Utilizing Lipid-extracted Microalgae Residue for Removal of Methylene Blue from Aqueous Solution

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Abstract. Recently, microalgae biomass have been highlighted as the most feasible feedstock for biodiesel production, particularly due to their fast growth rate and they are able to synthesize high content of lipid within their cells. However, after extraction of lipid from the microalgae biomass, the remaining microalgae residues are usually discarded as waste. Thus, the present paper is aimed to recycle the lipid-extracted microalgae residue as adsorbent to remove methylene blue (MB) from aqueous solution. It was found that the microalgae residue could remove 74 % of the MB with biomass dosage of 6.25 g/L, stirring speed of 200 rpm at initial MB concentration of 2 mg/L. The adsorption of MB on microalgae residue fitted well with the pseudo-second order kinetic model and the equilibrium adsorption data indicated that the adsorption behavior followed the Langmuir isotherm.

1. Introduction

Adsorption is a separation process where adsorbate like soluble organics, dyes, pesticides, etc., is concentrated from a bulk liquid or vapour attached on to the surface of adsorbent (porous solid) by either physical or chemical attraction. It is commonly used in environmental engineering practices for colour removal of wastewater caused by dyes or other substances but the effectiveness and the sustainability in terms of economic is depending on the type of adsorbent [1]. In the past years, there are a lot of studies on biosorption (adsorption using biomass products) and its potential as a process for treatment on metal or dye bearing in the wastewater. Biosorption can be defined as the passive uptake of organic or inorganic species from solution by either microbial biomass, such as bacteria, yeast and algae, or waste from industry such as pinewood, rice husk, etc. [1, 2].

Various adsorbent can be used to remove the colour or dye from water, such as activated carbon, organic substances like clay, sludge and zeolites, biosorbents like fungi and algae, solid waste from agriculture like leaves, seeds and fibres, industrial solid wastes and many more type of adsorbents [3]. Studies have proven that adsorption using activated carbon is the best technique for water treatment especially when dealing with dye that contains toxic pollutant as activated carbon has high sorption capacity. However, the activated carbon is usually expensive and may not economically feasible to be used in large scale [2].

On the other hand, the lipid extracted microalgae residue can be potentially recycled as an absorbent instead of discarded after the lipid extraction process. Microalgae biomass are potential precursors for biodiesel production by extracting their lipid and convert the lipid through transesterification reaction. However, after the lipid is extracted from the microalgae biomass, the remaining microalgae residue is usually discarded to the environment. However, the microalgae residue has the potential to be recycled as an absorbent due to the pore structure created in the biomass during lipid extraction process.

Thus, in the present paper, microalgae residue will be recycled and used as alternative adsorbent for the dye removal. The experiments were conducted under lab-scale and removal efficiency of MB by adsorption onto lipid-extracted microalgae residue were determined, as well as the associated adsorption isotherm and kinetic.

2. Methodology

2.1. Preparation of Dye Solution

MB (Sigma-Aldrich) powder was used as the basic dye in the present work. The MB solution was prepared in the concentrated stock solution, which was 1 L of 200 mg/L solution. From this stock solution, the sample solutions were prepared later through dilution process.

2.2. Microalgae Lipid-extracted Residue

The dried microalgae biomass, *Chlorella vulgaris*, was supplied by Centre for Biofuel and Biochemical Research, UTP. 70 g of the dried microalgae biomass was weighed and mixed with 400 ml of methanol and 200 ml of chloroform. Then, the microalgae and the solvent solution was stirred for 5 hours by using magnetic stirrer before it was filtered using filter funnel and filter paper. The microalgae residue on the filter paper was taken and dried by both air dry and oven before used in the subsequent adsorption process. The microalgae before extraction and after extraction were characterized to determine their morphology and functional groups by using Scanning Electron Microscopy (SEM) and Fourier-transform Infrared Spectroscopy (FT-IR), respectively.

2.3. Effect of Methylene Blue Concentration

Four concentration of MB was produced; 2, 3, 4 and 5 mg/L. The solution then was put inside the stirrer and stirred for 10 minutes. Then, 10 ml of solution from each of the solution was taken and read by UV-VIS spectrophotometer, as the initial reading. The stirring was proceeded and 1 g of adsorbent (lipid-extracted microalgae residue), was inserted into each of the concentration of the solution. The 10 ml sample collection was repeated in 20 minutes interval until the system reached equilibrium.

2.4. Effect of Biomass Dosage

Five solutions of MB solution were prepared with equal concentration based on the optimized value from Section 2.4. The initial reading was taken by taking 10 ml of sample using 10 ml cell square and absorbance was determined using UV-VIS spectrophotometer. The process was proceeded with addition of 0.5 g, 1 g, 1.5 g, 2 g and 2.5 g of microalgae residue into the solution, respectively. The stirring process continued and 10 ml sample collection was repeated in 20 minutes interval until the system reached equilibrium.

2.5. Effect of Stirring Speed

Four solutions of MB solution were prepared with equal concentration based on the optimized value from Section 2.4. The initial reading was taken by taking 10 ml of sample using 10 ml cell square and absorbance was determined using UV-VIS spectrophotometer. Then, 2.5 g of microalgae residue was added into each of the solution and the stirring proceeded with different stirring speed for each solution; 50, 100, 150 and 200 rpm respectively. The 10 ml sample collection was repeated in 20 minutes interval until the system reached equilibrium.

2.6. Removal Efficiency

The removal efficiency of MB was calculated by using equation (1) as follow:

$$\boldsymbol{Removal\ efficiency} = \left(\mathbf{1} - \frac{\boldsymbol{C_t}}{\boldsymbol{C_0}}\right) \times \mathbf{100}\ \% \quad (1)$$

where, C_0 is initial concentration and C_t is concentration at time t.

2.7. Characterization of Lipid-extracted Microalgae Residue

The morphology and functional groups of pure microalgae biomass and lipid-extracted microalgae residue were carried by using Scanning Electron Microscopy (SEM, Hitachi TM3030) and Fourier Transform Infrared Red (FT-IR, Thermo Scientifi Nicole iS10) at Centre for Biofuel and Biochemical Research, UTP.

2.8. Adsorption Isotherm Model

The adsorption isotherms, Freundlich and Langmuir, were used in the present work (table 1). The original and linearized equation for each Freundlich and Langmuir isotherm are shown in equation (2) and (3), where q_e is the amount of dye adsorbed at equilibrium time (mg/g), C_e is the equilibrium concentration of dye in solution (mg/L), q_m is the maximum adsorption capacity (mg/g), K_a is the isotherm constants for Langmuir (L/mg), K_f is the capacity of the adsorbent constant for Freundlich and n is the intensity of adsorption constant for Freundlich [4-7].

Table 1. The equation model and linearized model for Freundlich and Langmuir isotherm

Isotherm Models	Equation model	Linearized model	
Langmuir	$q_e = \frac{q_m K_a C_e}{1 + K_a C_e}$	$\frac{1}{q_e} = \frac{1}{K_a q_m}\frac{1}{C_e} + \frac{1}{q_m}$	(2)
Freundlich	$q_e = K_f C_e^{\frac{1}{n}}$	$lnq_e = lnK_f + \frac{1}{n}(lnC_e)$	(3)

2.9. Adsorption Kinetic Model

The adsorption kinetic was determined based on the result from Section 2.4, and was evaluated based on the highest R^2 value on the plotted graph depending on their equation (table 2). For the present work, only two types of kinetic was analyzed and compared, which were kinetic pseudo-first order and pseudo-second order, as shown in equation (4) and (5), where, q_t is the concentration of adsorbate on adsorbent at time t, q_e is the equilibrium concentration of adsorbate on adsorbent at time t, K_1 is the kinetic constant for pseudo-first order and K_2 is the kinetic constant for pseudo-second order [8, 9].

Table 2. The equation and linearized model for Pseudo-first order and Pseudo-second order kinetic.

Kinetic Models	Equation model	Linearized model	
Pseudo-first order	$\frac{dq_t}{dt} = K_1(q_e - q_t)$	$ln\left(1 - \frac{q_t}{q_e}\right) = lnq_e - K_1 t$	(4)
Pseudo-second order	$\frac{dq_t}{dt} = K_2(q_e - q_t)^2$	$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \left(\frac{1}{q_e}\right)t$	(5)

3. Results and Discussions

3.1. SEM Analysis

Samples of microalgae before and after lipid extraction were characterized to describe their morphology by using SEM. Figure 1 shows the result of SEM for microalgae before and after lipid extraction, respectively. From the figure, the sample of microalgae biomass after lipid extraction was ruptured and scatted. This is probably due to the extraction process itself as the lipid was extracted out from the cell body, as mention by Lee, *et al.* [10], that the cells were disrupted in extraction process.

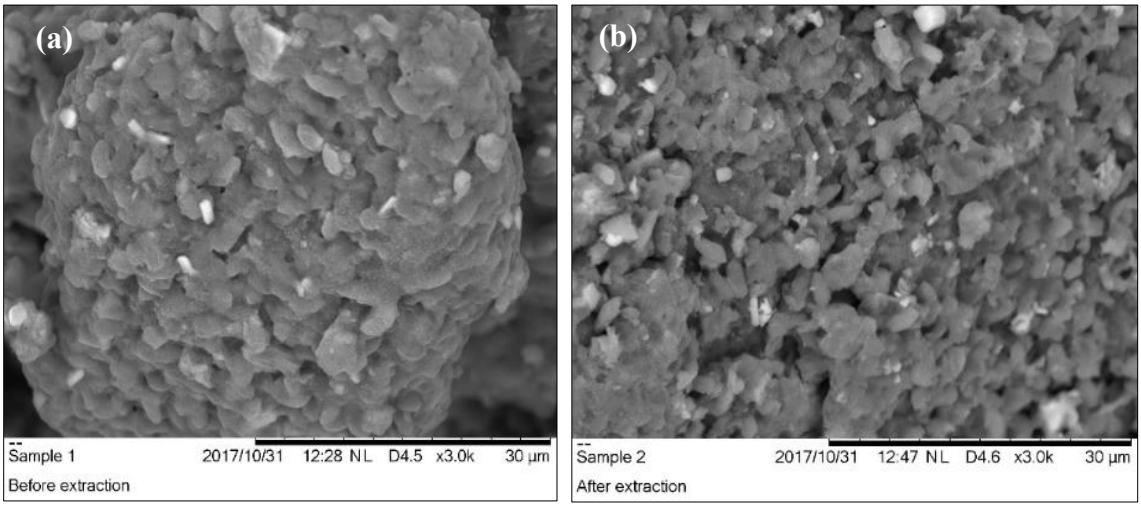

Figure 1. The SEM results for microalgae sample (a) before lipid extraction (b) after lipid extraction.

3.2. FT-IR Analysis

The FT-IR result shown in figure 2 compared microalgae functional groups before and after lipid extraction process. As can be seen, the trend of both graphs is similar while the peaks intensity has only minor differences (table 3).

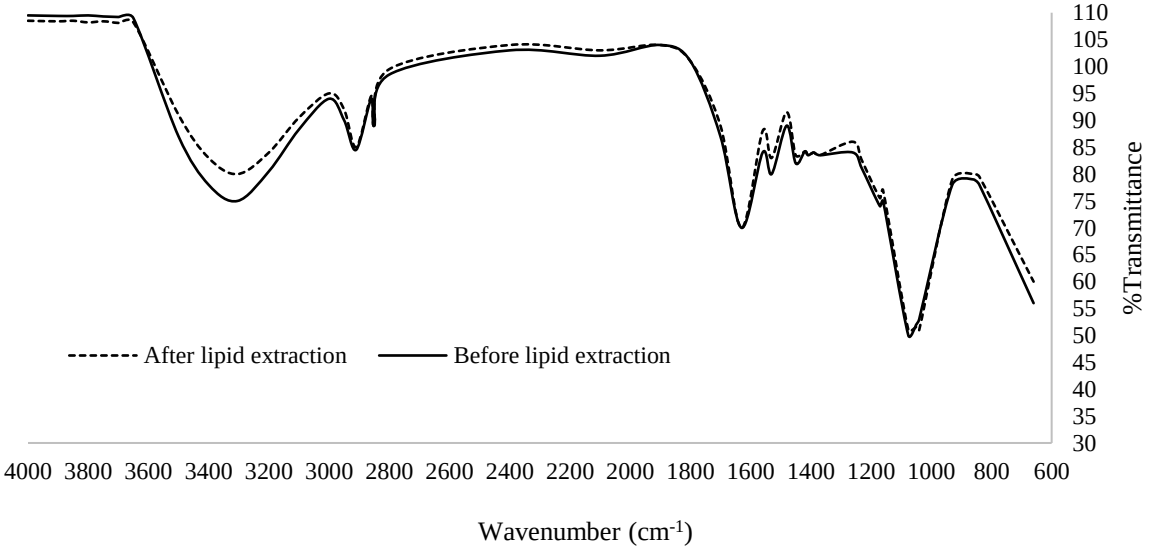

Figure 2. FT-IR result for microalgae sample before and after extraction.

The result indicated that there were no significant changes in functional groups occurred during the lipid extraction process. Even though there are slightly different in intensity of peaks, the values are still remained in the same range.

Table 3. Functional groups of microalgae samples

Peak value	Functional group	Frequency range
Before: 3304.82 After: 3287.54	Alcohol (O-H)	3200-3600
Before: 2919.51 After: 2919.33	Alkane (C-H)	2850-3000
Before: 2850.81 After: 2850.62	Carboxylic Acid (O-H)	2500-3300
Before: 1627.09 After: 1625.09	Alkene (C=C)	1620-1680
Before: 1538.43 After: 1537.76	Nitro (N-O)	1515-1560
Before: 1409.49 After: 1455.19	Alkane (-C-H)	1350-1480
Before: 1076.48 After: 1088.94	Amine (C-N)	1080-1360

3.3. Effect of MB Concentration

Figure 3 shows the MB removal efficiency (in percentage) for all four samples. From the figure, the highest MB removal efficiency was 73.6 % at 2 mg/L MB concentration, followed by samples 3 mg/L (69.5%), 4 mg/L (66.7%) and 5 mg/L (62%), respectively. From this finding, it can be concluded that the lower concentration of methylene blue solution resulted the highest removal efficiency for this type of adsorbent. Bulut and Aydın [11] also attained similar trend of results, which they indicated that the high driving force for mass transfer was one of the causes of increment in loading capacity of adsorbent in relation to dye ions. Other than that, the adsorption capacity, q, also can be calculated from the concentration by using Equation 6, where q_t is the adsorption capacity at time t (mg/g), C_0 is initial concentration (mg/L), C_t is concentration at time t (mg/L), V is volume of solution (L) and W is weight of adsorbent (g) [12]. Figure 4 shows the adsorption capacity for all four different concentrations, in which the higher concentration of MB producing higher adsorption capacity of MB adsorbed per unit mass of microalgae residue. The increment of the initial concentration of MB solution might increases the driving force of the concentration gradient, thus attained the results as shown in figure 4.

$$q_t = \frac{C_0 - C_t}{W} V \quad (6)$$

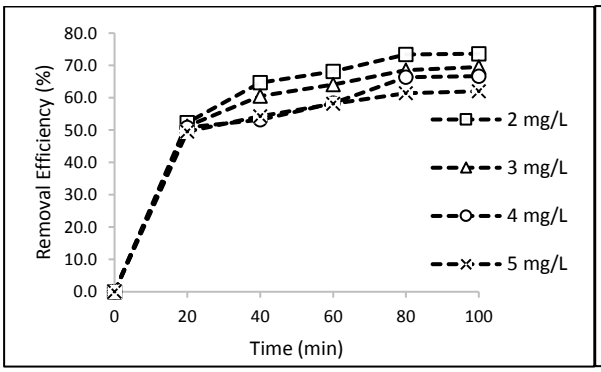

Figure 3. Removal efficiency of MB solution by microalgae residues

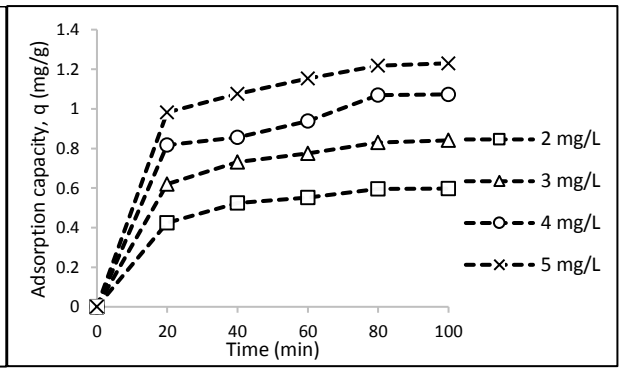

Figure 4. Adsorption capacity, q at different time.

3.4. Adsorption Isotherm

The results attained in Section 3.3 were fitted in the linearized isotherm equations. Table 4 shows that Langmuir and Freundlich isotherms are acceptable as both can fit the data which resulted to the R^2 value more than 0.99. However, Langmuir isotherm is chosen as it gives the higher R^2 value as compared to the Freundlich isotherm. According to Dural, *et al.* [13], the Langmuir isotherm indicates the physical monolayer adsorption, in which the adsorbed molecules do not interact as the uptake only occurred on the homogenous surface [14, 15]. In addition, the q_m attained in the present research was significantly higher as compared to banana peel (0.124 mg/g) [16] and orange peel (1.744 mg/g) [17]. The comparison also indicates the potential of microalgae residue to be utilized as alternative adsorbent and can be further transformed to activated carbon to attain higher adsorption capacity.

Table 4. Langmuir and Freundlich constants for MB adsorption by microalgae residue

Isotherm	Langmuir			Freundlich		
Parameter	q_m (mg/g)	K_a (mg/L)	R^2	K_F ((mg/g)(L/mg)$^{1/n}$)	n	R^2
Value	2.151	0.7158	0.9983	0.874	1.708	0.9926

3.5. Adsorption Kinetic

The data obtained in Section 3.3 were fitted into the linearized kinetic equations and table 5 summarizes the information related to the adsorption kinetic model. The second-order kinetic model was likely to be chosen as the R^2 values for all the concentration were the highest and constant compared to the first-order kinetic model. Beside the R^2 value, the Sum of Error Square (SSE) also can be used to determine the order of kinetic model [12]. The lower of SSE value means less error occurred and thus, determine the best fit of the adsorption kinetic model. Since second-order kinetic model also has the lowest SSE values (except at concentration at 3 mg/L), therefore the second-order kinetic is the best fit for adsorption of MB by using microalgae residue as adsorbent. Besides, the second-order kinetic also indicates the chemisorption is the rate-determining step that controls the adsorption process. This is further proved in the FT-IR analysis that the presence of multiple functional groups resulted to the binding of MB on the surface of the lipid-extracted microalgae residue instead of only depending on the van der Waals force.

Table 5. Adsorption kinetic model constants for methylene blue adsorption by microalgae residue.

Conc. (mg/L)	First-order kinetic model					Second-order kinetic model			
	$q_e$exp (mg/g)	$q_e$cal (mg/g)	K_1 (1/min)	R^2	SSE (%)	$q_e$cal (mg/g)	K_2 (1/Min)	R^2	SSE (%)
2	0.597	0.730	0.0626	0.9044	0.059	0.669	0.1302	0.9989	0.032
3	0.841	0.776	0.0492	0.9593	0.029	0.930	0.1012	0.9992	0.040
4	1.073	1.313	0.0580	0.8146	0.107	1.208	0.0625	0.9882	0.060
5	1.230	1.074	0.0519	0.9525	0.070	1.330	0.0914	0.9989	0.045

3.6. Effect of Biomass Dosage

The effect of dosage of biomass was studied by manipulated the biomass dosage, which were 1.25 g/L, 2.50 g/L, 3.75 g/L, 5.00 g/L and 6.25 g/L, respectively. The initial concentration of methylene blue solution was set to be constant at 5 mg/L with constant stirring speed, at 150 rpm. The result was obtained and the removal efficiency was calculated as shown in figure 5. From the figure, the higher biomass dosage could result the higher MB removal efficiency. By comparison, when the biomass dosage was 1.25 g/L, the final efficiency was 59% compared to the biomass dosage of 6.25 g/L which can achieved efficiency as high as 70.3%. This is probably due to more surface area are available for the adsorption process to occur [11].

3.7. Effect of Stirring Speed

The effect of stirring speed was studied on the efficiency in removal of MB. Four stirring speed were set and the initial concentration of methylene blue solution and biomass dosage was kept constant at 5 mg/L and 6.25 g/L, respectively. Figure 6 shows the result of the removal efficiency for each stirring speed. From the figure, it can be observed that the higher stirring speed could result higher MB removal efficiency. The MB removal efficiency at 50 rpm was about 63%, however, as the speed increased to 100 rpm, 150 rpm and 200 rpm, the removal efficiency increased to 66.5%, 70.3% and 74.1%, respectively. This is probably because the stirring speed may influence the distribution of the adsorbate to be adsorbed. Nevertheless, the results obtained differ with Doğan, *et al.* [15], as according to that study, the stirring speed did not give any different to the removal efficiency.

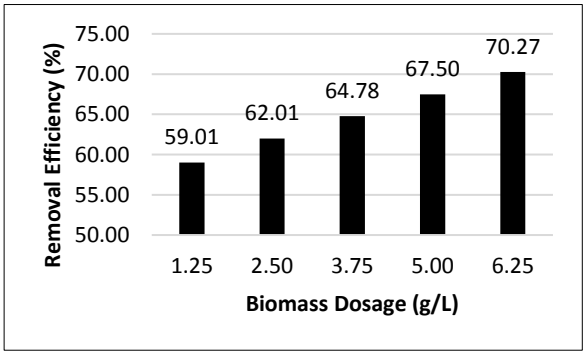

Figure 5. Removal efficiency of different biomass dosage at 100th minute.

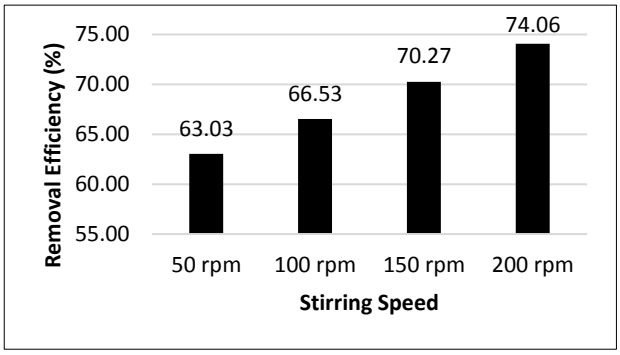

Figure 6. Removal efficiency of different stirring speed at 100th minute.

4. Conclusion

In the present work, it was proven that lipid-extracted microalgae residue could be used as alternative adsorbent to adsorb MB. As for the initial concentration of MB solution, the lower concentration resulted the higher removal efficiency, while for the adsorbent dosage, as the adsorbent dosage increased, the

removal efficiency increased. The higher stirring speed also resulted the higher removal efficiency. The highest MB efficiency attained was 74% with the biomass dosage of 6.25 g/L, stirring speed of 200 rpm. The adsorption isotherm determined in the present work was Langmuir isotherm whereas the adsorption kinetic model would be following the second-order kinetic model due to its high R^2 and SSE% values.

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