Kinetic and Adsorption Removal Study of Malachite Green Dye on Carbon Nanotubes Immobilized Biomass (CNTIB)

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Abstract

The adsorption of Malachite green (MG) onto Carbon Nanotubes Immobilized Biomass (CNTIB) was carried out in this research. The interactive effects of experimental variables such as biosorbent loading, MG dye concentration and pH were investigated. Optimized conditions for adsorption studies were achieved by employing the Response Surface Methodology (RSM) of Design expert 7 software using the Faced Centered Central Composite Design (FCCD). MG adsorption uptake was found to increase with an increase in biosorbent loading. Isotherm studies showed that the experimental data better fitted with the Langmuir isotherm for CNTIB when compared to Freundlich isotherm. Adsorption kinetics was found to follow the pseudo-second order kinetic model which suggests that chemisorption is the process through which adsorption took place. The biosorbent was produced by immobilization technique. Optimized conditions of parameters for biosorption studies (initial dye concentration, biosorbent loading and pH) as well as response were determined. 111.0mg/L of dye was removed out of an initial dye concentration of 112.5mg/L. Acidic pH was favourable for the adsorption of MG. Coefficient of determination (R²) for biosorption model was 0.989. The biosorbent produced was characterized using SEM to determine its morphology and available functional groups which would further enhance its biosorption ability.

Highlights

➢ Production of CNTIB for the first time was presented
➢ Application of CNTIB for the removal of MG dye from aqueous solution is reported for the first time
➢ Kinetics and Isotherm of adsorption process is also described

Keywords: MG, CNTIB, Biosorbent, Kinetics

1. Introduction

Textile industry consumes a large quantity of water and therefore generates a huge amount of wastewater (Hai et al., 2007). Normally, a number of sophisticated dyes and auxiliary chemicals are used in the textile industries to obtain a good quality product. Continuous usage of these chemicals have brought about an environmental concern (Coulibaly et al., 2004). Dye wastewater discharged by textile and dyestuff industries therefore need to be well treated before being released into the environment.

Physical, chemical and some biological methods have been used in dye removal. However, these methods are characterized by some major setbacks such as low efficiency and high cost amongst others. Several researchers have shown that biosorption (adsorption) can be a better substitute to the physico-chemical, microbial and or enzymatic biodegradation methods (Khan et al., 2013).
Cnts have been investigated as the best adsorbent for industrial pollutants even from dilute solutions. This is due to their large specific surface area and nano structure, outstanding thermal and chemical stability, excellent adsorption efficiency, environmental friendly nature, high reactivity, porosity, surface functional groups, binding strength, high affinity between sites and the particular pollutant of interest, high adsorptive capacities etc. (Chen et al., 2009).

The last three decades have also witnessed an extensive study of biomass being a perfect replacement for activated carbon. A wide diversity of microorganisms has been studied and results have shown that they were able to decolorize a wide variety of dyes. Among the various types of biomass, the fungal biomass has proved to be particularly suitable. Fungal biomass is highly effective yet very cheap. Considering the nature of CNTs and fungi biomass as excellent adsorbents in the removal of textile dye contaminants from industrial wastewaters, this research therefore comes up with a novel biomaterial such as a combination of non-living fungal biomass and CNTs in the treatment of dye wastewater. This new biosorbent will be referred to Carbon Nanotubes Immobilized biomass (CNTIB).

A basic understanding of the adsorption equilibrium and kinetics is very paramount for adsorption studies on dye wastewater. The equilibrium studies highlights a relationship between the concentration in the majority of the fluid phase and the material adsorbed at a constant temperature. The adsorption isotherms are used to express the amounts of dye adsorbed per unit mass. Moreover, it gives adequate information about the adsorption process. In this study, the Langmuir and the Freundlich isotherm models were used to describe the equilibrium adsorption characteristics of Malachite green using CNTIB by varying dye concentrations. Its validity assumes that a monolayer sorption of malachite green dye on a surface containing a finite number of sites took place in the studied system.

2. Materials And Methods

2.1 Materials and chemicals

The materials and chemicals used in this research were CNTs (from sigma), Methanol (CH₃OH), malt extract, distilled water, hydrochloric acid (HCl), Sodium hydroxide (NaOH), fungal strains (collected from Environmental Biotechnology lab of IUM) , other consumables and Malachite Green dye (MG) of analytical chemical grade. About 200 mg/L of MG was prepared as stock solution by dissolving 200 mg of MG dye in 1000 mL distilled water. The stock solution was then diluted with distilled water to different concentrations desired for the experiment. The supernatant fluids were then filtered off with whatman filter paper. The chemical formula, molecular weight and maximum wavelength and chemical structure of MG dye have been summarized in Table 1 and figure 1 respectively.

| Dye                  | Commercial name | IUPAC name                                                                 | λmax (nm) | Molecular weight |
|----------------------|-----------------|-----------------------------------------------------------------------------|-----------|------------------|
| Malachite green      | Basic green, aniline green, fast green, etc | N-[4-[4-(dimethylamino)phenyl]phenylimethylene]-2,5-cyclohexadien-1-yldiene]-N-methylmathanaminium chloride | 617       | 364.92           |

Table 1: Dye used for the present study

Figure 1: Malachite green structure

2.2 Instrumentation

The structure of the carbon nanotubes immobilized biomass (CNTIB) was examined using the scanning electronmicroscope (SEM) (model: JEOL JSM-6300F) under a voltage of 18 Kv and resolution of 5.0 nm. All pH measurements were made with a pH meter. The sample was dried in a drier and agitation of the system was done on a shaker. The residual MG concentration in the aqueous solution was ascertained by using UV/Vis spectrophotometer. The structure of the carbon nanotubes immobilized biomass (CNTIB) was examined using the scanning electron.
2.3 Preparation of adsorbent

2.3.1. Preparation of culture plates

Potato dextrose agar (PDA) of 3.9% (w/v) was diluted in an autoclavable glass bottle. The dilution was sterilized at 121°C for 15 minutes. The sterile PDA dilution was poured onto petri dishes in the laminar flow cabinet. The air bubble of PDA in petri dishes was removed by slight heating with flame. The plate with PDA was left into the laminar flow cabinet until solidification. Prepared PDA plate was preserved in a chiller for further use.

2.3.2. Cultivation of fungal biomass

Two different types of fungal strains: Aspergillus niger, and Penicillium sp were studied for immobilization of Carbon nanotubes. These strains were obtained from the lab stock at Bioenvironmental Engineering Lab, IIUM and maintained on potato dextrose agar (PDA) slants incubated at 32°C for three days. It was then stored at 4°C until required. To ensure the availability of sufficient stock culture for experimental procedure, the microorganism was monthly sub-cultured on new agar. The inoculum of each was prepared and their biomass was produced. Solid nutrients were used for the growth of fungi and for storage of cultures (Harley & Prescott., 1990). Aspergillus niger and Penicillium sp were grown in Bacto® potato dextrose agar (PDA) (39g/L) petri dishes and incubated for 3 to 7 days at room temperature (32 ±2°C). The PDA petri dishes with A. niger & Penicillium sp were always stored in sealed plastic bags to prevent the loss of moisture from agar.

2.3.3 Inoculum preparation

Spore suspension of fungal strain Aspergillus niger was used as inoculums for all laboratory scale fermentation because of its simplicity in preparation. The culture was grown on PDA medium in petri dishes at temperature 32°C for 3 to 4 days. Spore suspension inoculum was prepared by washing the fungal culture grown on PDA plate (Alam, Mansor, & Jalal, 2009). To prepare inoculum, all flasks, funnels, filter papers, distilled water and L-shaped glass rod were sterilized prior to use. About 10 mL of distilled water was poured each time onto the PDA plate and the spores were gently scrapped using sterile glass rod. Three plates of fungal culture were washed with 50 mL sterilized distilled water. It was left to shake on the incubator shaker for 24 hours. The suspended fungal spores were then filtered using Whatman number 1 filter paper into an Erlenmeyer flask. Based on biomass production, Aspergillus niger was chosen for immobilization of Carbon nanotubes.

2.3.4 Preparation of Carbon nanotubes immobilized biomass (CNTIB)

Aspergillus niger was cultivated in a liquid medium using a shake flask method. The liquid medium was 98 ml of (17g/1000ml) malt extract, (0.1-0.5) g of carbon nanotubes and 2mls of inoculum. The malt extract solution was prepared by dissolving 17g of malt extract powder into 1000mls distilled water. It was placed on a magnetic stirrer and allowed to make a uniform mixture. Varying quantities of carbon nanotubes were measured and poured into the liquid media in the Erlenmeyer conical flask. The pH of the sample ranging from 4 to 8 were recorded and then autoclaved at 121°C for 2-3 hours. The liquid medium was allowed to cool down to room temperature after autoclaving. The liquid medium was inoculated with inoculum of 2% (2 mls) Aspergillus niger fungus and then transferred to the incubator shaker at 150 rpm for three days. The growth of fungus (Aspergillus niger) was observed after two days and it grew as pellets. The pellets increased in diameter subsequently. After the fifth day of growth (120 hours), the carbon nanotubes immobilized biomass was harvested by filtering through a 150μm.

It was then washed thoroughly with distilled water to ensure that CNTs were tightly attached to the biomass. The filtrate which contained the unattached CNTs was passed through the pump, washed thoroughly with deionized water to remove the growth medium sticking on to its surface and filtered to get a clear solution.

Immobilized activated carbon was dried at 105°C in a drier. The weight of dried biomass was recorded, crushed and then stored in a dry container.

2.4 Batch adsorption experiments

Batch adsorptions studies were performed in laboratory scale using a 250 mL Erlenmeyer flasks containing known weights of adsorbent (CNTIB) and different concentrations of 20 mg/L, 40 mg/L and 60 mg/L, pH and temperature. The pH of the MG solution was adjusted at optimum values making use of appropriate quantities of 0.2 M NaOH and HCl. The adsorption capacity (amount of MG adsorbed per unit mass of adsorbent) was calculated as thus:

\[ q_e = \frac{(C_o - C_e)V}{m} \]  
\[ q_i = \frac{(C_o - C_i)V}{m} \]

The percentage MG removal was calculated thus:

\[ \text{Amount Removed} = \frac{(C_o - C_e)}{C_o} \]

Where \( C_o \) (mg/L), \( C_e \) (mg/L) and \( C_i \) (mg/L) are the initial concentration, equilibrium concentration and time t concentration of MG dye, respectively, V (L) is the solution volume of MG dye, and m (g) is the amount of the adsorbent.

3.0 Results And Discussions

3.1. Characterization of adsorbent

Figure 2 a, b and c shows the SEM images of Carbon nanotubes alone, fungal biomass only and carbon nanotubes immobilized biomass (CNTIB). From figures 2 a and b, the SEM images showed no existence for any matrix on their surfaces. However, from c, the results for the carbon nanotubes immobilized biomass of Aspergillus niger showed the attachment of fungal mycelia attached to carbon nanotubes. Furthermore, Figure 2c showed that there was an obvious entrapment after immobilization of Aspergillus niger on carbon nanotubes forming a matrix. It is clearly indicated that the powered carbon nanotubes were significantly attached to Aspergillus niger which is well disposed for adsorption of dye molecules. As shown, the branches of microbial biomass got a significant structural change as compared to the structures without entrapment.

3.2. Statistical analysis for dye removal

Using the experimental design software version 7, batch runs were conducted according to Face Centered Central Composite Design (FCCCD) (model design experiments) to visualize the effect of independent variables on the response along with the experimental conditions. The analysis demonstrated that the model was highly significant with a model F-value of 8.32 and \( \text{Prob} > F \) value of 0.0009. In general, \( \text{Prob} > F \) values of less than 0.0500 indicate the model is significant (Design Expert Software, 2007). The adequate precision ratio of the model was 8.85 which show an adequate signal for the model. It also implies that the model can be used to navigate the design space.
### Table 2: Analysis of variance (ANOVA) for Response Surface Model for dye biosorption

| Source                  | Sum of squares | DF | Mean square | Fvalue | p-Value Prob > F |
|-------------------------|---------------|----|-------------|--------|------------------|
| Model                   | 30153.2       | 9  | 3350.37     | 9.10   | 0.0009           |
| A-Ph                    | 1771.56       | 1  | 1771.56     | 4.81   | 0.0530           |
| B-Dye Conc.             | 1702.50       | 1  | 1702.50     | 4.62   | 0.0570           |
| C- Biosorbent loading   | 9541.92       | 1  | 9541.92     | 25.91  | 0.0005           |
| AB                      | 269.12        | 1  | 269.12      | 0.73   | 0.4126           |
| AC                      | 2178.66       | 1  | 2178.66     | 5.92   | 0.0353           |
| BC                      | 269.12        | 1  | 269.12      | 0.73   | 0.4126           |
| A²                      | 198.65        | 1  | 198.65      | 0.54   | 0.4795           |
| B²                      | 2994.92       | 1  | 2994.92     | 8.13   | 0.0172           |
| C²                      | 2994.92       | 1  | 2994.92     | 8.13   | 0.0172           |
| Lack of fit             | 3682.10       | 5  | 736.4217    | 674.09 | < 0.0001         |

R²=0.8912  
Adjusted R²=0.7932

### 3.2.1 Effect of interactive experimental variables

As discussed earlier, an experimental design (FCCCD) coupled with RSM was used to evaluate the effects of three process variables on the dye removal process.

#### 3.2.1.1 Effect of dosages (Biosorbent loading) and pH on biosorption

In Figure 4.15 and 4.16, the contour and 3D-surface plots were developed as a function of dosages and pH, while the dye concentration was at 112.5 mg/L. As shown from the Figures, the maximum amount of dye removed was about 111.0 mg/L. The amount of dye removed from the initial amount of malachite green dye increased with increase in biosorbent dosages. The lowest amount of synthetic dye removed (64.65 mg/L) was obtained at a pH of about 8.75, while the dosage was around 4.75 mg/L. Based on ANOVA results obtained, both pH and biosorbent loading (dosages) were found to have significant effects on dye removal from dye solution.

**Figure 3a:** Surface plot for dye removal from synthetic dye water as a function of Dosages (Biosorbent loading) and pH (Dye Conc. of 112.5mg/L)

**Figure 3b:** 3D-surface plot for dye removal as a function of Dosages (Biosorbent loading) and pH (contact time 6 hours)

### 3.2.1.2 Effect of dye concentration and dosages (biosorbent loading)

To investigate the interactive effect of dye concentration and biosorbent loading on dye removal, surface contour and 3D plots were drawn and studied. The plots are shown in Figure 4a and b. It was observed that the interactive effect of the two process variables seemed to be same as compared to that of previous interaction. The lowest residual concentration of dye (64.65 mg/L) was obtained at a dosage of about 6.5 g/L. An increase in biosorbent loading also resulted in further increase in the amount of dye removed.

**Figure 4a:** Surface plot for dye removal from synthetic dye water as a function of Dosages (Biosorbent loading) and Dye Concentration.
3.2.1.3 Effect of dye concentration and pH

To study the interactive effect of dye concentration and pH on removal of synthetic dye, the experiments were carried out with dye concentrations varying from 25mg/L to 200mg/L and under different range of pH 3-10 at constant time. The results were displayed in Figure 5a and b. The Figures clearly showed that the minimum amount of synthetic dye removed was obtained at pH 6.5 and at a dye concentration of 68.75mg/L.

3.2.1.4 Validation

To validate the robustness and applicability of the biosorption process, the initial values of process parameters were determined by the software for low and high levels. The actual values of biosorption parameters and the predicted values were presented in Table 3. The result showed that predicted values by the software and experimental values were close and this suggests that the model was validated.

Table 3: Validation of experimental model for dye biosorption

| Run No | Biosorbent loading, C (g/L) | Dye concentrations B (mg/L) | Predicted amount of dye removed (mg/L) | Actual amount of dye removed (mg/L) |
|--------|-----------------------------|----------------------------|----------------------------------------|------------------------------------|
| 1      | 6.5                         | 112.5                      | 29.30                                  | 25                                 |
| 2      | 6.5                         | 200                        | 100.5                                  | 112.5                              |
| 3      | 3                           | 200                        | 72                                     | 69.69                              |
| 4      | 10                          | 25                         | 25                                     | 25                                 |
| 5      | 6.5                         | 112.5                      | 112.5                                  | 112.5                              |
| 6      | 6.5                         | 112.5                      | 110                                    | 112.5                              |
| 7      | 6.5                         | 25                         | 20                                     | 25                                 |
| 8      | 6.5                         | 112.5                      | 97.5                                   | 112.5                              |
| 9      | 10                          | 200                        | 118                                    | 112.5                              |

3.3 Adsorption capacity

3.3.1 Adsorption isotherms

The Langmuir and Freundlich models were employed to describe the equilibrium adsorption. The Langmuir model assumes that adsorption occurs on homogenous surfaces for a known number of sites. The linear expression of the Langmuir model as described by (Periasamy, management, 1995).

\[
\frac{C_e}{q_e} = \frac{1}{q_{max} K_a} + \frac{C_e}{q_{max}}
\]  \hspace{1cm} (4)

Where \( q_e \) is the amount of solute adsorbed per unit weight of adsorbent (mg/g), \( q_{max} \) is the maximum adsorption capacity corresponding to the site saturation, \( C_e \) is the solute concentration at equilibrium (mg/L) and \( K_a \) is the Langmuir equilibrium constant (L/mg).

Furthermore, the essential characteristics of the Langmuir isotherm can be expressed by the separation factor or equilibrium parameter which is a dimensionless constant \( R_L \) as defined by Weber & Chakravorti (1974).

\[
R_L = \frac{1}{1 + K_a C_e}
\]  \hspace{1cm} (5)

Where \( K_a \) is the Langmuir equilibrium constant (L/mg) and \( C_e \) is the highest initial dye concentration (mg/L). The parameter \( R_L \) indicates the nature of the adsorption process. On the other hand, the Freundlich model is an empirical relation between the solute concentrations on an adsorbent surface to the concentrati on of the solute in the liquid it is in contact with. The non-linear Freundlich isotherm model is given as:

\[
q_e = K_f C_e^{1/n}
\]  \hspace{1cm} (6)

The non-linear equation may also be linearized and represented by the following equation:

\[
\ln q_e = \ln K_f + \frac{1}{n} \ln C_e
\]  \hspace{1cm} (7)
Where \( q_e \) is the amount of solute adsorbed per unit weight of adsorbent (mg/g), \( C_e \) is the solute concentration at equilibrium (mg/L), while \( K_f \) and \( n \) are Freundlich constants, which can be determined by plotting \( \log q_e \) versus \( \log C_e \). The value of \( n \) gives an indication of how favorable the adsorption process and \( K_f \) represents the adsorption capacity of the adsorbent (Tan & Ahmad 2008).

The constant of equilibrium \( K_a \) decreased as the concentration of dye increased. This depicts a decrease in the chemical attraction between the dye and biosorbent. Furthermore, the \( R_L \) a dimensionless constant of Langmuir isotherm model which is the separation factor was used to find out more about biosorption studies. The \( R_L \) value can either be unfavorable \((R_L > 1)\), favorable \((0 < R_L < 1)\) or irreversible \((R_L = 0)\) (Khatri & Singh, 1999). The \( R_L \) values of Langmuir isotherm model at different concentration are listed together with other isotherm constants in Table 4.

According to the Freundlich isotherm as shown in the table, there was a reduction in the \( K_f \) value with decreasing concentration. The Freundlich constant \( K_f \) for the biosorption of MG reduced from 0.852 to 0.678 and 0.126 at concentrations of 60 mg/L, 40 mg/L and 20 mg/L respectively.

This explains that there is a decrease in the binding capacity of the biosorbent with decrease in concentration. The values of the correlation coefficients \( R^2 \) can be used to determine the model that best describes the experimental process. From the experimental data for the biosorption of MG as shown in table 4, it can be concluded that the biosorption of MG on CNTIB was best described by Langmuir isotherm model. The overall correlation coefficients, \( R^2 \) of Langmuir isotherm model were found to be greater than those of Freundlich isotherm model. However, the values of the correlation coefficients for both models are fairly close, showing that both models were applicable in biosorption process. The value of the maximum adsorption capacity \((q_{max})\) is shown in table 4. At all the concentrations studied, comparing all \( R^2 \) values, those of the pseudo second-order kinetics were found to be more than 0.90. According to Wang & Chen (2002), pseudo-second order assumed that the rate limiting step was biosorption. There must be sharing or exchange of electrons between the biosorbent and the biosorbate.

### 3.3.2 Kinetic study

The adsorption kinetics describes the rate at which substances are adsorbed onto the surface of the adsorbent and also controls the equilibrium time. To study the kinetic of MG dye onto CNTIB, its experimental data were fitted using the pseudo-first-order and pseudo-second order kinetic models.

#### 3.3.2.1 Pseudo first order and second order model

The pseudo-first-order kinetic model has been widely used to predict sorption kinetics. Simonin (2016) defined the model as:

\[
\log(q_e - q_t) = \log q_e - \frac{k_1}{2} t
\]

where \( q_e \) and \( q_t \) are the amounts of adsorbate adsorbed at equilibrium and at any time, \( t \) (min), respectively (mg/g) and \( k_1 \) is the adsorption rate constant (1/min). The plot of \( \log (q_e - q_t) \) versus \( t \) gives the slope of \( k_1 \) and intercept of \( \log q_e \). At the concentration range of 20 mg/L to 60 mg/L, the linearized pseudo first-order and pseudo second-order kinetics model were plotted.

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**Table 4: Coefficients of Langmuir and Freundlich isotherms**

| Concentration (mg/L) | Langmuir isotherm model | Freundlich isotherm model |
|----------------------|-------------------------|----------------------------|
|                      | \( K_f \) (L/mg) | \( R_L \) | \( R^2 \) | \( K_f \) | \( n \) | \( R^2 \) |
| 20 mg/L 1.89          | 0.311                  | 0.772          | 0.83     | 0.126    | 1.175   | 0.869 |
| 40 mg/L 1.95          | 0.307                  | 0.685          | 0.942    | 0.687    | 1.165   | 0.809 |
| 60 mg/L 3.48          | 0.255                  | 0.634          | 0.984    | 0.852    | 1.275   | 0.998 |

**Figure 6a:** Langmuir plots Adsorption isotherms plot of MG

**Figure 6b:** Freundlich plots Adsorption isotherms plot of MG (CNTIB=3.5g/L, contact time=1h and T=302K).
ion 150 TIB at various concentrations. The results of meters as 50 100 200 50 100 200 50 100 200 50

The figure therefore provides a very similar correlation data.

4. Conclusion
This study investigated the equilibrium and adsorption of Malachite green on CNTIB at various concentrations. The results of biosorption evaluation using the active biosorbent for dye removal showed that the dye was decolorized with a dye uptake of 98% at a dye concentration of 60mg/L as shown in table 5. Isotherm studies showed that the experimental data better fitted with the Langmuir isotherm for CNTIB when compared to Freundlich isotherm. The kinetic study however indicated that the biosorption followed the pseudo-second order kinetic model which suggests that chemisorption is most likely the process through which biosorption took place.

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Table 5: Pseudo-first order and pseudo-second order kinetic model parameters for the biosorption of MG onto CNTIB at different concentrations

| Concentration (mg/L) | Pseudo-first order | Pseudo-second order |
|----------------------|--------------------|---------------------|
|                      | R²                 | K1                  | R²                 | K2                  |
| 20 mg/L              | 0.806              | 0.025               | 0.989              | 0.853               |
| 40 mg/L              | 0.973              | 0.028               | 0.991              | 0.545               |
| 60 mg/L              | 0.853              | 0.023               | 0.981              | 0.403               |