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Biosorption of Acid Dyes by Non-living Aquatic Macrophyte, *Hydrilla verticillata*

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ABSTRACT

The aim of this research was to develop a low cost adsorbent for the acid dye wastewater treatment. The non-living aquatic macrophytes, *Hydrilla verticillata* has been investigated as a function of initial pH, contact time and initial concentrations. Adsorption kinetic and equilibrium tests were carried out in flasks under batch operations. Optimum decolorization was observed at pH 3.0 and the equilibrium state was achieved after 80 min. The pseudo-first-order and the pseudo-second-order kinetics were investigated for the biosorption system. Isotherm data can be described reasonably well with the Langmuir isotherm. In addition, Scanning electron microscope of pre-treated biomass shows that surface of the biomass with $CaCl_2$ solution would remove the fine agglomerates and other surface impurities from the surface of biomass and Energy dispersive X-ray spectroscopy analysis released that pre-treated biomass was significant difference in ion exchange as K^+ and Cu^{2+} between un-treated biomass and pre-treated biomass. FTIR spectroscopy was employed to identify the chemical functional group present on the biomass. The characteristics bands of *H. verticillata* biomass are attributed to chemical bonds belonging to protein and polysaccharides functional groups. The experimental results in this study indicated that this low-cost biomaterial was an attractive candidate for the removal of acid dye from aqueous solutions at even low concentration.

Key words: Biosorption, adsorption, *Hydrilla verticillata,* acid dye, silk dye wastewater treatment

INTRODUCTION

The hand-made textile weaving industry of Thailand is a collection of household industries that individually produce only small volume of dye-colored wastewater. These industries, however, are scattered through out all the country, collectively creating widespread water contamination. Acid dyes, one group of organic pollutants, are extensively used in several industries such as textile, paper, printing and dye houses. The effluents of these industries are highly colored (Aksu and Tezer, 2000; Atmani *et al.*, 2009; Aksu, 2005) and discharge of dye containing effluents into the natural water bodies can pose hazardous effects on the living systems because of carcinogenic, mutagenic, allergenic and toxic nature of dyes. The presence of very small amounts of dyes in water (less than 1 mg L^{-1} for some dyes) is highly visible and undesirable (Bakshi *et al.*, 1999). Many treatment processes have been applied for the removal of dyes from wastewater such as: photocatalytic degradation (Sleiman *et al.*, 2007; Sohrabi and Ghavami, 2008), sonochemical

degradation (Abbasi and Asl, 2008), micellar enhanced ultrafiltration (Zaghbani *et al.*, 2008), cation exchange membranes (Mohammed and Naik, 2011; Wu *et al.*, 2008), electrochemical degradation (Fan *et al.*, 2006), adsorption/precipitation processes (Bello *et al.*, 2011; Zhu *et al.*, 2007), integrated chemical-biological degradation (Sudarjanto *et al.*, 2006), integrated iron (III) photoassisted-biological treatment (Sarria *et al.*, 2003), solar photo-Fenton and biological processes (Sarria *et al.*, 2003), Fenton-biological treatment scheme (Lodha and Chaudhari, 2007) and adsorption on activated carbon (Hameed and Daud, 2008; Lori *et al.*, 2008; Wu and Tseng, 2008). As synthetic dyes in wastewater cannot be efficiently decolorized by traditional methods, the adsorption of synthetic dyes on inexpensive and efficient solid supports was considered as a simple and economical method for their removal from water and wastewater (Forgacs *et al.*, 2004).

Biological processes such as biosorption (Ramakrishna and Viraraghavan, 1997), bioaccumulation (Aksu, 2003) and biodegradation (Chao and Lee, 1994; Khammuang and Sarnthima, 2009; Rajendran *et al.*, 2011a, b) have been proposed as having potential application in removal of dyes from textile wastewater. Among these, biosorption is more advantageous for water treatment because in this process, dead organisms are not affected by toxic wastes, they do not require a continuous supply of nutrients and they can be regenerated and reused for many cycles (Vieira and Volesky, 2000). The biological resources of dry biomasses as aquatic plant, algae and plant materials are available in large quantities and can be used for the various developments of biosorption material as coated, pre-treat, modified etc. *H. verticillata* are submerged aquatic weeds that can widely grow on the surface and form to mats in all bodies of water especially in Thailand. This study was carried out to determine the potential of immobilized living cells of *H. verticillata* to adsorb acid dyes in a batch scale.

MATERIALS AND METHODS

Preparation of biomass: *Hydrilla verticillata* was collected from Surin province and washed with tap water to remove dirt and then dried at 103°C in a hot air oven (Memmert model 600) for 24 h. After dry biomass was reduced by blender, the biomass was prepared for pre-treatment process as follows. A sample of 50 g of dry biomass was treated with pH 5.0 of 0.2 mol L^{-1} $CaCl_2$ solution (1000 mL) for 24 h under slow stirring. The calcium- treated biomass then was washed several times with deionized water to remove excess calcium and heated again in an oven at 103°C for 24 h and thereafter passed through a 125-1000 μm sieve giving the particle sizes of 250-500 μm.

Preparation of chemicals: All chemicals used in this study were prepared using deionized and distilled water. Acid blue was prepared by dissolving appropriate amounts of the tone of dye's trade name Singto Tee Klong, Phua Kiam Seen Co, Ltd. in distilled water. To adjust the pH, 0.1 M L^{-1} HNO_3 and NaOH solutions were used. Measurement of pH was performed using WTW multi 350i pH meter.

Biosorption of acid dyes: The biosorption of acid dyes onto pre-treated biomass of *H. verticillata* from aqueous solutions was investigated in batch biosorption equilibrium experiments. A set of 500 mL Erlenmeyer flasks containing 200 mL of the dye solution (100 mg L^{-1}) was used and 0.4 g of biosorbent was contacted with the dye solutions by the flask on a shaker (Heidolph Unimax 2010 model) with constant shaking at 125 rpm. Optimum biosorption conditions (pH, contact time and initial metal concentration) for these biomass were determined in only single component

system. The effect of initial pH was determined by agitation 0.4 g of the pre-treated biomass and 200 mL of dye solution concentration 40 mg L^{-1} using shaker at different the initial pH range from pH 2 to pH 9 at 25°C. The agitation contact time and speed were 120 min and 125 rpm. The pH of solutions was adjusted to desired value by using 0.1 mol L^{-1} HNO_3 and 0.1 mol L^{-1} NaOH. Similarly the effect of contact time was varied for the determination of the optimum biosorption time at optimum pH value. For this purpose, the flasks from an experimental set were removed at 5 to 120 min. Experimental controls test were also performed in the absence of the biomass to investigate the removal of acid dye which might occur via chemical precipitation and sorption on to the vessel walls. The effect of the initial acid dyes concentration on the biosorption was studied at optimum conditions for adsorption of dyes (obtain from above experiments). The concentration of each acid dyes in the adsorption medium was varied between 25 and 200 mg L^{-1}.

Characterization of biomass: The functional group of dried algal mass was determined using Fourier Transform Infrared Spectroscopy (FTIR) model NICOLET 6700 Spectrometer. The morphology of pre-treated algal biomass and after dyes-loaded were compared and determined by Scanning Electron Microscope and Energy Dispersive X-ray Spectroscopy (LEO 1450 VP).

Acid dyes analysis: The concentrations of dyes in the biosorption medium were measured colorimetrically using a spectrophotometer (UV-VIS 1601 model). The absorbance of the colours was read at 553 nm.

The removal efficiency and adsorption capacity were analyzed as following Eq. 1 and 2 as following:

$$\text{Removal}\,(\%) = \frac{(C_i - C_e)}{C_i} \times 100 \tag{1}$$

The adsorption capacity of metal ion can be analyzed based on the mass balance according as:

$$q = \frac{V(C_i - C_e)}{M} \tag{2}$$

where, q represents the amount of metal up taken per unit mass of the biomass (mg dye g^{-1} of the dry biosorbent), V is volume of the solution (L), M is the dry biomass of H. verticillata (g), C_i and C_e are the initial and final concentrations (mg L^{-1}), respectively.

RESULTS AND DISCUSSION

Effect of pH: In Fig. 1, the biosorption capacity of *H. verticillata* biomass increased from pH 2.0-3.0 and reached maximum at pH 3.0 and then declined sharply with further increase in pH, indicating that the optimal pH for biosorption of *H. verticillata* biomass is 3.0 under the experimental conditions and is in accordance with many researches as Sivaprakash and Rajamohan (2011) and Rezaee *et al.* (2006). Acidic conditions could be favorable for the biosorption between the acid dyes and the biomass, because a significantly high electro-static attraction could exist between the positively charged surface of the adsorbent under acidic conditions and the anionic dyes. At strongly acidic conditions the surface of the biosorbent gets positively charged thus provides attractive forces for negatively charged dye molecules. When the pH of the solution increased the number of negatively charged sites increased. The low biosorption capacity under
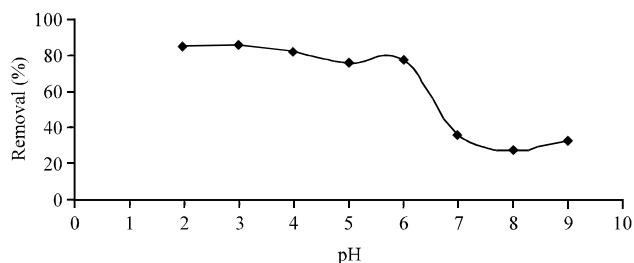
Fig. 1: The effect of pH for the biosorption of acid dye onto *H. verticillata* biomass (adsorbent dose 2 g L^{-1}; particle size 250-500 μm, initial dye concentration 100 mg L^{-1}, contact time 90 min, agitation speed 125 rpm and room temperature)
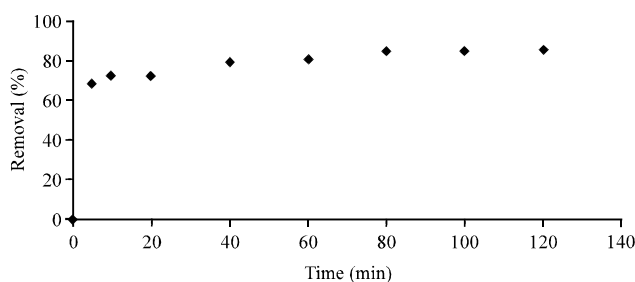

Fig. 2: The effect of contact time for the biosorption of acid dye onto *H. verticillata* biomass (adsorbent dose 2 g L^{-1}; particle size 250-500 μm, initial dye concentration 200 mg L^{-1}, agitation speed 125 rpm and room temperature)

alkaline conditions could be mainly attributed to that the increasing number of negative charge on the surface of the biomass could result in electrostatic repulsion between the adsorbent and dye molecules (Aksu and Donmez, 2003) and that the existence of excess OH^{-} ions may compete with the anionic dyes for the decreasing number of positively charged sites on the biomass surface. These results suggest that the one type of mechanism for the biosorption of acid dyes should be the electrostatic attraction between the positively charged biosorbent surface and the negatively charged dye anions.

Kinetic study: The kinetic of biosorption and contact time are the important parameters for successful use of the biosorbents for practical application and rapid sorption is among desirable parameters. The contact time on the biosorption of acid dyes onto *H. verticillata* biomass was shown in Fig. 2. The uptake of dye was occurred within 5 min and equilibrium was reached in 80 min and after then, it was nearly constant. Therefore, the optimum contact time was selected as 120 min for further experiments. The sorption kinetics was analyzed by applying the pseudo-second order Lagergren rate equation, where the binding capacity was assumed to be proportional to the number of active sites occupied on the sorbent. The pseudo-second order kinetic rate equation is:

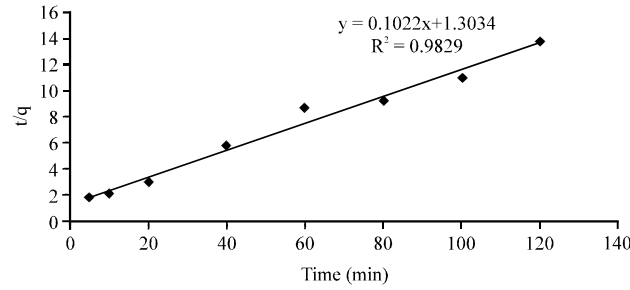$$\frac{t}{q_t} = \frac{1}{kq_e^2} + \frac{1}{q_e}t \qquad (3)$$

Fig. 3: The pseudo-second-order kinetic plot for the biosorption of acid dye onto *H. verticillata* biomass (adsorbent dose 2 g L^{-1}; particle size 250-500 μm, initial dye concentration 200 mg L^{-1}, agitation speed 125 rpm and room temperature)

where, q_e is the amount of dye sorbed at equilibrium (mg g^{-1}), k the rate constant of sorption (g mg^{-1} min) and q_i is the amount of dye sorbed on the surface of the sorbent at any contact time, t (mg g^{-1}).

A plot of t/q_t versus time was shown in Fig. 3. This clearly indicated that biosorption of acid dyes by *H. verticillata* biomass followed the pseudo-second order kinetics equation. The equilibrium dye sorption, q_e, for *H. verticillata* biomass was obtained 9.78 mg g^{-1}. The values of dye sorption rate, k, for dye sorption was 0.008 g mg^{-1} min. The obtained correlation coefficient values (R^2) point out that sorption onto *H. verticillata* biomass followed the pseudo-second order equation. The fit of the experimental data to this equation also indicates that the rate of sorption may be a chemical sorption involving valence force thought sharing or exchange of electrons between sorbent and sorbate.

Biosorption isotherm: A biosorption isotherm is characterized by certain constant values which indicates the surface properties and affinity of the biosorbent and can also be used to compare the biosorptive capacities of the biosorbent for different pollutants (Dursun *et al.*, 2005). The Langmuir isotherm is probably the most widely applied adsorption isotherm. A basic assumption of this model is that adsorption takes place at specific homogeneous sites within the biosorbent. The saturated monolayer isotherm is represented as:

$$q_e = \frac{K_L q_m C_e}{(1 + K_L C_e)} \tag{4}$$

where, q_m represents the maximum adsorption capacity, K_L is an constant value, Eq. 4 may be written into a linear form as follows:

$$\frac{C_e}{q_e} = \frac{1}{(K_L q_m)} \tag{5}$$

From the plot of C_e/q_e vs C_e, the slope $1/q_m$ gives q_m and intercept $1/K_L q_m$ gives K_L and Freundlich adsorption isotherm expressed in Eq. 6 and 7:
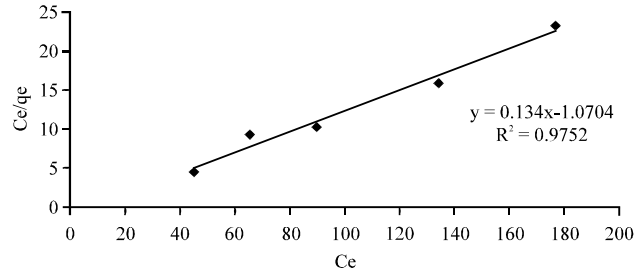
$$q_e = K_F C_e^{\frac{1}{n}} \tag{6}$$

Fig. 4: Langmuir adsorption isotherm obtained from acid dyes adsorption isotherm for *H. verticillata* biomass (acid dyes concentration of 25-200 mg L^{-1}, contact time 120 min, agitation speed 125 rpm and temperature of 25°C)
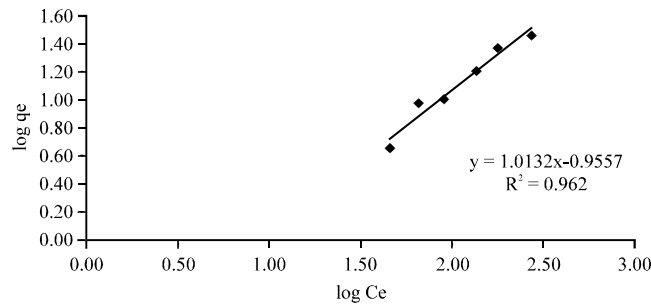

Fig. 5: Freundlich adsorption isotherm obtained from acid dyes adsorption isotherm for *H. verticillata* biomass (acid dyes concentration of 25-200 mg L^{-1}, contact time 120 min, agitation speed 125 rpm and temperature of 25°C)

where, Eq. 6 may be written into a linear form as Eq. 7:

$$\log q_e = \log K_F + \left(\frac{1}{n}\right) \log C_e \qquad (7)$$

where, K_F is an constant value:

$$\frac{mg}{g}\left(\frac{L}{mg}\right)^{\frac{1}{n}}$$

and n is a constant value.

The linearized Langmuir and Freundlich isotherm presented in Fig. 4 and 5. The experimental data could better be described by the Langmuir isotherm than the Freundlich isotherm (Table 1). The Langmuir maximum sorption capacity of *H. verticillata* biomass was found to be 7.46 mg g^{-1}. The q_m of the Langmuir model was assume to be the maximum amount of the ions which form a complete monolayer onto the surface of biosorbent. Figure 5 shows the Freundlich isotherm obtained for the biosorption of acid dyes onto *H. verticillata* biomass using Eq. 7. The values of K_F and 1/n were found to be 2.60 and 1.01. The 1/n values were not between 0 and 1, indicating that the biosorption of acid dyes onto *H. verticillata*. biomass was unfavourable at studied conditions. The Freundlich adsorption isotherms are not fit well with relatively low R^2 values (Table 1).

Table 1: Biosorption characteristics of Freundlich's model for acid dyes using *H. verticillata*

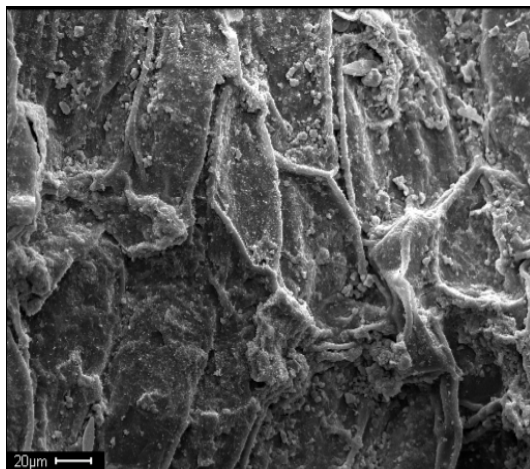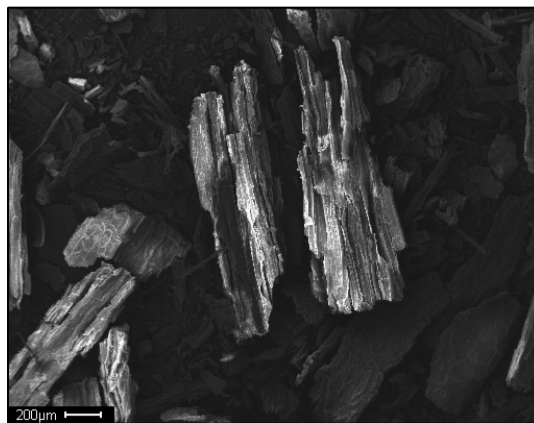| Langmuir	Adsorption	Isotherm	Freundlich	Adsorption	Isotherm
q_m	K_L	R^2	K_F	$1/n$	R^2
7.46	0.1252	0.9752	2.60	1.0132	0.9620

Fig. 6: SEM image of un-treated biomass

Fig. 7: SEM image of pre-treated biomass

Biomass characterization: The microstructure of *H. verticillata* biomass (250-500 micron) was examined by using SEM-EDX and image was showed in Fig. 6. SEM micrograph of *H. verticillata* biomass indicates that the biosorbent had a multi-pore and cylinder or fiber- like structure. However, there were some differences between the detailed structures of un-treated and pre-treated biomass (with $CaCl_2$) according to Fig. 6 and 7. The arrangement of cylinders or fibers of un-treated biomass showed shrinkage. They tended to collapse and cylinder or fibers-like structure was not clearly seen from un-treated biomass. The microstructure of the pre-treated biomass is shown in Fig. 7. Much more uniform biomass structure was observed. The surface of pre-treated
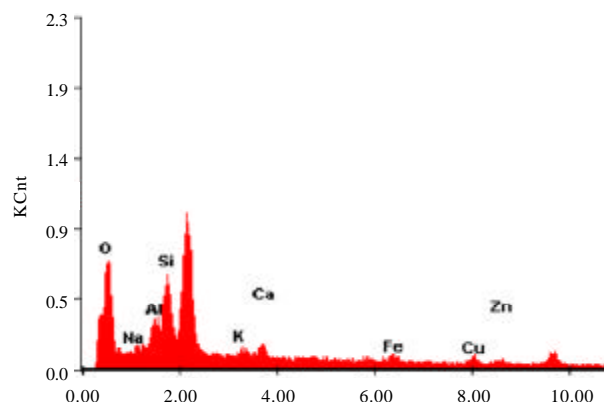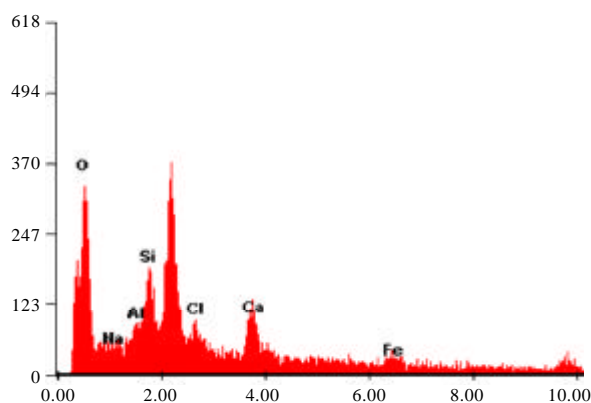
Fig. 8: EDX graph of un-treated biomass

Fig. 9: EDX graph of pre-treated biomass

biomass was also clearly cleaner than un-treated biomass. The more uniform structure might be due to a treatment of biomass by $CaCl_2$ solution. The calcium ions may clean out some impurities in biomass structure.

The EDX analysis was performed on the un-treated biomass and pre-treated biomass. The result of un-treated biomass was shown in Fig. 8, presented the different characteristic spectra of the un-treated biomass and the spectra were identified O, Si, Na, Ca, Al, K, Fe and Cu. In Fig. 9, the results showed that after washing the biomass with a high concentration of $CaCl_2$, Ca ions would wash away the different metal ions found on the surface of the biomass so that majority of sites were occupied by calcium ions. The pre-treated biomass was composed of various elements such as O, Na, Al, Si, Cl, Ca and Fe. The results also showed the change in relative peak intensities were observed between un-treated biomass and pre-treated biomass, especially peak of K and Cu onto the surface of biomass.

IR spectrum of *H. verticillata* biomass: The effective biding sites can be identified by FTIR spectral comparison of the un-treated biomass, pre-treated biomass and metal-loaded biomass for
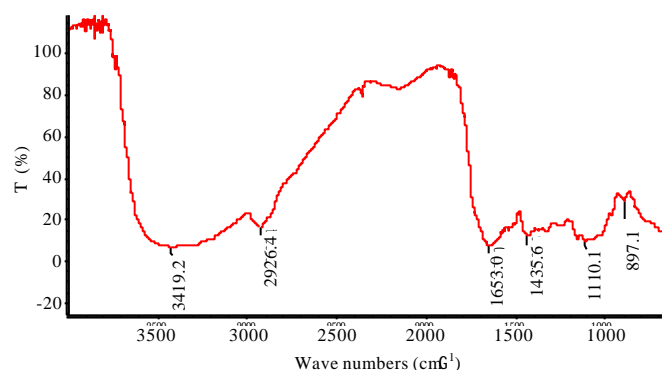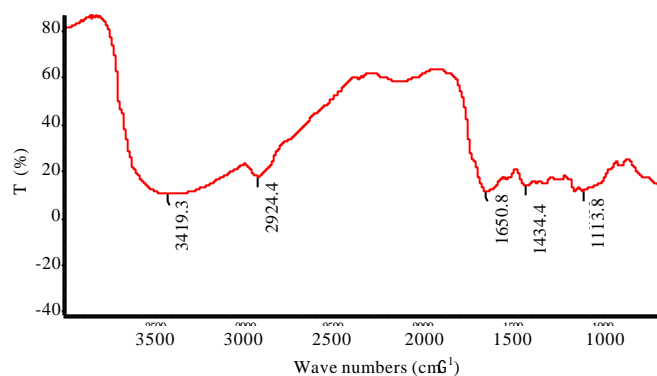
Fig. 10: IR spectrum of pre-treated biomass

Fig. 11: IR spectrum of dye-loaded biomass

H. verticillata biomass. The absorbance spectrum of *H. verticillata* biomass with that pre-treated with $CaCl_2$ was shown in Fig. 10. Some intense characteristic bands were obtained from the functional groups presented in proteins and polysaccharides (carboxylic and amine): the peak at 1110.1 and 1160.7 cm^{-1} can be attributed to the C-O stretching vibration. The peaks at 1635.9, 1647.0 and 1653.0 cm^{-1} were due to C-N stretching vibration. The peaks at 3419.2 cm^{-1} can be attributed to the N-H stretching vibration. For dye-loaded biomass was shown in Fig. 11, the IR spectra were found to be 1113.8, 1434.4, 1650.8, 2924.4 and 3419.3 cm^{-1} with functional groups of C-O, C = O, C-C and N-H bend vibration, respectively. The result also indicated that the functional groups were slightly decreased for dye-loaded biomass.

CONCLUSIONS

The results showed that the pre-treated biomass may be used as an inexpensive and effective for the removal of acid dyes from aqueous solution. The biosorption process was affected by experimental condition such as, pH, contact time and initial metal ion concentration. The kinetics of dye biosorption were examined and found to have a fast removal rate stage, followed by a second slower removal rate stage, until reaching equilibrium. Analyses of two kinetic models show that the kinetics of acid biosorption is better described by a pseudo-second-order model. The biomass has higher uptake capacity for dye-sorption followed the Langmuir isotherm adsorption model with high coefficient of the regression (R^2) than Freundlich adsorption isotherm model. The dye-biosorbent interactions were confirmed by FTIR and heterogeneous, smooth and porous

structure were observed by SEM-EDX technique. Scanning electron microscope of pre-treated biomass shows that surface of the biomass with $CaCl_2$ solution would remove the fine agglomerates and other surface impurities from the surface of biomass. EDX analysis showed the change in relative peak intensities between un-treated biomass and pre-treated biomass, especially peak of K and Cu onto the surface of biomass. The characteristics bands of *H. verticillata* biomass can be attributed to chemical bonds belonging to protein and polysaccharides functional groups (carboxylic and amine compound). Furthermore, *H. verticillata* biomass can be evaluated as an alternative biosorbent to treatment the textile wastewater containing acid dye.

ACKNOWLEDGMENTS

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