Article

Removal of Methylene blue, *Escherichia coli* and *Pseudomonas aeruginosa* by Adsorption Process of Activated Carbon Produced from *Moringa oleifera* Bark

Md. Shahin Azad 1, 2, Syaza Azhari1, Mohd Sukri Hassan*1

1 Faculty of Science & Technology, Universiti Sains Islam Malaysia (USIM), Bandar Baru Nilai, 71800 Nilai, Negeri Sembilan, Malaysia
   e-mail: mohdsukri@usim.edu.my, syaza@usim.edu.my

2 Department of General Educational Development, Faculty of Science & Information Technology, Daffodil International University (DIU), Dhanmondi, Dhaka, Bangladesh
   e-mail: shahinazad26@gmail.com

Abstract- The utilization of biopolymer derived from *Moringa oleifera* bark using ZnCl2 and H2SO4 as activating agents for eliminating Methylene blue, *Escherichia coli* and *Pseudomonas aeruginosa* from producing wastewater. In this study, Methylene blue and both bacteria were effectively adsorbed by activated carbon with lowest dosage. The activated carbon was prepared from natural-by product of *Moringa oleifera* bark by pyrolysis in a furnace at 700°C for 1 h. The characteristics of activated carbon have been determined using Scanning Electron Microscopy (SEM), Brunauer-Emmett-Teller (BET), pHzpc (zero point charge), and FTIR spectroscopy. The obtained result were closely fitted with Freundlich isotherm model and adsorption kinetics follow the pseudo-second order model with the highest value of correlation coefficient (R2~1). Adsorption quantity was dose dependent and bacteria were maximum adsorbed using 10 mg of activated carbon as well as 25mg for methylene blue. The maximum adsorption capacity showed within 1 hour. The bacterial load was reduced by 98% for *E. coli*, 96% for *P. aeruginosa* as well as methylene blue reduced 94.2% from aqueous solution using batch adsorption methods. Adsorption process controlled by film diffusion mechanism. These result proposed that the activated carbon of *Moringa oleifera* can be used as a good adsorbent for the removal of Methylene blue, *E. coli* and *P. aeruginosa*.

Keywords- *Moringa oleifera* bark; Film diffusion; Dye uptake; *E. coli*; *P. aruginosa*.

I. INTRODUCTION

Synthetic dye is used in many manufacturing industries for the colouring of products such as textile, paper, and paint [1]. Around 50% of total dyes is released from textile industries [2]. Now-a-days, synthetic dye controls most of the chemical industries [3]. Methylene blue is a basic dye which is a heterocyclic aromatic compound. If consumed, it can cause irritation of mouth, throat with symptoms of jaundice in humans [4]. Methylene blue dye enter into water system can cause human cancer of breast and skin.

There are several common physical, biological and chemical methods for eliminating colouring matter from aqueous solution. These include reverse osmosis, adsorption [5], assimilation [6-8], precipitation, coagulation and ion exchange ([9], [10]). Among all methods, Adsorption is one of the major processes for dye removal from wastewater [11]. Generally, commercial activated carbon is used as adsorbent in adsorption process due to high uptake capacity, eco-friendly and non-toxic nature [12] but it is a costly process [13]. In recent times, researchers are looking into green materials for the wastewater treatment as alternative options of commercial activated carbon. There are some natural plants used as adsorbents for the removal of dye, heavy metal and bacteria from wastewater such
as oil palm [13], groundnut shell [14], Moringa oleifera leaf [15], rice husk [16], Moringa oleifera seed which can be used to remove dye from industrial wastewater. Among them Moringa oleifera plant is a multi-purpose tree which is used for food and medicinal activities [17]. Various parts of Moringa oleifera such as stem, bark, and roots have antimicrobial activities [18, 19]. Moringa oleifera leaves have both macro- and micronutrients such as β carotene, protein etc. It’s leaves possess hypolipidaemic and antiatherosclerotic activities [20]. Moringa oleifera pods have free radicals scavenging abilities [21]. Its acts as a natural antioxidant [22] like some other plants which are reported as natural sources of antioxidants [23, 24].

Activated carbon is a good adsorbent due to its ability to bind with a variety of molecules [25]. It is well known that activated carbon can be used to remove bacteria and bacteria toxin [26]. It is also used for developing the water quality used for consumption purposes. The active part of adsorbents can remove organic molecules like methylene blue from wastewater. The main aim of this study to produce activated carbon as adsorbent from Moringa oleifera bark to remove methylene blue, E. coli, and P. aeruginosa from wastewater.

II. EXPERIMENTAL PROCEDURE

A. Materials

All chemicals were of analytical rating. Methylene blue solution were prepared using deionized water. Moringa oleifera bark was collected from outside of USIM campus and bacteria was received from the microbiological lab of USIM.

B. Instrumentation

Functional groups on surface of adsorbents were determined using Fourier-transform infrared spectroscopy (PERKIN ELMER). The surface properties of activated carbon were identified using (SEM-HITACHI, Japan) and Brunauer-Emmett-Teller (BET: BELSORP, Japan). Centrifuge, water distillatory, pH meter, Shaker, UV/Vis. Spectrophotometer (Cany 50 conc. VARIAN, Agilent Technologies, USA), analytical balance, were used in this study.

C. Sample collection and preparation

Moringa oleifera bark sample was collected from natural sources in-front of Akasia avenue, Nilai, Negeri Sembilan, Malaysia. Firstly, the bark was washed using water and dried under the sun for 6 hours. Then it was again washed to remove dirt from its surface and dried in an oven at 110 °C for 13 hours. The Moringa oleifera bark pieces were reduced into smaller particle sizes using blender. The samples (100 g) were then chemically activated using 500 ml zinc chloride (10% ZnCl₂) and Sulphuric acid (0.5M H₂SO₄) with ratio of 1:5 for 12 hours. Then the samples were dried in the oven at 110 °C for 12 hrs. It was grinded and sieved at 400μm. Then the sample was kept in the desiccator for further use. The precursor was placed in the furnace at 700 °C with heating rate of 10 °C/min for 60 min. During the carbonization process, purified nitrogen was flown. It was cooled in normal temperature. These activated carbon samples were washed several times with distilled water. The washed samples were dried in the oven at 110 °C for 15 hours. These were then sieved with a sieve of 400μm. The activated carbon sample was kept in a polypropylene bottle to avoid exposure to moisture. These prepared activated carbon sample from Moringa oleifera bark were used for the experiments as the adsorbent in adsorption process.

D. Characterization of the Prepared Activated Carbon

The characterization of the Moringa oleifera was obtained by Fourier Transform Infrared Spectroscopy (FTIR). It was range from 400 to 4000 cm⁻¹. IR spectra were detected by disk method. The surface functional groups on the activated carbon were analysed by FTIR spectroscopy. The morphologies of the adsorbents were determined using Scanning Electron Microscope (SEM). The surface area, pore volume and pore size of activated carbon were measured by Brunauer-Emmett-Teller (BET). The system were measured by a Quanta chrome NOVA automated gas sorption system.

III. RESULT AND DISCUSSION

A. Characterization of Activated carbon

1) Surface Morphology: Figure I shows the FESEM micrographs of the Moringa oleifera bark activated carbon before adsorption under (a) 60x magnification and (b) 500x magnifications. Large and well-developed pore are obtained from prepared activated carbon under optimum condition (at 700 °C temperature, activation time 60 min as well as char: ZnCl₂ and H₂SO₄ impregnation ratio 1:5). It might be occurred due to use chemically active agent. Surface area and pore volume development could be happened also during pyrolysis process. This produced well developed pore was the important factor to adsorb the maximum methylene blue from wastewater. This was due to the sudden
burst of the thermal expansion from pyrolysis. Since it would develop the surface area of adsorbents after the activation process [27]. The activated carbon formed irregular, large caved-in shaped cavities on its surface.

![Figure I. FESEM micrograph of Moringa oleifera bark (Activated carbon) (a) 60x and (b) 500x magnification](image)

2) Surface Area and Porosity: In this experiment, the surface characteristic of the prepared adsorbents were analysed. The BET surface areas and pore volume were found to be relatively high. The largest pore was of the surface area of 435.1738 m²/g when pyrolysis was done at 700 °C and pore volume was 0.189245 cm³/g. The development of surface quality of adsorbents can occur due to the elimination of impurities from surface using acid. The pore size for single pore (diameter) was analysed as 68.488 Å. Activated carbon can consist of mineral, which clogs up in the pores [28]. According to (Nuithitikul et.al.2010) [29], the minerals are observed as unwanted contaminants such as N, P, Si, Na, K, Zn and Fe. These elements could be removed from adsorbents using acid (H₂SO₄) and ZnCl₂ treatment for reducing ash.

3) Surface Functional Groups: The FTIR spectra analysis is essential to determine various surface function of Moringa oleifera bark. FTIR spectrum of Moringa oleifera bark shows the characteristic peaks obtained with slightly shifted after adsorption, but it was a more intense peak. The peaks were detected at 3371 cm⁻¹ (O-H), 3183 cm⁻¹ (C-H), and 1501 cm⁻¹ stretching (C=C of aromatic carbon). Carbonyl groups (C=O) were not detected in the produced adsorbent. The presence of oxygen (C-O) functional group peak was detected at 1126 cm⁻¹. It could be indicative of polarity.

B. Adsorption Process

1) Adsorption process of Methylene blue: The dye adsorption by activated carbon of Moringa oleifera bark was detected using batch adsorption process. It was conducted by 100ml of methylene blue solution in a 250ml glass beaker at different initial concentrations (5-50 mg/L). The various doses of activated carbon (0.005-0.05 g) and different contact time were evaluated at time intervals (10-120 min). This study was conducted by adding 0.025gm dried adsorbent per 100ml methylene blue solutions in glass beaker. Samples are shaken in a shaker at 25±2 °C with 200rpm speed for 2 hours. The pH was controlled at 6 to get best adsorption capacity. After time interval to take solution and completing filtration, methylene blue was analyzed using a spectrophotometer (UV-VIS). The sample solutions were taken at equilibrium to evaluate the remaining concentration.

2) Adsorption process of Bacteria: This experiment was conducted to determine the activity for reduction of E. coli and P. aeruginosa bacteria. Both bacteria were collected from microbiological lab in FST of USIM. Approximately, E. coli (1.15 x 10⁷ cells) and Pseudomonas (1.73 x 10⁷ cells) in 1 ml of nutrient broth were separately mixed with (1, 3, 5, 10 mg) of activated carbon. Then all mixtures were placed in the incubator with agitation for 1 hour. Initial bacteria cell of both bacteria and after 10, 20, 40, 60 min duration alive cells counted for 5mg of activated carbon. Those mixtures were separated using centrifuge for 5 min. After centrifugation, it was then placed onto nutrient agar in petri disk for counting the bacteria.

C. Adsorption Mechanism of Moringa oleifera

There are some important factors for dye adsorption onto activated carbon, such as (i) Van der Waals attraction, (ii) hydrogen bonding (iii) pore filling and (iv) ion exchange [30]. On the other hands, the π-π and σ-π interactions depends on the structure of MB and adsorbents. These types of interaction are less important for adsorption of MB [31]. The surface area of adsorbents can influence
the Van der Waals attraction. Generally produced activated carbon contains high surface area and pore volume. That’s why pore filling happens easily in porous activated carbon. Van der Waals forces and pore filling are important adsorption mechanisms. But methylene blue (C16H18ClN3S) has high molecular weight which could not easily enter the inner pores of adsorbents. However, the nitrogen atom of methylene blue can participate in bonding as a hydrogen bond with oxygen atoms on outer surface of adsorbents. Furthermore, Methylene blue could bind with acidic groups of adsorbents by ion exchange methods. The hydrogen bonding and ion exchange methods are very vital adsorption mechanisms for methylene blue. This type of adsorption process is correlated with oxygen-containing functional groups. Generally, methylene blue adsorption onto activated carbon occurs by active site on the surface, film diffusion and adsorption into the interior pore of the adsorbents and monolayer build-up of adsorbate. Film diffusion is better than inter-particle diffusion in this process. There are several factors which affect the adsorption process.

D. Adsorption Isotherms of methylene Blue

Adsorption isotherms are related to the distribution of solute from the solution to adsorbent surfaces. That can be carried out by several mathematical relationships. Langmuir and Freundlich and Dubinin-Radushkevich models are shown in Figures II to IV respectively. The values of linear regression coefficients (R²) of various models are shown in the Table 1. From the table, Freundlich model is closely fitted with data. The symbol of b is designated for the adsorption affinity of activated carbon. qmax is considered as the total active parts of adsorbents. But q is defined for the binding parts which are filled up by solute [32]. The theoretically qmax is found to be 129.87 for Methylene blue ions that is not comparable with experimental calculated qmax (108.12). On the other, constant symbol k is designated the strength of adsorption. Lower value k indicated higher adsorption [33]. Data would be favourable when n value is (1<n<10). All data of Moringa oleifera was well fitted of Freundlich isotherm with coefficient regression.

The properties of the adsorption process can be described from Dubinin-Radushkevich isotherm (D-R). However, the mean adsorption energy (E) is an important factor about these properties [34]. When the value of (E) is less than 8 kJmol⁻¹, it indicates that the adsorption process is due to physical forces. On the other hand, the value of E ranges from 8-16 kJmol⁻¹ which indicates that adsorption method is due to chemical bonded. According to the Table I, E values (2.4) suggested that Moringa oleifera shows physical adsorption. At initial stage, the adsorbent contain available vacant active sites and shows good adsorption capacity [35].
### TABLE I

**ISOTHERM PARAMETERS FOR THE ADSORPTION OF METHYLENE BLUE ONTO ACTIVATED CARBON OF MORINGA OLEIFERA BARK**

| Activated Carbon | Langmuir model | Freundlich model | Dubinin-Radushkevich |
|------------------|----------------|-----------------|----------------------|
|                  | Adsorbate      | $R^2$           | $q_{\text{max}}$ (mg/g) | $b$ (L mg$^{-1}$) | $R^2$ | $1/n$ | $k$ | $R^2$ | $q_{\text{max}}$ (mg/g) | $E$ (kJ mol$^{-1}$) |
| Moringa oleifera bark | Methylene blue | 0.587 | 129.87 | 0.103 | 0.803 | 0.348 | 6.94 | 0.441 | 51.07 | 2.4 |

### E. Kinetics of Adsorption

All constant value ($k_1$, $k_2$, $K_d$, $R^2$) of adsorption kinetics were shown in Table 2 and Figure V to VII. There are large differences for $q_e$ values between experimental (108.12) and calculative values (232) for pseudo-first-order equation. So, this graph does not fit with first-order model. On the other hand, the experimental value ($q_e$) and calculative value (108.69) were closed for pseudo-second-order model with coefficient regression constant ($R^2$~1). The results indicate that there are several steps involved in the adsorption process. The rate of adsorption for methylene blue was evaluated by external transport or intra-particle transport. Most of the line developed did not pass through the origin as shown figure 8. It is indicated that the adsorption process was controlled by film diffusion [36]. So, the adsorption of methylene blue by *Moringa oleifera* bark was conducted by film diffusion than internal transport.

![Fig. V First order adsorption kinetics plot of Methylene blue](image1)

![Fig. VI Second order adsorption kinetics plot of Methylene blue](image2)

![Fig. VII Intra-particle diffusion of Methylene blue adsorption](image3)
TABLE II
KINETICS PARAMETERS FOR THE ADSORPTION OF METHYLENE BLUE ONTO ACTIVATED CARBON OF MORINGA OLEIFERA BARK

| Activated Carbon Of Moringa oleifera | Heavy Metal | $q_{exp}$ (Mg/g) | First Order | Second Order | Intra–Particle Diffusion |
|-------------------------------------|-------------|-----------------|------------|-------------|-------------------------|
|                                     |             | $q_{cal}$ (Mg/g) | $K_1$ (min$^{-1}$) | $R^2$ | $K_1$ (gmg$^{-1}$ min$^{-1}$) | $R^2$ |
| MB                                  | 108.12      | 0.955           | 232        | 0.018       | 0.999                   | 108.69 | 0.0128 | 7.267       | 0.516 |

F. Some Important Factor for Adsorption of Adsorbents

1) Effect of Initial Dye Concentration: This experiment was conducted at ranging (5-50mg/L) in batch adsorption process with time interval (10-120) min. Generally, 100 ml of solution was mixed with 0.025g of activated carbon into each 250mL flask. The flask was then placed in shaker at constant temperature (25 °C). The rotation speed of shaker was 200 rpm. After reaching equilibrium point then it was tested by UV-vis analyser.

![Fig. VIII Effect of Initial Concentration for Methylene Blue](image1)

The effect of initial concentration graph shown as Fig. VIII With increasing the initial concentration, the removal percentage of methylene blue from aqueous solution was decreased but the adsorption capacity of adsorbents was also increased. At lower initial concentration (5mg/L), about (94.2%) percent of methylene blue was removed and with the increase of the initial concentration up to 50mg/L, the removal percentage of methylene was about 50% and the adsorption capacity was about 108 mg/g. It can occur due to possession of high surface area and active functional group onto activated carbon. The higher mass transfer of the adsorbents was due to the increase in the driving force which was the initial concentration of dye [37].

2) Effect of adsorbent dosage: (a) The effect of adsorbents dosage in the range of 0.005–0.05 g on the removal percent for MB dye was conducted in Fig. VIII. From this figure, the removal percent increases from 25 to 100% for MB with increasing the adsorbent dose from 0.005 to 0.05 g. It was found that 94.2% from aqueous solution was removed with use of 0.025g of activated carbon. It can be said to have occurred due to available active sites present on activated carbon for the adsorption. Further increasing the adsorbent dose, the removal percentage was not significant. This may have happened due to it reaching the equilibrium state between binding adsorbate and unadsorbed adsorbate in adsorption process.

![Fig. IX Effect of dosage for Moringa oleifera](image2)

(b) The effect of dosage of activated carbon on bacteria were studied as shown in Fig. X and XI. The stem bark of agricultural products can show antibacterial activities [38]. The number of cell for both bacteria decreased while using adsorbents. The efficacy of activated carbon on bacteria was dose dependant. The removal percentage of *E.coli* were obtained as 29.3%, 62.7% and 87.8% respectively using 1,3,5 mg activated carbon of *Moringa oleifera*.
at 37 degree for 1 hour. On the other hand, the removal percentage of Pseudomonas aeruginosa incubated with 1, 3, 5 mg adsorbents were obtained as 59.8%, 74.1% and 90% respectively. E. coli was decreased as 98% and Pseudomonas was reduced as 96% using 10mg of adsorbent.

Fig. X Effect of adsorbent dosage for E.Coli of Moringa oleifera

Fig. XI Effect of adsorbent dosage for Pseudomonas of Moringa oleifera

3) Effect of solution pH: The 0.025gm of activated carbon was added with 5mg/L for 100 mL solution at pH range (2-12) into flask. Figure 12 shows the plot of the initial pH against with final pH to detect the zero point charge (pHzpc). The pH(pzc) of Moringa oleifera was found to be around (5.4). If the pH of a solution is changed, the dissociation of the functional group of activated carbon also will be changed. That’s why the adsorption capacity of adsorbents can be changed.

The adsorption mechanism of MB+ can be conducted by the electro static forces [39]. With the increasing pH value, the concentration of H+ ion in solution decreases. The negative surface charge will be an increase on the activated carbon above the pH(pzc) value of (5.4). For this reason, adsorbents can attract more as the cationic form of MB+. It can easily adsorb onto the adsorbents. The Methylene blue can be also adsorbed on adsorbents by ion exchange mechanism. The main functional groups of adsorbents are the carboxyl, hydroxyl, carbonyl, and methoxyl group. All are responsible for ion exchange onto their surface at appropriate pH. At pH 6, the adsorption of methylene blue was found to be maximum as shown in Fig.13.

4) Effect of contact time: (a) The adsorption study of methylene blue was essential to determine the contact time required to reach the equilibrium position. The adsorption of methylene blue by Moringa oleifera bark was increased with time up to 80min then it became constant at the end of the experiment (120min). It is suggested that the metal binding rate of adsorbents was more during initial stages. After some time it gradually increases and remains almost constant after 80 to 120 min as
shown in Figure 14. When the dye was contacted with active site of adsorbents then it soon involved cation complexation.

![Figure XIV](image1)

**Fig. XIV** Effect of Contact time for adsorption capacity of M. Blue

(b) This experiment was also investigated to clarify binding time of bacteria using activated carbon. As shown in Figure 15 - 16, the 5mg of adsorbent was incubated with 1ml of *E. coli* and *Pseudomonas aeruginosa* during (10-60) minute. However, binding activity of activated carbon with *E. coli* and *Pseudomonas aeruginosa* has increased with the increases of time. After reaching 40 min, the removal percentage of bacteria did not show more significance. These result indicate that activated carbon can be effective adsorbent to adsorb the *E. coli* and *P. aeruginosa* bacteria.

![Figure XV](image2)

**Fig. XV** Effect of adsorbent rate for *E. coli* of Moringa oleifera

![Figure XVI](image3)

**Fig. XVI** Effect of adsorbent rate for *Pseudomonas* of Moringa oleifera

**G. To evaluate the number of Bacteria using Disk methods**

About the cells (1.15x10^7) of *E. coli* and (1.73x10^7) of *Pseudomonas aeruginosa* were mixed with activated carbon (1, 3, 5, 10mg) respectively. These mixtures were incubated at 37°C with agitation for 1 hour and after then centrifuged for 5 min. After separating, it was then placed onto nutrient agar of disks to determine the number of bacteria. All disk were placed into the incubator at 37°C for 24 hours. After incubation, the number of bacteria were counted using magnifying glass. The results are shown in Fig 17 and 18. Summary of microbiological report are shown in Table 3. From the Table 3, The number of bacteria were counted 120, 55, 5, 0 for *E. coli* using (1mg, 3mg, 5mg and 10mg) activated carbon respectively and 165, 145, 95 and 0 for *Pseudomonas aeruginosa* bacteria using activated carbon of *Moringa oleifera* respectively.

![Figure XVII](image4)

**Fig. XVII** Number of bacteria for *E.Coli* of *Moringa oleifera* by Disk methods
IV. CONCLUSION

This study evaluated the use of activated carbon derived from Moringa oleifera as a low-cost adsorbent for the removal of Methylene blue, E. coli and P. aeruginosa in wastewater treatment. The adsorption of methylene using the activated carbon of Moringa oleifera bark was closely fitted by the Freundlich model. The methylene blue adsorption of activated carbon showed physical adsorption by D-R model (E<8). The adsorption kinetics parameter better fit pseudo-second order model ($R^2 > 0.99$) than pseudo-first-order model and intra-particle diffusion. The increase in dose of activated carbon enhanced the bacterial adsorption capacity and at 10 mg of adsorbents, it shows maximum adsorption capacity of 98% for E. coli and 96% for P. aeruginosa as well as 94.2% removed methylene blue using 25mg of activated carbon. The result suggested that the activated carbon of Moringa oleifera can be used as a good adsorbent to remove contaminant from wastewater.

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TABLE III

| Types of Bacteria | Escherichia coli | Pseudomonas aeruginosa |
|-------------------|----------------|-----------------------|
| Quantity of AC    | Num. of Bacteria lost (disk) | After 10 min. Cell removal | After 20 min. Cell removal | After 40 min. Cell removal | After 60 min. Cell removal | Num. of Bacteria lost (disk) | After 10 min. Cell removal | After 20 min. Cell removal | After 40 min. Cell removal | After 60 min. Cell removal |
| Moringa Oleifera Bark | 1 mg | 120 | 29.3% | 165 | 59.8% |
|                    | 3 mg | 55 | 62.7% | 145 | 74.1% |
|                    | 5 mg | 5 | 24.1% | 82.5% | 87.8% | 95 | 15.1% | 35.1% | 85.9% | 90% |
|                    | 10 mg | 0 | 98% | 0 | 96% |

Fig. XVIII Number of bacteria for Pseudomonas of Moringa oleifera by Disk methods
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