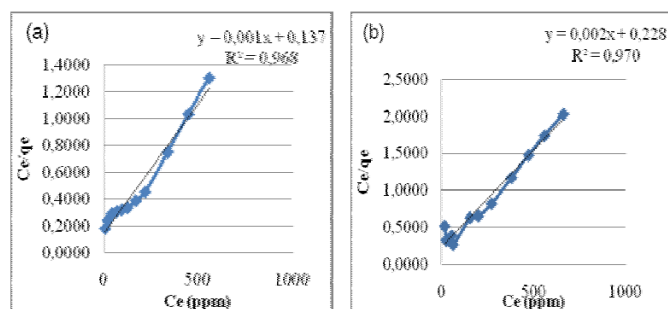


Figure 8. Langmuir isotherm for the adsorption Hg(II) ions on: (a) cysteine-chitosan (b) chitosan

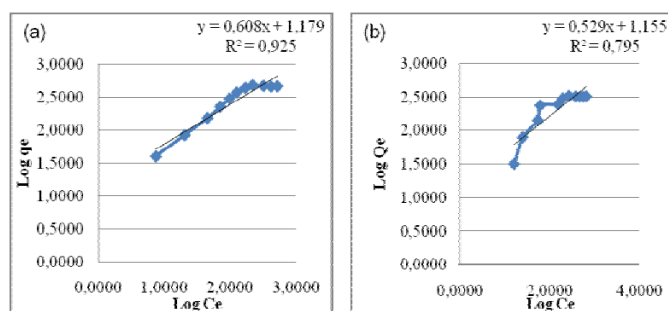


Figure 9. Freundlich Isotherm for the adsorption Hg(II) ions on: (a) cysteine-chitosan (b) chitosan

Langmuir model assumes that adsorption of metal ion occurs at homogenous surface with monolayers adsorption without reaction between adsorbed ion. In order to get balance data, early concentration of metal ion was varied, whereas adsorbent mass in each sample was made constant. The Langmuir isotherm equation is as follows:

$$\frac{c_e}{q_e} = \frac{bc_e}{K_L} + \frac{1}{K_L} \quad (5)$$

$$q_{\max} = \frac{K_L}{b} \quad (6)$$

The calculation result indicates a better correlation with Langmuir isotherm model with the value of R^2 close to 1, both for adsorbent chitosan-Cysteine ($R^2 = 0.972$) and chitosan ($R^2 = 0.970$). The closeness between adsorbate and adsorbent can be predicted by using Langmuir parameter that is separation factor, R_L :

$$R_L = \frac{1}{1 + bC_0} \quad (7)$$

in which C_0 is early concentration of metal ion and b is constant of Langmuir isotherm. $R_L > 1$ is not beneficial, $R_L = 1$ linear, $0 < R_L < 1$ is beneficial, $R_L = 0$ Irreversible.

This indicates the balance of isotherm is very compatible with Langmuir model and adsorption process is monolayer adsorption to the surface of adsorbent with the amount of identical limited sites, distributed homogeneously through the surface of adsorbent.

Freundlich isotherm model with the assumption that the adsorption of monolayer and process of adsorption occur at the heterogenous surface with adsorption capacity and concentration of Hg (II) at balance. The Freundlich isotherm equation is as follows:

$$\lg q_e = \lg K_F + \frac{1}{n} \lg C_e \quad (8)$$

K_F is relative adsorption of capacity and n is size of characteristic and strength of the adsorption process and distribution of active sites. The result of calculation shows low correlation with Freundlich isotherm model indicated by the value of $R^2 < 0.99$, both for chitosan-Cysteine adsorbent ($R^2 = 0.925$) and chitosan ($R^2 = 0.795$). The value of n based on mathematical calculations between 1 and 10 indicates effective adsorption.⁹

Table 1. Kinetic parameters obtained from Lagrangian models in the adsorption of Hg (II) onto cysteine-chitosan (CC) and chitosan ($C_0 = 30$ ppm, $w_{ads} = 0.1$ g, volume = 0.1 L)

Adsorbent	Pseudo-first order				Pseudo-second-order			
	$K_1(h^{-1})$	$q_e (mg g^{-1})$		R^2	$K_2 (g mg^{-1} h^{-1})$	$q_e (mg/g)$		R^2
		Calcd.	Exp.			Calcd.	Exp.	
CC	0,6541	15,3462	27,5417	0,766	0,245	28,5714	27,5417	0,997
Chitosan	0,4514	6,8865	23,7667	0,710	0,882	23,8095	23,7667	0,999

Table 2. Adsorption equilibrium constants obtained from Langmuir and Freundlich isotherms in the adsorption of Hg(II) onto cysteine-chitosan (CC) chitosan

Adsorbent	Langmuir model						Freundlich model			
	$q_{max} (mg g^{-1})$		$K_L, dm^3 g^{-1}$	B, $dm^3 mg^{-1}$	R_L	R^2	$q_{max}, mg g^{-1}$	n	K_F	R^2
	Calcd.	Exp.								
CC	1000,0068	483,5414	6,4103	0,0064	0,3184	0,972	813,1152	1,6447	15,1008	0,925
Chitosan	500	331,4083	4,3859	0,0088	0,2068	0,970	422,1502	1,8904	14,2889	0,795

Conclusion

The results of this study indicate that the effectiveness of cysteine as modifier to chitosan is at the comparison of mol 1:3. pH solution of metal ion and time of adsorption fully affect the adsorption capacity of cysteine-chitosan. The balance of metal ion Hg (II) adsorption by cysteine-chitosan is quicker than chitosan that is 4 hours at pH 5.5. Based on the data of the findings, adsorption of metal ion Hg(II) by cysteine-chitosan follows kinetic model of pseudo-second order and Langmuir adsorption isotherm with the capacity of maximum adsorption is 4.99 mmol g^{-1} .

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