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Optimisation of Process Variables by Response Surface Methodology (RSM) for Malachite Green Dye Removal Using Spent Tea Leaves Biochar

NUR IR IMANI BINTI ISHAK

F15A0152

A report submitted in partial fulfilment of the requirements for the degree of Bachelor of Applied Science (Bioindustrial Technology) with Honours

Faculty of Bioengineering and Technology

Universiti Malaysia Kelantan

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DECLARATION

I hereby declare that the work embodied in this final year project report is the result of the original research except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for higher degree to any universities or institutions.

_____

Student,

Name:

Date:

Approved by:

_____

Supervisor,

Name:

Date:

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LIST OF ABBREVIATIONS

ANOVA	Analysis Of Variance
CCD	Central Composite Design
CCF	Central Composite Face-Centered
C.V	Coefficient of Variance
Df	Degree of Freedom
DOE	Design Of Experiments
FTIR	Fourier Transfer Infrared
F-Value	*Fisher's* Value
UIPAC	International Union of Pure and Applied Chemistry
MG	Malachite Green
RSM	Response Surface Methodology
RPM	Rotation Per Minutes
SEM	Scanning Electron Microscope
STL	Spent Tea Leaves
UV-Vis	Ultraviolet Visible
2D	Two Dimensional
3D	Three Dimensional

LIST OF SYMBOLS

G	Gram
mg/L	Milligram per litre
Min	Minute
mL	Millilitre
Nm	Nanometre
pH	Acidity / Alkalinity
R^2	Determination Coefficient (Correlation Coefficient)
R^2_{adj}	Adjusted Coefficients of Determination
A	Alpha
ºC	Degree Celsius
µL	Microlitre
µm	Micrometre
%	Percentage

Pengoptimuman Pemboleh Ubah Proses Melalui Kaedah Gerak Balas Permukaan Untuk Penyingkiran pewarna Malakit Hijau (MG) Menggunakan Bioarang Daun Teh Terpakai

ABSTRAK

Disebabkan oleh penyediaan yang mudah, daun teh terpakai mendorong kepada berlakunya masalah pelupusan dan menyebabkan pengumpulan sisa pertanian. Kaedah konvensional untuk rawatan sisa buangan adalah mahal dan tahap kecekapan penyingkiran adalah rendah. Maka, penggunaan sisa pertanian kepada produk nilai tambahan yang dikenali sebagai biopenjerap adalah dicadangkan disebabkan oleh kebolehpercayaannya dan kemampuannya. Tujuan kajian ini adalah untuk menggunakan bioarang diperoleh daripada daun teh terpakai sebagai biopenjerap untuk penyingkiran Malakit Hijau (MG) dan aplikasi kaedah gerak balas permukaan untuk mengoptimumkan pemboleh ubah proses. Semua analisis telah berjaya dilakukan dengan mengaplikasikan reka bentuk komposit berpusat (CCD). Kapasiti penjerapan bagi biorang daun teh terpakai (STL) telah dikaji melalui gabungan kesan parameter seperti dos bahan penjerap, kepekatan awal pewarna dan masa sentuhan. Penjerapan maksimum pewarna MG, pada kadar 98.76 % telah dicapai dengan menggunakan bioarang STL. Analisis statistik telah dilakukan menggunakan ANOVA yang menunjukkan hubungan korelasi parameter eksperimen yang baik dengan nilai R^2 adalah 0.9854. Data eksperimen adalah sesuai dengan model polinomial empirikal tertib kedua. Pengoptimuman berangka menunjukkan keadaan operasi optimum bagi dos bahan penjerap adalah 0.18 g, 46.92 mg/L kepekatan awal pewarna dan 56.16 minit masa sentuhan pada kebolehinginan 1.000. Pencirian fizikal pewarna MG, serbuk STL dan bioarang STL telah dikaji menggunakan analisis FTIR dan SEM. Bioarang daun teh terpakai telah didapati sangat berkesan untuk penyingkiran pewarna MG daripada larutan akueus.

Kata Kunci: Daun teh terpakai, bioarang, penyingkiran pewarna, Malakit Hijau (MG), Kaedah Gerak Balas Permukaan.

Optimisation of Process Variables by Response Surface Methodology (RSM) for Malachite Green Dye Removal Using Spent Tea Leaves Biochar

ABSTRACT

Due to simple preparation, spent tea leaves lead to disposing problem and causes accumulation of agricultural wastes. Conventional methods for the effluent treatment are expensive and have low removal efficiency. Hence, the utilisation of agricultural waste to an added value product which known as biosorbent is suggested due to its reliability and affordability. This study is aimed to use biochar derived from the Spent Tea Leaves (STL) as a biosorbent for Malachite Green (MG) dye removal and the application of Response Surface Methodology (RSM) to optimise the process variables. All analysis was successfully done by applying Central Composite Design (CCD). The adsorptive capacity of spent tea leaves biochar were investigated under combined effects of parameter such as adsorbent dosage, initial dye concentration and contact time. Maximum MG dye adsorption of 98.76 % was achieved using STL biochar. The statistical analysis was performed by ANOVA which indicated good correlation of experimental parameter with R^2 of 0.9854. The experimental data was fitted to the empirical second-order polynomial model. Numerical optimisation showed the optimum operating conditions of adsorbent dosage was 0.18 g, 46.92 mg/L of initial dye concentration and contact time of 56.16 minutes with desirability of 1.000. Physical characterization of MG dye, STL powder and STL biochar was studied using FTIR and SEM analysis. Spent tea leaves biochar was found to be very effective for the removal of MG dye from aqueous solution.

Keywords: Spent tea leaves, biochar, dye removal, Malachite Green (MG), Response Surface Methodology.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Environmental pollution has become a global phenomenon because of its adverse impact on human health and animals (David &Rajan, 2014). Industrialization and urbanization is the main factor of this pollution matters. Water pollution happened because of the spillage of dyeing industries to the water bodies. Considering the fact that the dyeing process was recognized as one of the most environmentally unfriendly industrial process, the best alternative and eco-friendly method is needed in order to put this problem to a minimum level.

Malachite Green (MG) dye is applied in textile, leather, cotton, silk, paper, food and acrylic industries. It is also used in the treatment of parasites, fungal, and bacterial infections in fish and act as an antiseptic to wounds and ulcers (Mohammadi e*t al.*, 2014). Nowadays, MG dye has become a highly controversial compound due to its genotoxic and carcinogenic properties (Wan Ngah *et al.,* 2010). Therefore, it is important to eliminate the MG dye from effluents before being discharged into the water bodies.

Adsorption is the most effective ways to treat the wastewater from several inductries. Agricultural wastes as adsorbent have recently gained importance due to its comparable removal efficiency and it is cost effective. In this context, agricultural wastes that have a huge possibility to be expanded as a cost effective adsorbent include banana peel, rice husk, wheat bran, corn seed and spent tea leaves.

Tea is an aromatic beverage drink by almost all the people in the world. It is approximately in the range of 18-20 billion cups of tea are being drink everyday (Ghosh e*t al.*, 2015). Due to the simple preparation, the spent the leaves lead to disposing problem (Hameed, 2009). Therefore, it is necessary to utilize it as an eco-friendly adsorbent to treat the dye effluents from several industrial activities.

Biochar is a carbon-rich product which heated to the point of thermal decomposition without the presence of oxygen from organic biomass. Production of biochar not only can utilise the natural resources but at the same time convert the waste into value-added product. Biochar have high adsorption capacities to adsorb cations and anions from aqueous solutions. Biochar is proven with a great affinity for the adsorption capacity compared to other adsorbent (Howard, 2014).

Response Surface Methodology (RSM) usually used for modelling and analysing the interaction effect of experimental factors towards the response based on the series of statistical technique (Kaith *et al.*, 2014). Nowadays, this method is applied in chemical engineering and applied science to optimise experimental variables (Garg e*t al.*, 2009). The most widely process modelling applied in experimental design are Central Composite Design and Box-Behken Design.

1.2 Problem Statement

Water pollution is one of the leading environmental issue triggering serious problems for living organism. The main source of environment contamination is dye pollutants that come from different industries. Hence, the effluents should be treated to specified level before being discharged. Over the years, most industries use activated carbon as adsorbent agent for dye removal. However, this method has high generation cost and environmentally unfriendly. Therefore, an alternative to remove MG dye is using agricultural waste like spent tea leaves biochar as adsorbent. Spent tea leaves biochar have significant capability to eliminate MG dye from effluents as it improve total surface area of tea leaves which lead to higher pores volume.

1.3 Research Objectives

The objectives of this study are:

1. To study the removal of Malachite green (MG) dye from aqueous solution using spent tea leaves biochar as adsorbent.
2. To investigate the combined effects of parameters which are initial concentration of Malachite green (MG) dye, adsorbent dosage and contact time on adsorption process.
3. To develop predictive models for the optimisation of Malachite green (MG) dye removal using Response Surface Methodology (RSM).

1.4 Scope of Study

The scope of this study is to determine the removal of Malachite green (MG) dye using spent tea leaves biochar as adsorbent. The adsorption process was studied under various parameters which are initial concentration of MG dye, adsorbent dosage and contact time. The concentration of dye in the solution was determined using UV-Vis Spectrophotometer. Surface morphology of the biochar was studied using Scanning Electron Microscope (SEM). Fourier Transfer for Infrared (FTIR) was used to study the physical characteristics of spent tea leaves biochar. The experimental data obtained was analysed using Response Surface Methodology (RSM) in Design Expert Software.

1.5 Significant of Study

The findings of this study will be a significant endeavour in contribution to the community. This study is likely to utilise the natural resources to remove the dye from effluents. Furthermore, this study could be importance to overcome disposing problem of the spent tea leaves. Nevertheless, conversion of spent tea leaves into biochar can reduce the waste disposal cost and even convert these wastes into value-added product. Moreover, this study should increase the awareness towards the environment of using the non-toxic, environmental friendly and low-cost agricultural waste as an adsorbent. Last but not least, this study potentially will educate the community regarding the green chemistry approaches for the nature sustainability.

CHAPTER 2

LITERATURE REVIEW

2.1 Malachite Green (MG) Dye

2.1.1 Characteristics of Malachite Green (MG) Dye

Malachite green (MG) dye is water soluble cationic dyeing material which exists in green crystalline powder. Besides, it is classified as triphenyl methane dye. Malachite green (MG) is extensively used for dyeing purposes. Malachite green is referred to chloride salt which have the chemical formula of $[C_6H_5C\ (C_6H_4\ (CH_3)_2)_2]Cl$ as shown in Figure 2.1 Table 2.1 shows the properties of Malachite green (MG) dye.

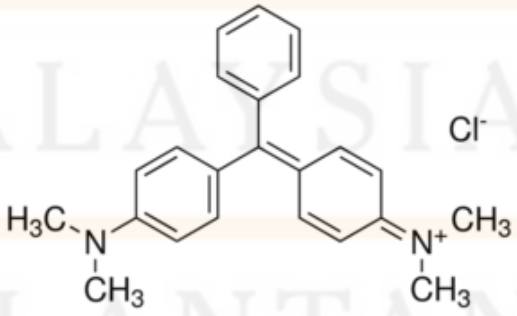


Figure 2.1: Chemical Structure of Malachite green (MG) (Raval e*t al.*, 2017).

Table 2.1: Properties of Malachite Green (MG) Dye

Properties	**Description**
Chemical Name	Malachite Green Chloride
IUPAC Name	[4-[[4-(dimethylamino)phenyl]-phenylmethylidene]cyclohexa-2,5-dien-1-ylidene]-dimethylazanium;chloride
Molecular Formula	$C_{23}H_{25}N_2Cl$
Molecular Weight	364.91 g/mol
Appearance	Green crystalline

(Source: Raval*et al.*, 2017).

2.1.2 Benefits of Malachite Green (MG) Dye

There are several industries used MG dye as the colouring agent including textile, leather, cosmetics, paper, plastics, and food products. Nowadays, MG dye used as a therapeutic agent in aquacultures and animal husbandry to treat fungal, bacterial, parasites infection. Besides, MG dye is widely used as an antiseptic to treat wounds and ulcer for external application only (Mohammadi *et al*., 2014). Moreover, Malachite green (MG) dye also been used as an active agent to fight infections in fish eggs in commercial aquaculture. In addition, MG and its reduced form, lecomalachite, may persevere in edible fish tissues for the longer shelf life (Shedbalkar & Jadhav, 2011).

2.1.3 Harmful Effects of Malachite Green (MG) dye

Despite various benefits, Malachite green (MG) dye also has harmful effects towards the living organism.MG dye can cause detrimental effect on marine fauna organ such as liver, gill, kidney, intestine and gonads. Any skin contact with MG dye will cause redness and pain. It also can cause permanent damage for human eyes, if it gets into the eyes. When human breathe in or consumed MG dye, it might cause pain and irritation or even can cause cancer (Santhi *et al.*, 2015). Thus, MG cannot be consumed by human because it can cause severe impact on human liver, brain, kidney, nervous system, and reproductive system.

2.2 Adsorbent

2.2.1 Tea Leaves

Tea plant is came from the Theaccae family which grown in about 30 countries worldwide. Tea is the beverage that most widely drinks in the world after water. Tea is available in various types including Oolong, green, and black tea. The mass of tea produced in a year is approximately 78% of black tea, 20% of green tea and 2% of Oolong tea (Peng *et al.*, 2013). After being consumed, most of the spent tea leaves are disposed as waste products. Hence, the spent tea leaves become plentiful and thus it is a good alternative to recycled it in order to avoid accumulation of agricultural wastes and converse our environment.

2.2.2 Importance of Tea Leaves

Recent medical research has stated that tea plant has a lot of health benefits including anticancer, antibacterial, anti-inflammatory properties and it helps in weight loss. The consumption of tea will prevent several devitalizing human diseases such as keeping up of cardiovascular and metabolic health. Moreover, polyphenolic compound in tea plant has good relationships with cardiovascular health especially atherosclerosis and coronary heart disease. The polyphenolic compounds in tea plant which consist of catechins and flavins are responsible in the prevention of chronic diseases such as cancer and cardiovascular disease (Khan & Mukhtar, 2013).

2.2.3 Biochar

Biochar is the product of organic material that undergo without the presence of air which known as pyrolysis process. Nowadays, the application of biochar is widely used by public to mitigate climate change, improve soil condition, reduce the pollution and for bio material utilization purposes. According to previous studies, it is proved that biochar able to increase the growth of plants in term of modifying the soil properties includes pH, moisture holding capacity and cation exchange capacity (CEC). In additional, biochar also it can improve the availability of plant nutritional value and increase the rate of seed germination (Edenborn *et al*., 2017).

Biochar is produced using several techniques from different types of feedstock and result in substance with specific properties which give corresponding impacts on soil (Edenborn*et al.*, 2017). The characteristics of biochar are specifically depends on the type of biomass used and the thermal decomposition condition. Consequently, preparing the suitable condition has been the crucial thing in the production of biochar(Tran e*t al.*, 2015). Hence, biochar can be said as a prospective applicant to treat the wastewater due to its porous structure which led to high surface area, large pore volume and the ample of functional groups.

Unlike others adsorbent, biochar is a stable carbon-enriched and has porous structure comparable to activated carbon. However, biochar appears to be the latest alternative which is cost effective and tend to have high efficiency of adsorption rate compared to activated carbon. This is because production of the activated carbon required a lot of energy in term of temperature and auxiliary activation method. In comparison, the preparation of biochar is cheaper as well as lower energy requirements. Moreover, the sources of biochar are abundant which can be easily obtained from the biomass and agricultural waste (Tan *et al*., 2015).

Table 2.2 shows the percentage removal of several dyes using Spent Tea Leaves (STL). Untreated STL tend to yield lower percentage of dye removal compared to the treated STL. The treated STL has potentially high percentage due to the modification of the STL such as acid-alkali treatment or conversion into activated carbon.

Table 2.2 Removal of dye using Spent Tea Leaves (STL)

Type of Dye Used	Treatment	Percentage of Removal	Reference
Reactive Blue (RB 19) Reactive Red (RR 120) Reactive violet (RV 15) Reactive Green (RG 19)	Untreated STL at 25ºC	Below 7%	(Zuorro *et al.*, 2013)
Reactive Blue (RB 19) Reactive Red (RR 120) Reactive violet (RV 15) Reactive Green (RG 19)	Activated STL at 30ºC for 1 hour	68.5% - 98.4%	(Zuorro *et al.*, 2013)
Malachite green (MG)	Untreated	89%	(Indolean *et al.*, 2017)
Malachite green (MG)	Treated with acid & alkali	92% - 95%	(Indolean e*t al.*, 2017)
Methylene blue (MB)	Untreated STL at 30ºC	43.4% - 95.5%	(Hameed, 2009)

2.3 Adsorption Process

The most commonly conventional method used for the removal of dyes are oxidation, ozonation, membrane filtration and microbial degradation (Ranjan, 2015). Among of these techniques, the most effective technique is adsorption. Currently, adsorption process is used due to its simplicity, high sorption capacity, non-toxicity, and environmental friendly technique to remove dye from effluents. Figure 2.2 shows the adsorption mechanism which involved the adsorbate and the adsorbent.

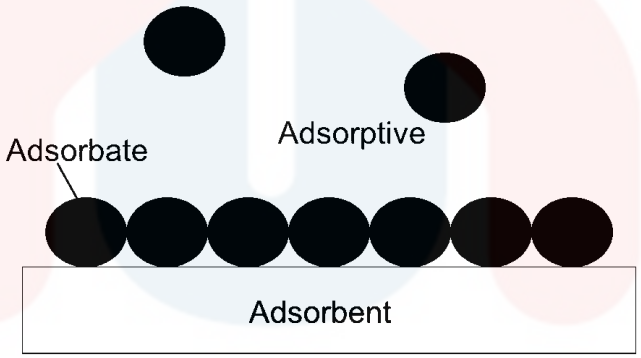


Figure 2.2: Adsorption Mechanism

Adsorption process is the building up of molecules on a surface layer. It can be occurred between liquid-liquid phase, liquid-solid phase, gas-solid phase or gas-liquid phase. All adsorption process is depends on the equilibrium between the fluid and solid phase. The adsorption process depends on the molecular weight, shape or polarity of the molecules. Usually, adsorption process is applied for purification to remove small amount of pollutants or for bulk separation. This process involved two main things which are adsorbate, the substance that being adsorbed on the surface of another substance and adsorbent is the substance that adsorption process occurred (Safa, 2015).

2.3.1 Effect of Initial Dye Concentration

Initial concentration of adsorbate is the main influence that contributes to the percentage removal of dyes. When initial dye concentration increased it will caused decreasing in the percentage removal of dyes. This condition is due to the saturation of adsorption site on the surface of adsorbent. The percentage of dye removal tends to be elevated in the beginning of the adsorption process is because of the high driving force for mass transfer which lead to a higher loading capacity of the adsorbent (Polipalli & Pulipati, 2013). However, the subsequent lower in the percentage removal of dye might due to the insufficient binding sites on the adsorbent surface.

2.3.2 Effect of Adsorbent Dosage

The adsorbent dosage is also affects the percentage of dye removal. The percentage removal of dyes increased as the adsorbent dosage increased. When the adsorbent dosage is increased, it will lead to larger surface area or adsorption site (Liu *et al*., 2011). Until to one extend, there will be no significant increasing in percentage of removal. This is because the dye concentration has achieved the equilibrium state. However, when the adsorbent dosage is increased, it will result in decreasing in adsorption capacity. This is might due to the movement is against the concentration gradient of the adsorbent (Rajamohan e*t al.*, 2014).

2.3.3 Effect of Contact Time

Adsorption process also depends on the contact time corresponding to adsorbent and adsorbate. Usually, adsorption rate is occurred efficiently at the beginning of the process. The higher adsorption rate was caused by the larger exchangeable sites on the adsorbent surface at the initial stage of the adsorption process. The adsorption capacity is consistently increased at the first uptake of the process. Unfortunately, as the contact time increased, the adsorption rate will decreased and almost constant. This is because equilibrium time has achieved due to the accumulation of adsorbate on the adsorption site (Al-Rashed & Al-Gaid, 2012).

2.4 UV-Vis Spectrophotometer

UV-Vis spectrophotometer is used to determine liquid concentration for certain period of time. This instrument is extensively used in research and experiment. It is applied in qualitative analysis for the identification of chemicals and quantitative determination of various organic and inorganic solutions. Apart from that, this instrument can be applied to evaluate the adsorption of light pass through the ultraviolet and visible light wavelength via aqueous sample. The measurement can be performed by scanning over a range in the spectrum or either at single wavelength (Royal Society of Chemistry, 2009).

2.5 Physical Characterization of Biochar

2.5.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is an analytical used mainly to identify organic materials. FTIR is an ideal tool to identify the functional group that able to remove dyes. FTIR spectrometry is based on interference of waves of light or radiation. FTIR spectrometer may be considered to consist essentially of three parts namely, an optical system, a computer system, and software for controlling the hardware and processing the recorded data. FTIR analysis was carried out within the range of 500-4000 cm^{-1} to determine the functional groups which involved in the adsorption process (Tasumi & Sakamoto, 2015).

2.5.2 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is a potential tool used to study the morphology solid particles. It is used to identify the structural variations in biochar particles after thermal treatments. Practically, the SEM images were used to determine the precise information about the porous of biochar. Apart from that, SEM consists of two condenser lenses, a set of deflectors, an electron gun, and an electron detection system. The source of electrons will be delivered by the electron gun and accelerates them to energies which ranges from 1 to 30 kV. Furthermore, SEM displays the images with high resolution which is a high quality images (Hafner, 2007).

2.6 Response Surface Methodology (RSM)

Response Surface Methodology (RSM) is a combination of mathematical and statistical method which commonly used for modelling and analysing situation for the optimization process of various parameters. It is used for the utilisation in the sorption process which involved the process variable and response function. Moreover, RSM is considered efficient because it is low cost generation and able to optimise complex experiment (Jain *et al.*, 2011).This is because optimisation studies using RSM as an experimental design able to help in reducing generation cost of expensive analysis including reduce the total of experimental run and can save a lot of time compared to other method (Ghosh *et al.*, 2015).

In application, RSM helps to predict a model for the process, determine feasible interactions, and identify the optimum operational conditions. It has been widely used in various field of studies such chemistry, biochemistry, chemical engineering, and many more. Generally, the optimisation process involve three major step include (1) designing the experiment, (2) evaluating the coefficients in experimental model, and (3) predicting the response and determining the acceptability of the model (Mondal *et al.*, 2013). It is an useful tool for modelling and analysing multi-factor experiment (Kaith *et al.*, 2014). The most commonly used of Response Surface Methodology (RSM) are Box-Behken design and Central Composite design.

2.6.1 Central Composite Design (CCD)

The crucial aspect when carried out RSM is the choice of suitable design for the experimental process which contributes huge impact on the constructing the response surface and also its accuracy on the estimation. To predict the interaction between the factors, there is a requirement, dependable and common design which can be effectively applied. Moreover, this technique executes the lowest set of trial for an experimental runs, enhancing statistical inference feasibility and indicating variables interaction (Rosales *et al.*, 2012). Figure 2.3 shows the Central Composite Face-Centered (CCF) design.

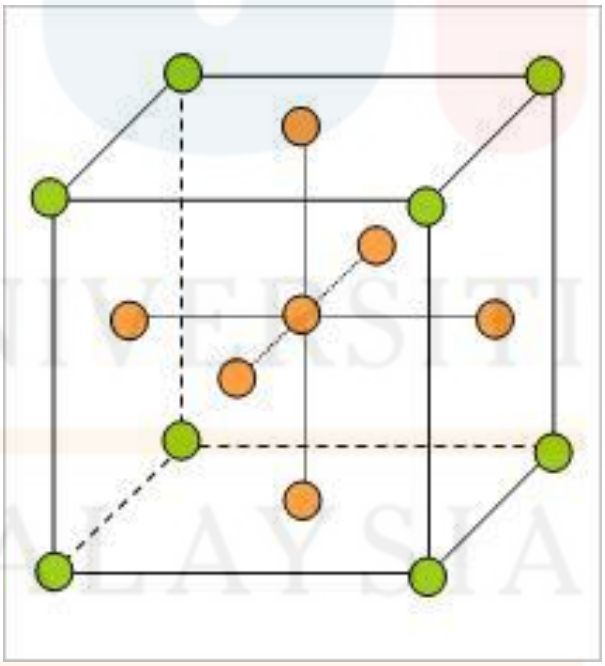


Figure 2.3: Central Composite Face-Centered (CCF) (Sibanda & Pretorius, 2013).

In this CCD design, there is a component that plays the utmost important role which is two-level factorial. This two-level factorial is consists with axial point, factorial point and center runs. The factorial point is represented the variance-optimal design for the first order. Meanwhile, the center runs is to provide the information regarding the existence of curvature in the system. The function of the additional axial point is to allow the effectiveness approximation of the actual quadratic terms. From this perspective, face-centered type of central composite design is used to fit the model with second-order response surface (Sibanda & Pretorius, 2013).

Central Composite Design (CCD) is the most popular model used in Response Surface Methodology (RSM). It has been widely employed mathematical technique which involved the several of nonlinear model for the optimization of process parameter of adsorption process. In addition, CCD also used to identify the regression model equations and operating condition of complex process (Sadhukhan *et al.*, 2016). Other than that, CCD is appropriate in illustrating the quadratic surface and it helps to optimise the functional factors (Kumar & Ahmad, 2011). CCD model also can estimate the curvature due to the existence of an imbedded factorial and fractional factorial design with point that augmented with a group of axial point (Singh e*t al.*, 2010).

Table 2.3 shows the application of RSM in adsorption process for the removal of several dyes using different types of adsorbent. The most commonly used model in the application of RSM is Central Composite Design (CCD). There is also the correlation coefficient (R^2) for each adsorption process. This value will determine the goodness of the fit of the model.

Table 2.3 Application of RSM in Adsorption Process for the Removal of Dye

Type of Absorbent	**Type of Dye**	**Model of RSM**	**R^2 Value**	**Reference**
Spent Tea Leaves	Cu (II) by alkali modified	CCD Model	0.9983	(Ghosh *et al.*, 2015)
Chemically Rice Husk	Crystal Violet	CCD Model	0.9945	(Saha *et al.*, 2012)
Defatted Papaya Seed	Copper (II) and Lead (II)	CCD Model	0.987	(Garba *et al.*, 2016)
Aleppo Pine Cones	Methylene Blue	BBD Model	0.965	(Elmoubarki *et al.*, 2017)
Wheat Bran	Methyl Red	BBD Model	0.998	(Ranjan, 2015)
Bauhinia purpurea leaves	Methylene Blue	CCD Model	0.970	(Polipalli & Pulipati, 2013)
Plumbagozeylancia Leaves	Malachite green (MG)	CCD Model	0.998	(Pallavi *et al.*, 2018)
Eucalyptus Wood Biochar	Malachite green (MG)	BBD Model	0.994	(Singh *et al.*, 2016)
Eggshell Biochar	Malachite green (MG)	CCD Model	0.9542	(Mohamad *et al.*, 2017)

CHAPTER 3

METHODOLOGY

3.1 Material and Chemicals

The samples that used for this study was spent tea leaves. The spent tea leaves were collected from the cafeteria at University of Malaysia Kelantan, Jeli Campus. The chemicals used were Malachite green (MG) dye and distilled water.

3.2 Apparatus and Equipments

The apparatus used for this study were conical flask (250 mL), beaker (250 mL & 500 mL), measuring cylinder (5 mL, 10 mL & 50 mL), volumetric flask (50 mL & 1000 mL), micropipette (1000µL), blue tips, crucible and lid, cuvette, spatula, air-tight zip bag, aluminium foil, filter papers, tissue paper and hand gloves. The equipments used were oven, blender, analytical balance, sieving machine, furnance, shaker, UV-Vis Spectrophotometer, Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). The software used for this study was the Design Expert (Version 7.0).

3.3 Preparation of Spent Tea Leaves Biochar

The material that used for this study was spent tea leaves. The spent tea leaves were collected from the cafeteria at University of Malaysia Kelantan, Jeli Campus. The spent tea leaves were rinsed using distilled water to remove any dirt or impurities. Then, the samples were placed in an oven at 80ºC for 24 hours until the spent tea leaves completely dry. After drying process, the spent tea leaves were grinded to form powder. Next, the spent tea leaves were sieved to obtain a standard particle size of the tea leaves powder. After that, the tea leaves powder was heated using furnace at 600ºC for 4 hours. When the tea leaves completely turned dark grey, the biochar were collected in air-tight zipper bag and stored in dry environment.

3.4 Physical Characterization of Biochar

For this study, two types of samples used which were spent tea leaves powder and spent tea leaves biochar to analyse the change in properties of the adsorbent. Fourier Transform Infrared Spectroscopy (FTIR) was used for the identification of functional group of organic compound present in the biochar. Based on this technique, the type of the functional groups was identified by studying the peak between the particular gap and band. Besides, the morphology character of the porous spent tea leaves biochar was characterized using Scanning Electron Microscopy (SEM).

3.5 Preparation of Malachite Green (MG) Dye Solution

1 g of MG powder was weighed using analytical balance and dissolved in 1000 mL volumetric flask with distilled water to produce 1000 mg/L standard MG solution. The solution was shaken well until all the MG completely dissolved. Different concentrations of MG solutions were prepared from the standard MG solution using dilution method. The stock solution was kept inside a reagent bottle to prevent from degradation of light.

3.6 Preparation of Malachite Green (MG) Calibration Curve

Six different concentration of MG dye (0.5 mg/L, 10 mg/L, 20 mg/L, 30 mg/L , 40 mg/L & 50 mg/L) were prepared from standard MG solution using dilution method. The absorbance reading of each concentration of MG solution was determined by using UV-Vis spectrophotometer with the wavelength of 617 nm. The measurement was repeated 3 times and the average was calculated. The average reading then was used to plot the calibration curve for MG solution. The linear calibration curve was used to calculate the concentration of MG solution after adsorption process.

3.7 Batch Adsorption Studies

Batch adsorption studies were carried out using spent tea leaves biochar powders as adsorbent. The constant parameters in this study were agitation speed of orbital shaker (150 rpm), pH of 7, volume of MG solution (50 mL) and the temperature fixed at 37ºC. Meanwhile, three manipulated parameters were used includes adsorbent dosage, initial dye concentration and contact time.

For the manipulated parameter, the range of initial concentration of MG dye is 25 mg/L to 100 mg/L. The adsorbent dosage used was between 0.02 g to 0.20 g. The contact time used was between 10 minutes to 60 minutes. Next, these values were inserted into Design Expert Software. The experimental design used in Response Surface Methodology (RSM) is Central Composite Design (CCD).

In CCD, the "Numeric Factors" was represented the manipulating parameter which is 3 while "Categoric Factors" was remained 0. After that, the detail about each of the parameters with maximum and minimum value was included using CCD model as shown in Table 3.1.

Table 3.1 Process Variables and their Levels

Variables	Name	Units	Actual Factors		
			Low	Middle	High
A	Adsorbent Dosage	G	0.02	0.11	0.20
B	Initial Dye Concentration	mg/L	25.0	62.5	100.0
C	Contact Time	Min	10.00	35.00	60.00

3.8 Analytical Method Using UV-Vis Spectrophotometer

In this study, Hach DR 6000 UV-VIS Spectrophotometer was used to measure the absorbance reading of MG solution. The wavelength used for the determination of the absorbance reading of MG solution was 617 nm. The concentration of each MG solution was determined using the calibration curve of MG dye. The percentage of MG removal was calculated using Equation 3.1. The percentages of MG removal were inserted as the response in Design Expert Software.

$$\text{Percentage of MG Removal (\%)} = \frac{ci - cf}{cf} \text{ x } 100\ \% \quad (3.1)$$

Where, ci is initial concentration MG dye (mg/L)

cf is final concentration MG dye (mg/L)

3.9 Experimental Design Using Response Surface Methodology (RSM)

Design of experiments (DOE) is an efficient procedure for planning experiments so that the data obtained can be analysed. The main objectives of this experimental design are to evaluate the effect of the various variables towards the response and to find out the optimum operating parameters. According to this study, the parameters used including adsorbent dosage (g), initial dye concentration (mg/L) and contact time (min) were deliberately changed in order to determine the interaction of variables towards the percentage of MG removal (%).

Apart from that, the experimental design of the adsorption of Malachite green (MG) dye was carried out using the response surface methodology (RSM). It was applied for the utilization in the sorption process which involved the process variable and response function. Furthermore, RSM helps to predict a model for the process, identify possible interactions, and determine the optimum operational conditions (Mondal*et al.*, 2013).

In this study, the Central Composite Design (CCD) was used for the optimization process of the removal of Malachite green (MG) dye by spent tea leaves biochar as adsorbent. CCD model is an ideal design tool for sequential experimentation. This is because, it allows doing the testing on the lack of fit when there is an adequate number of experimental values are available. Then, the experimental data obtain is analysed using Design Expert Software (Version 7.0).

The utilization of RSM for the assessment of the parameters shows the relationship between the response and the variables. The percentage of MG removal (%) was inserted as the response in Central Composite Design (CCD). The CCD model was proceed with the face centered option whereas alpha (α) equal to 1 was chosen. In this manner, there were 20 experimental runs conducted. These experimental runs were repeated three times in order to obtain the accurate reading of the MG dye concentration after adsorption process using UV-Vis Spectrophotometer. A series of experimental runs generated using CCD model were shown in Table 3.2.

Table 3.2: Experimental runs using CCD model

Std Run	Factor 1 A: Adsorbent Dosage (g)	Factor 2 B: Initial Dye Concentration (mg/L)	Factor 3 C: Contact Time (min)
1	0.11	62.50	10.00
2	0.11	62.50	35.00
3	0.20	100.00	10.00
4	0.11	62.50	35.00
5	0.11	25.00	35.00
6	0.11	62.50	35.00
7	0.11	100.00	35.00
8	0.20	62.50	35.00
9	0.11	62.50	60.00
10	0.20	100.00	60.00
11	0.20	25.00	60.00
12	0.20	25.00	10.00
13	0.11	62.50	35.00
14	0.02	25.00	10.00
15	0.02	100.00	60.00
16	0.02	25.00	60.00
17	0.11	62.50	35.00
18	0.11	62.50	35.00
19	0.02	62.50	35.00
20	0.02	100.00	10.00

3.10 Optimisation Studies

Optimisation of MG adsorption was carried out using Design Expert software (version 7.0). In this optimisation study, the desired goal for each process variables and response was chosen. The performance of the process variables were evaluated by analysing the response of MG removal efficiency. Response surface methodology (RSM) was applied in order to determine the interaction of various parameters and also to obtain the optimum condition for the MG removal using spent tea leaves biochar.

After 20 experimental runs were conducted then the percentage of adsorption were calculated. Next, the data were inserted into the CCD model at the response option and the optimisation result was generated. There may be two or more maximums because of curvature in the response surfaces and their combination into the desirability function. Apart from that, the optimisation study was included the analysis of adsorption variables in term of effect of the adsorbent dosage, initial dye concentration and contact time.

Moreover, the interaction of each parameter towards the adsorption process was also analysed through ANOVA from the CCD as well. The two-dimensional contour and three-dimensional curves of the response surface were developed. Thus, the optimum point of the study was figured out. Other than that, there is also the development of regression model equation based on the CCD model.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Calibration Curve

Calibration curve was conducted to interpret the instrumental response to an analyte and estimate the concentration of analyte of a sample. Theoretically, a batch of standard held a known quantity was prepared, then the absorbance reading of each standard was measured and the relationship of absorbance reading and analyte concentration was validated. The measurements obtained then converted using the relationship to determine the quantity of analyte present. Table 4.1 shows the average absorbance readings of different MG concentration (mg/L).

Table 4.1: The average absorbance readings of different MG concentration

MG Concentration (mg/L)	Average Absorbance Reading
0.5	0.0020 ± 0.0000
10.0	0.0323 ± 0.0020
20.0	0.0620 ± 0.0020
30.0	0.0997 ± 0.0032
40.0	0.1357 ± 0.0021
50.0	0.1717 + 0.0025

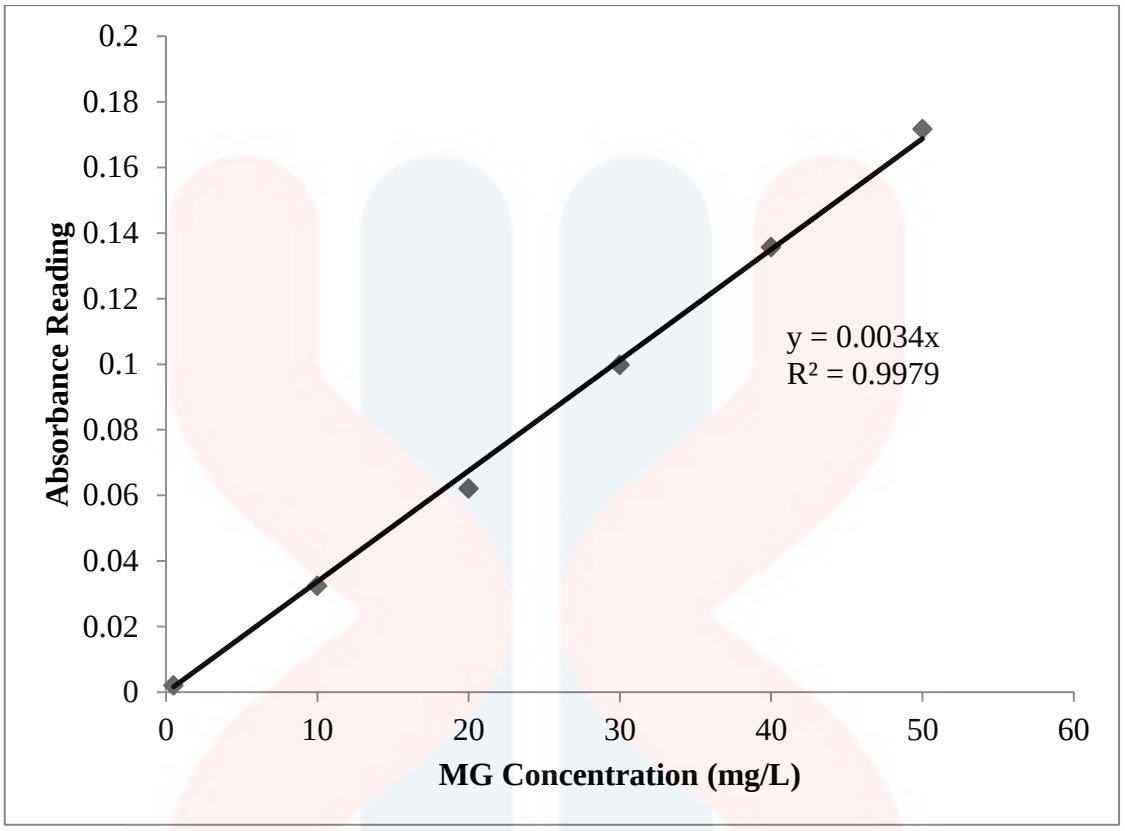


Figure 4.1: Calibration Curve for Malachite Green (MG) Dye

Figure 4.1 shows the calibration curve for Malachite Green (MG) dye. Based on the figure, the relationship between the absorbance reading and the MG concentration was linear relationship. When the relationship of the calibration was linear, the slope was a degree of sensitivity. The larger the slope with a steeper gradient line, it demonstrates more sensitive measurement. The relationship can be described by the equation of the line, y = 0.0034x, where 0.0034 is the gradient of the line. Meanwhile, the coefficient of determination, R^2 = 0.9979. This number indicates how well the data fit with the experimental data. The absolute line would have the R^2 value of 1. The nearer the R^2 value to 1, the more fit the linear curve with the experimental data.

4.2 Adsorption Studies

The experimental design used in this study was Central Composite Design (CCD) with 3 factors of input variables. There are several types of CCD options that can be studied including rotatable, spherical, orthogonal quadratic, practical and face-centered. Each of the CCD options will generate different value of alpha (α), which determines the level of factor. Besides, the level used in CCD model can be in three levels or five levels design. In this study, a face-centered CCD model was chosen and α distance is setting to one, α = 1. This indicates that each factors of this study has only three levels which are low, middle and high. To replace the factor values, coded factors such as -1 and +1 were used in the experimental design. Coded factors were used to determine the relationship of each factor on the outcome of a process on a simplified numerical basis. The coded value of experimental parameters design with coded factor was shown in Table 4.2.

Table 4.2: Experimental parameters design with coded factors

Factors	**Name**	**Units**	**Coded Factors**		
			Low	**Middle**	**High**
A	Adsorbent Dosage	g	-1	0	+1
B	Initial Dye Concentration	mg/L	-1	0	+1
C	Contact Time	min	-1	0	+1

In adsorption studies, the interaction of three factors towards the percentage of MG removal including adsorbent dosage, initial dye concentration and contact time were selected as factors in the CCD model. Furthermore, there are several constant variables used to generate the appropriate conditions for the adsorption of MG dye. The constant variables that used in this study were the volume of aqueous dye solution (50 mL), temperature (37 ºC), pH of 7 and agitation rate of 150 rpm.

The purpose of agitation during the adsorption was to increase the kinetic movement of both adsorbate and adsorbent. Hence, it can increase the effective collision between particles which able to increase the efficiency of adsorption process. In adsorption kinetic studies, the adsorption rate increase significantly with the agitation speed. However, if high stirring speed were used, there might be a destruction occurred on the adsorbent structure (Kusmierek & Swiatkowski, 2015).

Theoretically, high temperature tends to result in greater adsorption capacity of dye. This is because of the ability of the particles to move at high temperature. However, the structure of the adsorbent might be damaged at high temperature and thus lead to failure of the adsorbent properties (Ngadi *et al*., 2013). The removal of Malachite Green dye using coffee waste was found to be optimum at 37 ºC with pH 7 (Rajeshkannan *et al*., 2011). Therefore, this optimum condition would be the most preferred in this study.

A total number of 20 experimental run were employed using RSM and the order of the experiment was arranged randomly. In this study, the percentage of MG removal was chosen as a response. This response will determine how much of the dye attached to the surface of biochar and was removed from the aqueous solution.

Table 4.3 shows the percentage of MG removal obtained from the experimental runs. The highest percentage of MG removal obtained was 98.76 % which operated in 0.20 g of adsorbent dosage, 100 mg/L of initial dye concentration and 60 minutes of contact time. All the factors condition was in the high level coded. This may indicated that high level of the parameter was more effective in adsorption process. This behaviour was caused by the increasing in vacancy of the binding site. If the adsorbent dosage was high, it will lead to greater amount of active site for binding process and result in higher percentage of dye removal (Liu *et al*., 2011).

The lowest percentage of MG removal was 69.33 %. It was reached when the lowest design parameter of adsorbent dosage which was 0.02 g with the highest design parameters at 100 mg/L of initial dye concentration and contact time of 60 minutes were used. The differences between the lowest and the highest percentage of removal were only due to the adsorbent dosage. On the other hand, the highest and lowest percentage of MG dye removal, although have similar condition of initial dye concentration and contact time but different in adsorbent dosage, thus the result demonstrate a huge gap for almost 29.43 %.

It was identified that the increment from 0.02 g to 0.20 g in the adsorbent dosage reduced the percentage of MG removal from 98.76 % to 69.33 %. This declined trend of the percentage of MG removal may be due to the inaccessibility of the dye molecules which can cover all the adsorption sites. In other words, a smaller surface area on the adsorption site cannot attain saturation state at lower dosages of the biochar (Huang *et al*., 2018). Moreover, this situation also may due to the spilt in the flux or the concentration gradient between the solute and solution on the surface of the adsorbent (Uddin *et al*., 2017).

Table 4.3: Experimental Response with coded factors

Std Run	Factor 1 A: Adsorbent Dosage	Factor 2 B: Initial Dye Concentration	Factor 3 C: Contact Time	Response: Percentage of MG Removal (%)
1	0	0	-1	87.67
2	0	0	0	92.06
3	+1	+1	-1	94.67
4	0	0	0	92.12
5	0	-1	0	89.78
6	0	0	0	92.65
7	0	+1	0	91.11
8	+1	0	0	96.09
9	0	0	+1	98.04
10	+1	+1	+1	98.76
11	+1	-1	+1	94.07
12	+1	-1	-1	73.63
13	0	0	0	92.53
14	-1	-1	-1	76.60
15	-1	+1	+1	69.33
16	-1	-1	+1	97.48
17	0	0	0	92.36
18	0	0	0	91.11
19	-1	0	0	84.06
20	-1	+1	-1	71.93

4.3 Actual Response versus Predicted Response for MG removal using CCD Model

Table 4.4 shows the actual values, predicted values and the standard error of this study. The actual values were the experimental data obtained from the experimental works, while predicted values were the generated data from the CCD model using approximating functions. The percentage of error among the actual and predicted value was calculated using Equation (4.1).

$$\text{Percentage of Error (\%)} = \frac{|Actual\ value - Predicted\ Value|}{Actual\ Value} \text{ x } 100\% \quad (4.1)$$

Based on Table 4.4, the highest actual percentage of MG removal was 98.76% whereas the highest predicted percentage of MG removal was 98.45% with 0.31 % differences. Meanwhile, for the lowest actual percentage of MG removal was 69.33% compared to the lowest predicted percentage of MG removal which was 70.69% with the differences of 1.36%. Thus, it can be considered that there was only a slightly differences between the experimental and theoretical percentage of MG removal.

According to Table 4.4, the percentage of error among the actual and predicted values all were within 0.16 % to 2.45 %, the value was considered to be low and acceptable. This is because the lower the percentage of error, the more accurate the data will be. This also indicates that the actual values was nearer to the predicted value and have only slightly differences towards the predicted value. Thus, the actual data can be fit well with the quadratic model from the CCD model.

Table 4.4: Actual values, predicted values and standard error for MG removal using CCD model

Run Order	Coded Factor			MG Removal (%)		Standard Error (%)
	A (Adsorbent Dosage)	B (Initial Dye Concentration)	C (Contact Time)	Actual	Predicted	
1	0	0	-1	87.67	86.55	1.28
2	0	0	0	92.06	92.80	0.80
3	+1	+1	-1	94.67	96.21	1.63
4	0	0	0	92.12	92.80	0.74
5	0	-1	0	89.78	90.03	0.28
6	0	0	0	92.65	92.80	0.16
7	0	+1	0	91.11	88.88	2.45
8	+1	0	0	96.09	94.87	1.27
9	0	0	+1	98.04	97.18	0.88
10	+1	+1	+1	98.76	98.45	0.31
11	+1	-1	+1	94.07	94.92	0.90
12	+1	-1	-1	73.63	72.77	1.17
13	0	0	0	92.53	92.80	0.29
14	-1	-1	-1	76.60	77.40	1.04
15	-1	+1	+1	69.33	70.69	1.96
16	-1	-1	+1	97.48	96.43	1.08
17	0	0	0	92.36	92.80	0.48
18	0	0	0	91.11	92.80	1.85
19	-1	0	0	84.06	83.30	0.81
20	-1	+1	-1	71.93	71.57	0.50

Figure 4.2 was represented the interpretation of the regression equation of the actual and predicted values. The actual values were distributed relatively near to the predicted values. This is because the experimental data and the predicted model were located so closed and indicating a correspondence between each other. The model also considered to be reasonable because it shows the correlation between the theoretical and experimental data. Thus, it implies that the suggested model fitted well to the regression equation and CCD model also can be effectively employed for the optimization process (Chen *et al.*, 2011).

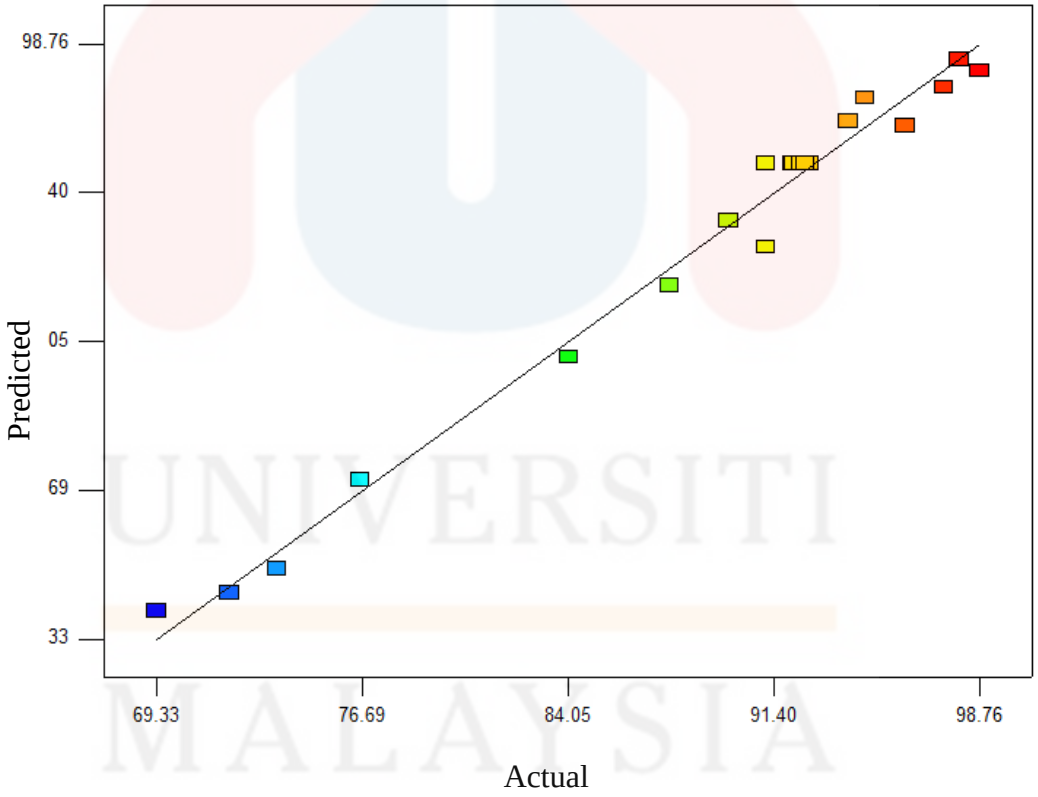


Figure 4.2: Linear correlation between predicted and actual values

4.4 Analysis of Residual for the Response

Figure 4.3 shows the residual values for each experimental run. The analysis of residual was an ideal diagnostic tool used to estimate adequacy of the fitted model for predicting the response. Based on Figure 4.3, it can be deduced that the residual values were evenly distributed from one another. This proves that the experimental data have smaller number of residual values and it considered to be acceptable. This is because the residues do not exceed the limitation throughout the experimental works. Hence, the suggested model was satisfactory as the residual values for predicting the response were lower than 3 %.

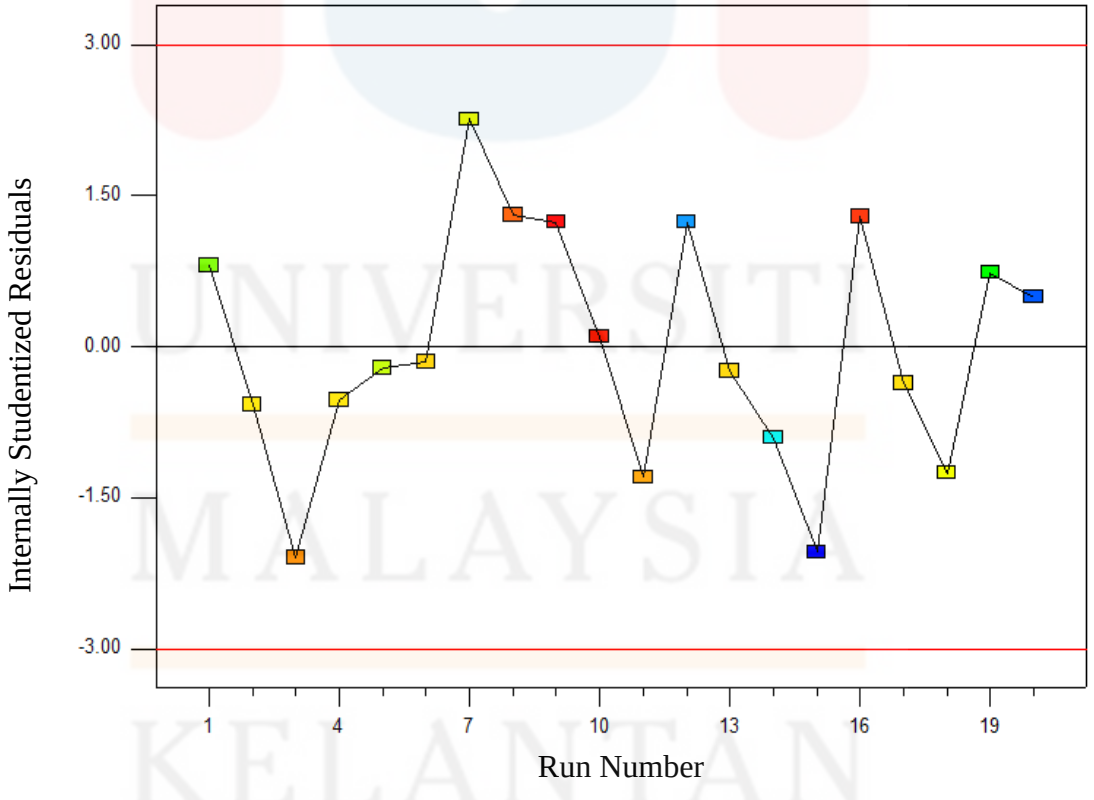


Figure 4.3: Analysis of Residual for the Response

4.5 Statistical Analysis

There were various types of model which can be generated from RSM model including linear, 2FI, quadratic and cubic model that might be fit to the response of this study. Based on the model summary statistics, the best model that fit the response was the quadratic model whereas the cubic model was aliased which mean that the cubic model was far away to fit the response which considered as the outlier model. The model summary of statistics generated by RSM for MG removal throughout the analysis was shown in Table 4.5.

Table 4.5: Model summary statistics generated by RSM for MG removal

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	Press	Remarks
Linear	7.51	0.4043	0.2926	-0.3200	2001.65	
2FI	4.61	0.8180	0.7341	-0.4599	2213.75	
Quadratic	1.49	0.9854	0.9722	0.8657	203.35	Suggested
Cubic	1.44	0.9918	0.9741	-7.8040	1335.25	Aliased

Based on the quadratic model, the standard deviation for the response was 1.49. For the correlation coefficient, R^2 value, it is an important source for the verification of the developed model. The R^2 value for the quadratic model was 0.9854. In statistical study, the nearer the R^2 value to 1, the better the model will be (Salman, 2014). Based on the study, the adjusted R-Squared and predicted R-Squared generated by the model were 0.9722 and 0.8657, respectively.

The goodness-of-fit of the model was analysed by coefficient of the estimation of R^2 (correlation coefficient) and adjusted coefficient of estimation R^2 adj. The greater result of the correlation coefficient R^2 = 0.9854 was considered relatively high which indicates the plausibility of the model in approximating the percentage of MG removal. The model can be explained by the response variability of 98.54 %. Therefore, the model implies a satisfactory agreement regarding the experimental value and the predicted values for the percentage of MG removal.

From the summary statistics table generated using RSM for percentage of MG removal, the model outcomes is focused on increasing the value "Adjusted R-Squared" and the "Predicted R-Squared". Apart from that, quadratic model also being selected for both lack of fit test and sequential model sum of squares. In term of lack of fit test, the model shows insignificant lack of fit where as for the sequential model sum of squares are sufficient and the model was not aliased. It can be stated that among of all the sources, quadratic model is suggested by the CCD for the response of MG removal.

Analysis of variance (ANOVA) is a statistical approach that separates the number of variation in asset of data into component partition with definite sources of variation to test the hypothesis on the variables of the model (Francesc & Julia, 2014). In this study, the analysis of variance (ANOVA) is plays an important role for the statistical significance testing of the curvature in the response surface quadratic mode at the confidence level of 95 %. Table 4.6 shows the ANOVA testing results for the response surface quadratic model.

Table 4.6: ANOVA for Response Surface Quadratic Model

Source	Sum of Squares	Df	Mean Square	F Value	P-value Prob> F	Remarks
Model	1494.18	9	166.02	74.79	< 0.0001	Significant
A-Adsorbent Dosage	326.04	1	326.04	146.87	< 0.0001	Significant
B-Initial Dye Concentration	4.20	1	4.20	1.89	0.1991	Not Significant
C-Contact Time	282.81	1	282.81	127.40	< 0.0001	Significant
AB	418.04	1	418.04	188.31	< 0.0001	Significant
AC	3.82	1	3.82	1.72	0.2188	Not Significant
BC	205.54	1	205.54	92.59	< 0.0001	Significant
A^2	41.35	1	41.35	18.63	0.0015	Significant
B^2	33.84	1	33.84	15.24	0.0029	Significant
C^2	1.50	1	1.50	0.67	0.4307	Not Significant

The values of the mean squares were evaluated by dividing the sum of squares of each variation sources by their degree of freedom, and a 95 % of confidence level ($\alpha = 0.05$) was used for the determination of the statistical consequences in all testing. By referring to Table 4.6, it suggested that the model was profoundly acceptable where it can be proven by the low probability value ($P < 0.0001$) with the *Fisher's F value* of 74.79. The F-value indicates that the model is applicable and the value of "Prob>F" which was less than 0.05 will demonstrate that the model terms are adequate. Thus, the model can be effectively applied with less error as it generates larger F-value with the associated of smaller P value.

The statistical significance of the model equation is assessed by the F-test ANOVA. The significance of each coefficient is resolved by F-values and P-values (Zhu *et al.*, 2013). The coefficient for the main consequence of the experimental parameters was statistically significant (P <0.0001). Then, the correlation coefficient (R^2) between the relationship of experimental value and predicted value was further checked for the determination of the goodness of fit of the suggested model. Table 4.7 shows the standard deviation and quadratic model for R^2 towards percentage of MG removal.

Table 4.7: Standard deviation and quadratic model for R^2 towards MG removal

Standard Deviation	1.49
Mean	88.80
C.V. %	1.68
PRESS	203.65
R-Squared	0.9854
Adj R-Squared	0.9722
Pred R-Squared	0.8657
Adeq R-Squared	25.874

In this case, the standard deviation for the response was 1.49 which considered to be satisfactory. The R^2 estimation of the recommended model was 0.9854, which revealed that the regression is statistically significant. The value of estimated multiple correlation coefficient (Pred R-Squared = 0.8657) was feasible with the estimation of the adjusted multiple correlation coefficient(Adj R-Squared = 0.9722). On the other side, the percentage of coefficient variance was (C.V. = 1.68 %). This demonstrates a better precision of the experimental works.

4.6 Development of Regression Model Equations

The development of the polynomial regression equation was generated according to the sum of square of the sequential model based on the highest polynomials order when the additional terms were sufficient (Sahu *et al.*, 2010). A polynomial regression modelling was applied in order to find out the responses and interaction of the corresponding coded factors where A represent for adsorbent dosage in g, B for initial dye concentration with the unit of mg/L and C for contact time in minutes towards the percentage of MG removal. The empirical formula for polynomial regression model that indicated MG removal, Y_1 with independent process parameters are expressed in Equation (4.2).

$$\text{MG removal, } Y_1 (\%) = 92.86 + 5.71A - 0.65B + 5.32C + 7.23AB + 0.69AC - 5.07BC - 3.88A^2 - 3.51B^2 - 0.74C^2 \quad (4.2)$$

Based on this study, CCD model generates empirical model expressed by a second-order polynomial equation in term of coded factor which reflects the interaction of variables towards response. Equation for coded factor in Equation (4.2) contains both coefficient with one factor and two factors. The sign of positive and negative before the terms represent the synergetic and antagonistic interaction for the experimental factors (Garba & Afidah, 2014). The expression of single factor in a term indicates one variable

impact, two factors represent double variables impacts and the quadratic effect signified a second order term of variables expression (Ahmad & Alrozi, 2011).

Coefficient with single factor indicates for the effect of the particular effect and the interaction effect with other factors. Meanwhile, coefficient with two factors represents the quadratic effect of factor. Prediction towards the percentage of MG removal by biochar can be made through the analysis of the polynomial coded equation. In coded equation, when position sign is present, it indicates that extend of the particular parameters, the higher the percentage of MG removal. Vice versa, negative sign means that the increasing of the particular parameter will adversely affect the percentage of MG removal.

By referring to Equation (4.2), the independent parameter A (adsorbent dosage) and C (contact time) shows a positive effect towards the efficiency of MG removal response while parameter B (initial dye concentration) will negatively affected the response. However, in term of the interaction, the interaction between pair of factors (A and B) and (A and C) show positive effect as indicated by the positive coefficient of AB and AC whereas the interaction pair of factors (B and C) shows negative effect which indicated by the negative coefficient of BC.

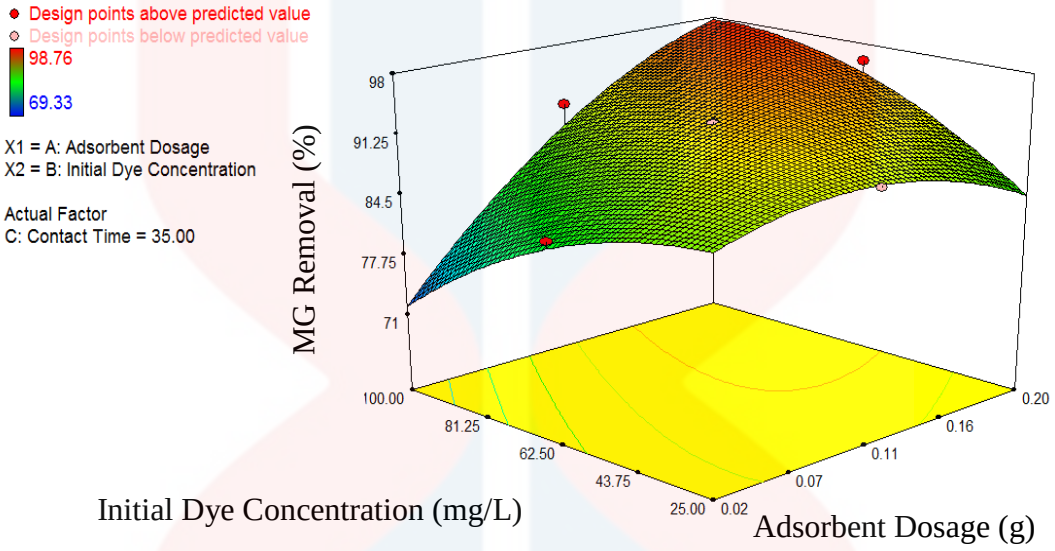
4.7 Optimisation of Adsorption Variables for MG Removal

Response surfaces graph was a valuable tool used for the prediction of MG dye removal efficiency and giving aid in the identification of the type of interaction between the process variables. The consequence of each model parameter was identified based on the *Fisher's F-value* and *p-value.* The *F-value* was the test that carried out to distinguish between the curvature variance and residual variance whereas probability > F (p-value) was the possibility of believing the obtained F-value if the theory was valid (Sarrai *et al*., 2016).

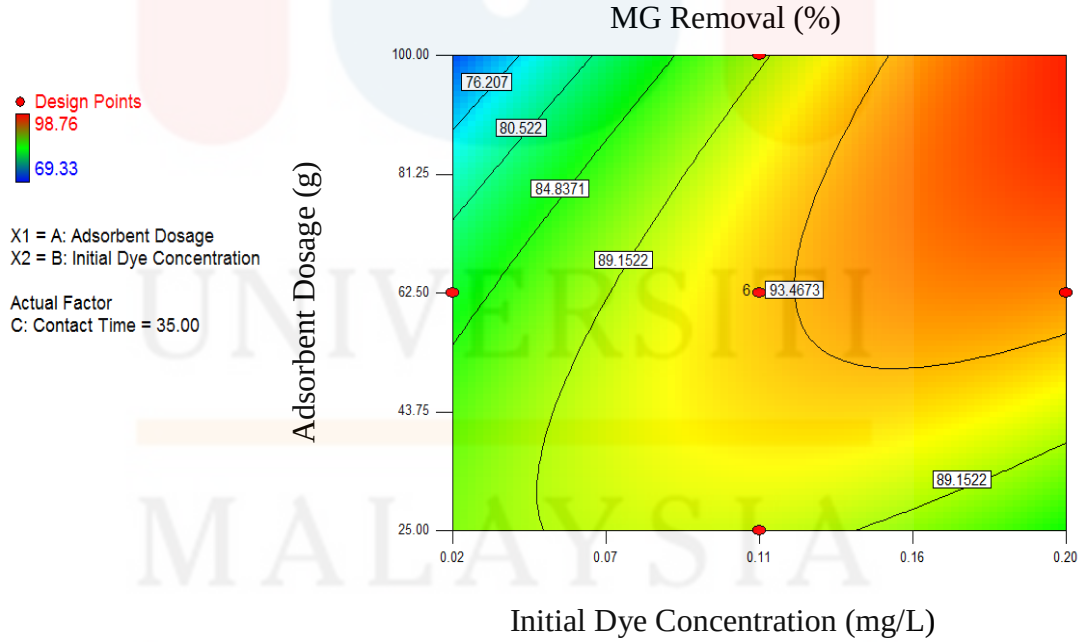
The optimisation studies were performed to evaluate the optimum experimental parameters. Proper combinations of the three experimental factors towards the percentage of MG removal through a 3D response surface graph were shown in Figure 4.4 (a), Figure 4.5 (a) and Figure 4.6 (a). There were low and high level values of the process variables drawn in the entire 3D response surface graph. The optimum condition of the operational variables was represented by the curved shape on the 3D response surface graph.

2D contour plots were shown in Figure 4.4 (b), Figure 4.5 (b) and Figure 4.6 (b) to study the interaction among the autonomous factors and their corresponding impact on the reaction.2D contour plot was a graphical rendition of a 3D response surface of two independent experimental variables by controlling auxiliary variable at fixed level. Moreover, these plots were considered to be useful in interpreting both of the synergy impacts of the experimental parameters towards the reaction (Chowdhury *et al.*, 2013).

4.7.1 Interaction of Adsorbent Dosage (A) and Initial Dye Concentration (B) on MG Removal Percentage



(a)



(b)

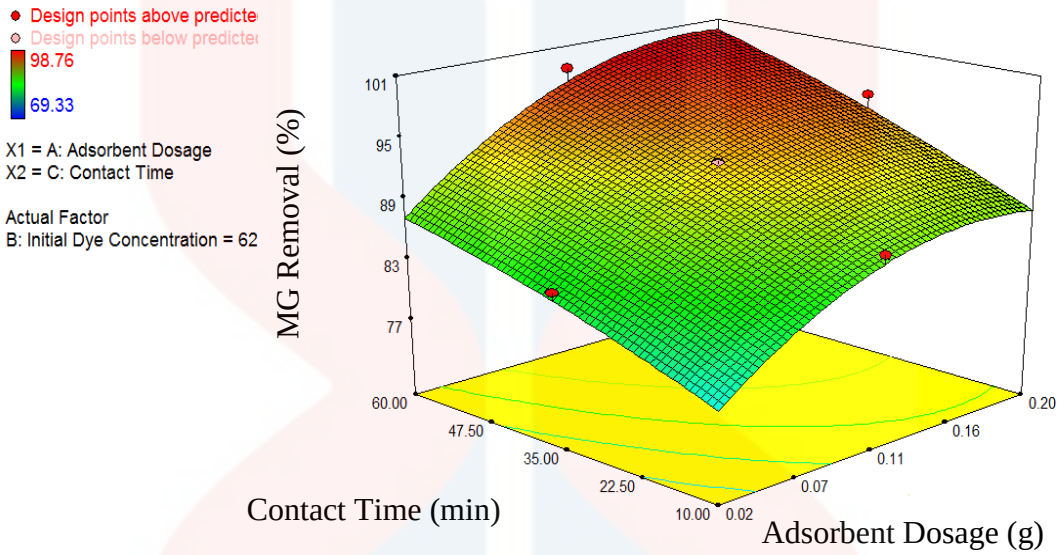
Figure 4.4: (a) 3D Response Surface and (b) 2D contour plot of interaction of adsorbent Dosage (A) and Initial Dye Concentration (B) on MG dye removal (%)

Figure 4.4 (a) and Figure 4.4 (b) demonstrates the interaction effect of adsorbent dosage and initial dye concentration on the percentage of MG removal. It has been found that the efficiency of adsorbent to remove the dye gradually escalated with high initial dye concentration. At lower concentration gradient of dye, the adsorbent able to react completely with the dye and thus facilitated the adsorption capacity whereas at higher concentration gradient of dye, there will be extra dye molecules which were not being removed from the solution caused by the saturation of the adsorption site (Abdel-Ghani & El-Chaghaby, 2014).

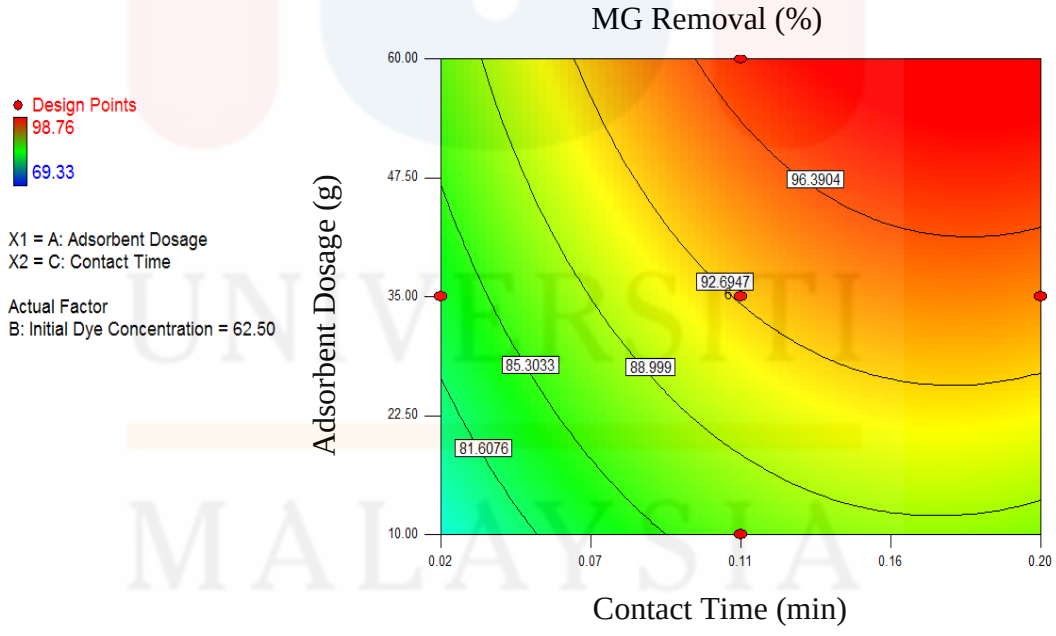
The biosorption of MG dye increased from 76.21% to 93.47% with the increment of adsorbent dosage from 0.02 g to 0.20 g. Such behaviour was obvious because when the adsorbent dosage is increased, it will lead to large number of binding site available for the MG dye adsorption. The high concentration of initial dye would decrease dye removal efficiency. This must be caused by the declined of the binding site on the adsorbent surface due to the raising of the dosage of dye remove per unit weight of the biosorbent (Alkhalssi *et al.*, 2014).

The efficiency of MG removal was significantly relies upon the concentration of MG dye aqueous solution. The data shows that, as the initial dye concentration increased, it will decline the efficiency of MG dye removal. This is because of the saturation of the binding site of the adsorbent. At low concentration of dye solution, there will be a lot of vacant of active site of the adsorbent. Meanwhile, at high concentration of dye solution, the surface of the adsorbent become lack of binding site for the adsorption process (Polipalli & Pulipati, 2013).

4.7.2 Interaction Effect of Adsorbent Dosage (A) and Contact Time (C) on MG Removal Percentage



(a)



(b)

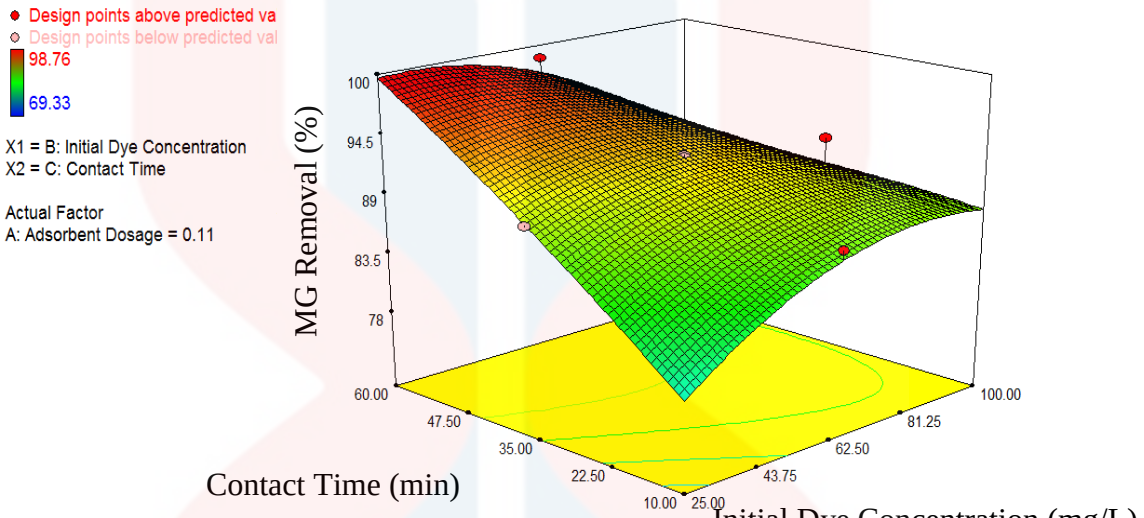
Figure 4.5: (a) 3D Response Surface and (b) 2D contour plot of interaction of Adsorbent Dosage (A) and Contact Time (C) on MG removal (%)

Figure 4.5 (a) and Figure 4.5 (b) show the relationship between adsorbent dosage and contact time towards the removal of MG dye using spent tea leaves biochar. The interactions to evaluate the adsorbent dosage and contact time were shown operating conditions with adsorbent dosage and contact period at the central levels, 0.11 g and 35 minutes, respectively. By referring to the figure, as both of the variables increased which were adsorbent dosage and contact time, the increased the removal efficiency will be. The increase of dye removal efficiency can be associated with the higher MG dye removal percentage (Jothirani *et al*., 2016).

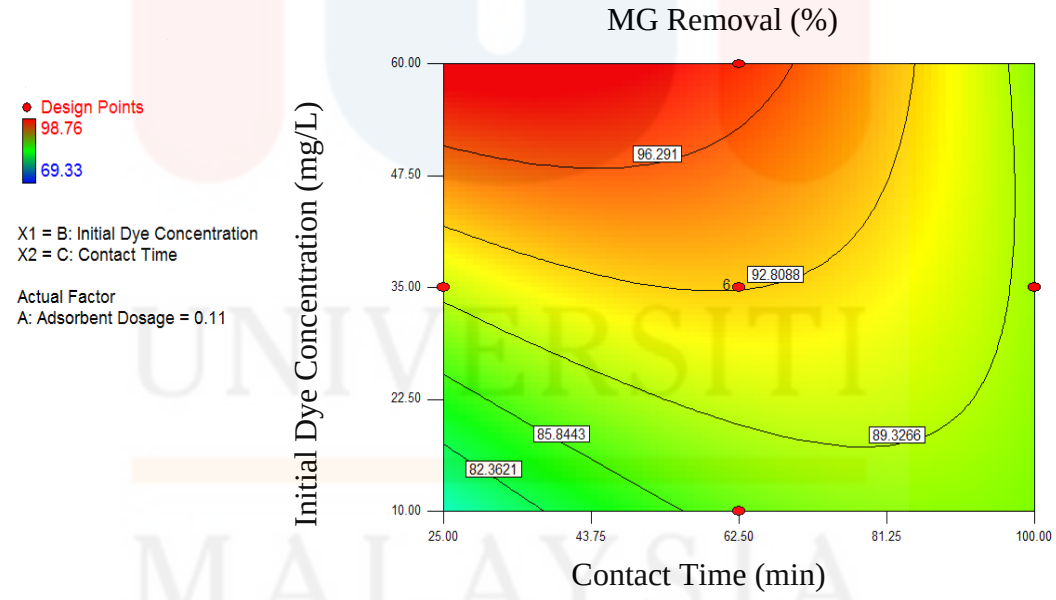
Adsorbent dosage will generate greater binding site on the surface of the adsorbent for the dye adsorption whereas the contact time will contribute sufficient time for the molecules to bind on the active site of the adsorbent. At the equilibrium stage, most of the dye molecules will be adsorbed on the adsorption site and in the intermolecular pore of the adsorbent as adsorption is a surface related phenomenon. Hence, at higher dosage, the adsorption of dye is higher caused by the availability of more empty binding sites as compared to lower dosages which has less binding sites to adsorb the same amount of dye in the adsorbate solution (Idan *et al*., 2017).

The increase in active sites will lead to higher percentage removal but with lowered dye uptake per unit of adsorbent. This was due to the abundance of active sites available on biosorbent for dye to be adsorbed at a higher dosage, thus leading to a higher interaction between dye particle and adsorbent. However, saturated dye binding sites occurred with the increasing of biosorbent dosage. In addition, saturation effect also reduces the efficiency of MG dye removal when maximum sorption capacity is reached (Abdel Ghani & El-Chaghaby, 2014).

4.7.3 Interaction Effect of Initial Dye Concentration (B) and Contact Time (C) on MG Removal Percentage



(a)



(b)

Figure 4.6: (a) 3D Response Surface and (b) 2D contour plot of interaction of Initial Dye Concentration (B) and Contact Time (C) on MG dye removal (%)

Figure 4.6 (a) and Figure 4.6 (b) illustrate 3D response surfaces graph and their corresponding contour plot of the reaction between initial dye concentration (B) and contact time (C) on percentage of MG dye removal. If the contact period and initial dye concentration were increased it will lead to higher of percentage of MG removal. It was easily understandable from 3-D response surface graph that the highest percentage of MG removal, 96.291 % which occurs between 60 minutes of contact time and 62.50 mg/L of initial dye concentration.

The percentage of MG removal was significantly elevated with the increasing of contact time between the adsorbent and MG dye solution. It can be deduced that the reaction time positively affected the percentage of MG removal. This is because the prolonged of reaction time will lead to high probability of the effective collision between the dye aqueous solution and the adsorbent. Consequently, high concentration of the initial dye will generate huge capacity for adsorption process. This is due to concentrated condition of the initial dye aqueous solution which requires greater driving force for mass transfer (Che Marzuki *et al*., 2015).

The increased rate of percentage removal of MG dye was caused by the availability of large number of active site on the adsorbent at the beginning of adsorption of MG dye solution. The longer the contact time, it will lead to the increase of the adsorption of MG dye and remains constant after equilibrium is reached (Safa, 2014). However, contact time became less important near the equilibrium stage. This is because, when the adsorption process reached the equilibrium stage, only a small increase in MG dye removal is observed due to limited adsorbent surfaces for dye entrapment (Mahdad e*t al.*, 2016).

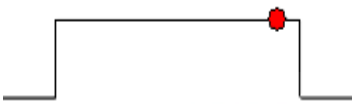
4.8 Numerical Optimisation using the Desirability Function

In numerical optimisation, the interested goal of every factors and response was selected. The possible goals included maximize and minimize or within the range. The goals were combined into an overall desirability function. Desirability is an objective function which lies in the range of zero to one or exceeds the limit goals. A random staring point was chosen until a relatively high gradient to a maximum point. This procedure will increase the chances for searching the optimum operating condition (Saha *et al.*, 2012). Table 4.8 shows the selection of factors and goal for the optimisation of Spent Tea Leaves (STL) biochar.

Table 4.8: Selection of factors and goal for numerical optimisation

Factors	**Goal**	**Lower Limit**	**Upper Limit**
A: Adsorbent Dosage	In range	0.02	0.20
B: Initial Dye Concentration	In range	25.0	100.0
C: Contact Time	In range	10	60
MG dye removal	Maximize	69.33	98.76

Table 4.8 shows the selection of factors and goals for the numerical optimisation procedure. The factors were selected “in range” for adsorbent dosage (0.02-0.20 g), “in range” for initial dye concentration (25-100 mg/L) and also “in range” of contact time of (10-60 minutes) to analyse the operational optimum condition efficiently. The purpose of this desirability procedure was to determine the optimum condition for the percentage of MG removal by utilising minimum adsorbent dosage, initial dye concentration and contact time.



0.02 0.20

Adsorbent Dosage = 0.18 g

25.0 100.0

Initial Dye Concentration = 46.92 mg/L

10.00 60.00

Contact Time = 56.16 min

69.33 98.76

MG Removal = 98.92 %

Desirability = 1.000

Figure 4.7: Desirability ramp of the selected goals

Figure 4.7 shows the desirability value for the numerical optimisation of the chosen goals. There were 45 solutions found according to the desirability. However, only the best optimisation condition was selected by the experimental model. The optimum operating conditions predicted by the model was found out to be at 0.18 g adsorbent dosage, 46.92 mg/L of initial dye concentration and contact time of 56.16 minutes. Meanwhile, the percentage of MG removal and desirability that suggested was 98.92 % and 1.000, respectively. The acquired value of desirability ramp was demonstrated the suggested function which represented the experimental model and optimum conditions.

4.9 Physical Characterization of Spent Tea Leaves Biochar

4.9.1 Physical Appearance of Spent Tea Leaves Biochar

In this study, the sample used to produce biochar was spent tea leaves. It was produced through pyrolysis process which means a process whereby a direct thermal decomposition of organic material at elevated temperatures under limited oxygen condition (Mittal *et al*., 2012). According to Xu *et al*. (2011), biochar is a find-grained and porous substance and has been widely applied in dye removal from aqueous solution. Therefore, biochar was preferred rather than raw powder due to the porous properties which lead to high surface area that would increase the ability of adsorption of malachite green.

Biochar was identified as a "supersorbent" for neutral organic compounds. It is the carbonaceous products of pyrolyzing biomass without activation. Due to the higher availability of its raw materials, biochar can be produced from direct burning which makes the production of biochar much more economical and environmentally friendly compared to the production of activation carbon. Other than that, biochar was an effective adsorbent for either both of cationic and anionic dyes. Hence, it may be an excellent substitute for activated carbon for wastewater treatment approaches by considering that it can be readily prepared with lower cost and environmental friendly (Tan *et al*., 2015).

(a) **(b)**

Figure 4.8 (a) Appearance of Spent Tea Leaves (STL) powder and (b) Spent Tea Leaves (STL) Biochar

Figure 4.8 (a) shows the appearance of Spent Tea Leaves (STL) powder and (b) Spent Tea Leaves (STL) biochar. As depicted in Figure 4.8, the colour of spent tea leaves (STL) powder was brown, while the spent tea leaves (STL) biochar was in dark grey and black due to high content of carbon after the thermal decomposition process. Apart from that, it can be stated that the STL biochar was more fine compared to the STL powder. As can be seen from the figure, after undergo the burning process, from large amount of the STL powder, it can only produced small yield of STL biochar due to the high temperature applied during the burning process.

4.9.2 Fourier Transforms Infrared (FTIR) Analysis

Fourier Transforms Infrared (FTIR) was used for the identification of organic compound exist in the sample by the absorption of ultraviolet light of each wavelength. The obtained absorption spectra of the compounds were the unique reflection of the molecular arrangement of the compound in the adsorbent. The functional group present in the adsorbent can be analysed by studying the peak between the frequencies. FTIR spectra were analysed to identify the characteristic functional groups of the adsorbent. In this study, FTIR analysis was conducted for the Malachite Green (MG) dye (Figure 4.9), spent tea leaves powder (Figure 4.10 (a)), spent tea leaves biochar (Figure 4.10 (b)) and spent tea leaves biochar after adsorption of MG dye (Figure 4.10 (c)).

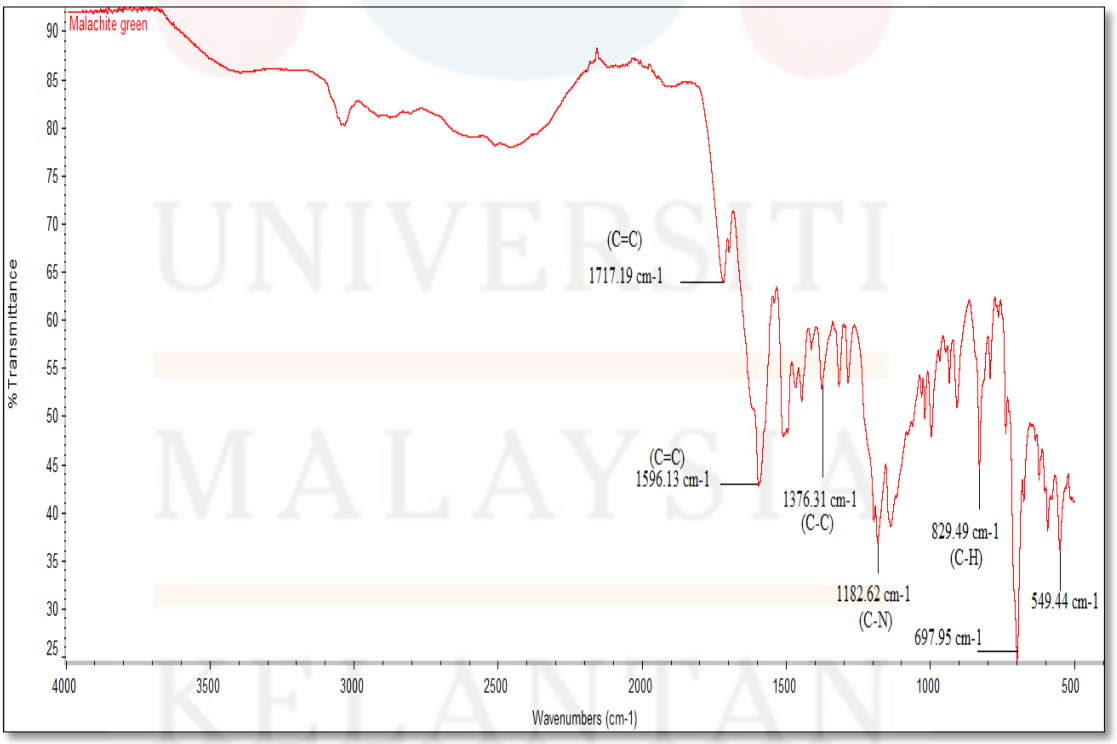


Figure 4.9: FTIR of Malachite Green (MG) Dye

By referring to Figure 4.9, the functional group found inside the Malachite Green (MG) dye was predicted. Malachite Green (MG) dye is a triarylmethyl cations. Most of the compound exist in MG dye was aromatic compound and miscellaneous compound which content naturally inside the dye. Specific peaks between 2000 cm^{-1} and 500 cm^{-1} indicates the present of benzene rings which is supporting by the peak at 1717.19 cm^{-1} and 1596.13cm^{-1} that corresponding to C=C aromatic stretching and peak at 1376.31 cm^{-1} was due to C-C aromatic stretching vibration. The same observations were reported (Sartape *et al*., 2017).

The spectrum also shows a peak at 1182.62 cm^{-1} that was assigned as a strong C-N bond which also corresponds to aromatic compound. In addition, a medium bending of alkene group represented the formation of C-H bond at 829.49 cm^{-1}. At peaks of 697.95 cm^{-1} and 549.44 cm^{-1}, there was a strong stretching of halo compound. These groups of halo compound can be the strong stretching of C-Br bond or C-l bond. Based on the studies, the peak signified the presence of –Br on the dye which similar to (Kumar *et al*., 2017). According to Coates (2000), within a range of 850 cm^{-1} – 670cm^{-1}, if more than one peak obtained in region of the C-H bending vibrations can support the presence of an aromatic structure.

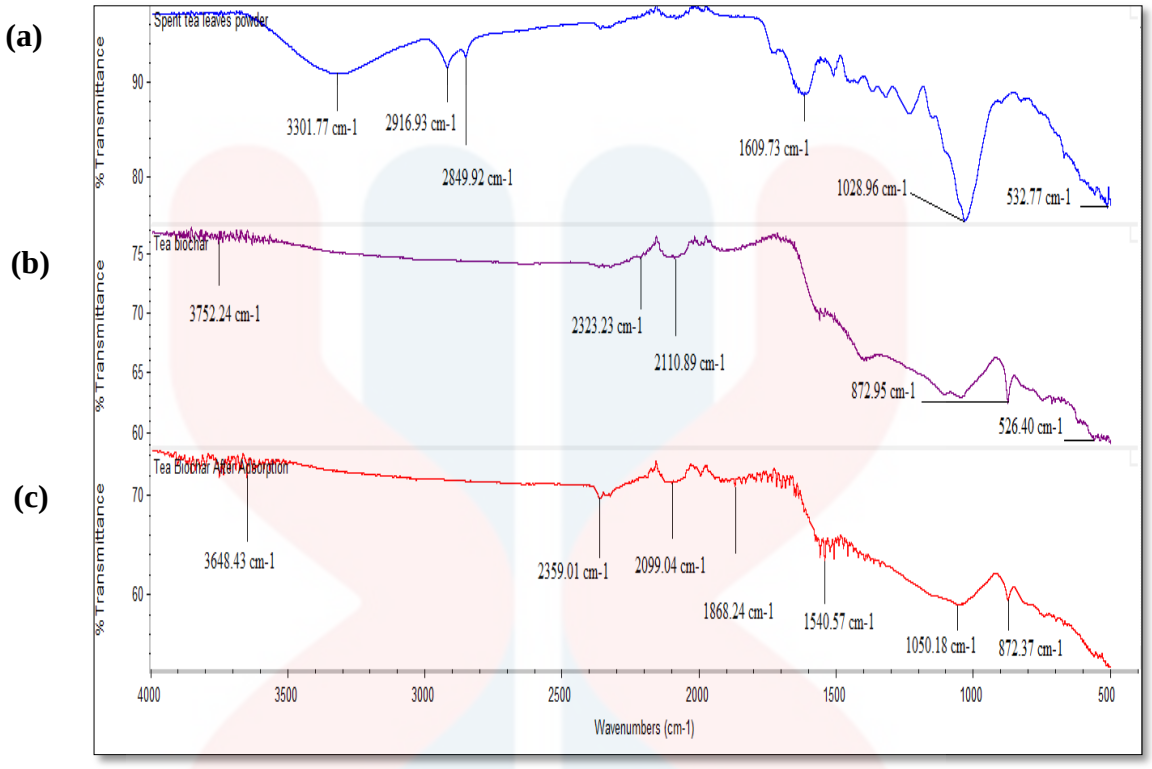


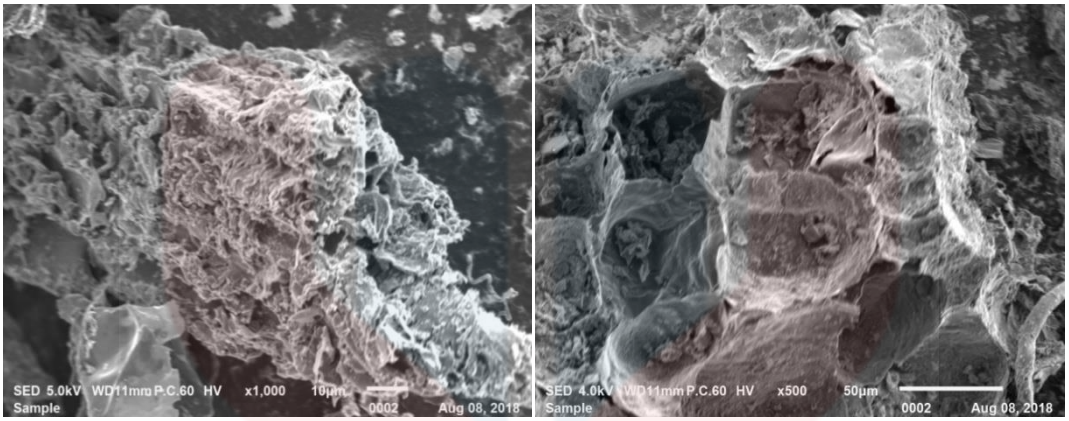
Figure 4.10 (a) FTIR of spent tea leaves powder and (b) spent tea leaves biochar and (c) spent tea leaves biochar after adsorption of MG dye

Figure 4.10 (a) shows the FTIR spectra of spent tea leaves (STL) powder. Based on the figure, the STL powder was analysed in the range of 4000-500 cm^{-1} of infrared spectral regions. Several obvious peaks can be observed in the infrared spectra of STL powder. There was a broad adsorption of (N-H) stretching vibrations peaks at 3301.77 cm^{-1} indicated the existence of amides group on the surface of the spent tea leaves powder. Other functional groups present in the powder including the C-H stretching of alkane group at 2916.93 cm^{-1} band. Next, the peak value around 1609.73 cm^{-1} was assigned to N-H stretching of amine salts. Apart from that, there was a broad peak at 1028.96 cm^{-1} which indicated the CO-O-CO bond of anhydride group and strong absorption bands at 532.77 cm^{-1}.

The pyrolysis process that produced biochar had created some changes for the compound present in STL biochar. Figure 4.10 (b) shows the FTIR spectra of Spent Tea Leaves (STL) Biochar. However, there were some peaks which exist in the STL powder disappeared in the STL Biochar. This might due to the thermal degradation in order to produce the biochar. A medium O-H bond stretching vibration of hydroxyl compound was found at 3725.24 cm^{-1}. The peaks located at 2323.23 cm^{-1} was attributed to the present carbon dioxide of O=C=O bond. Meanwhile, the medium stretching at 2110.89 cm^{-1} was represented C-C multiple bond stretching of monosubstituted in alkyne group. A sharp peaks at 872.47 cm^{-1}which was attributed to a strong bending of alkene (C=C). Lastly, strong absorption bands at 526.40 cm^{-1} which slightly reduced from the STL powder.

Figure 4.10 (c) shows the FTIR spectra of Spent Tea Leaves (STL) Biochar after adsorption of MG dye. Compared to the infrared spectra of STL biochar before the adsorption process of MG dye, there are some new peaks appeared, and some peaks shifted in the infrared spectra of STL before the adsorption process of MG dye. The peak was observed at 1868.24 cm^{-1} as the stretching C=O bond of carboxyl group. At 1540.57 cm^{-1}, there was a bending vibration mode of nitro compound, N-O may be due to the dye compound which attached to the STL biochar after the adsorption. Other than that, at of 1050.18 cm^{-1} was found a broad stretching of anhydride group. Those 3 new peaks distinguished between the samples of STL biochar before and after undergo adsorption process of MG dye.

4.9.3 Scanning Electron Microscopy (SEM)



(a) **(b)**

Figure 4.11 SEM images of Spent Tea Leaves (STL) Biochar

For the surface morphology of the samples, Scanning Electron Microscopy (SEM) was applied. SEM images of Spent Tea Leaves (STL) biochar were shown in Figure 4.11 (a) and Figure 4.11 (b). The availability of pores and internal surface was clearly displayed in the SEM image of the STL biochar in Figure 4.11 (a) whereas the coverage of the surface and the pores for the adsorption of MG dye solution was shown in Figure 4.11 (b).

The SEM images of the STL biochar showed that plane cleavage surface as the pyrolysis process would have stabilized the volatile hydrocarbons, smoothing the surface of biochar. The surface structure was like a "similar to honeycomb" with small pores and uneven surface structure. There were many mesopores and microspores on the surface of biochar, such that increased the surface areas which provides suitable binding site for MG dye molecule. Usually, biomass wastes contain low lignin and high volatile matter content which effect the pore creation in biochar (Lehmann *et al*., 2011).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this study, spent tea leaves were undergoing pyrolysis to produce biochar and used as biosorbent for the removal of MG dye from aqueous solution. Three independent process variables namely adsorbent dosage, initial dye concentration and contact time were selected as input parameters while the percentage of MG removal was considered as response. The most important effect on the biosorption performance was found to be the biosorbent dosage followed by the contact time. The effect of initial dye concentration was negligible within the experimental range.

Adsorption studies were performed using Response Surface Methodology (RSM) by employing central composite design (CCD). RSM also appeared to be very effective approach for studying the combined effects of process variables on the response factor. The effect of the operating factors was investigated by analysing the response surface graph and contour plots. The interaction of each parameter was studied according to the second-order polynomial model. The model was determined as a quadratic model with R^2 of 0.9854.

The optimum operating conditions for the removal of MG dye using STL biochar were determined at 0.18 g adsorbent dosage, 46.92 mg/L of initial dye concentration and contact time of 56.16 minutes. At this optimum conditions, the percentage of MG removal up to 98.92 % with desirability of 1.000. Physical characterizations of MG dye, STL powder and STL biochar were studied using FTIR analysis and SEM analysis. Thus, it can be concluded that STL biochar can significantly be an alternative for the removal of MG dye from aqueous solution.

5.1 Recommendations

There are several recommendations can be considered in order to enhance the efficiency of the removal of MG dye from the aqueous solution using spent tea leaves biochar. Activated carbon from spent tea leaves might be potential adsorbent due to high availability of specific active site for the adsorption process of MG dye. In term of the process variables, other combination of parameter can be used including pH, temperature and agitation rate. Thus, the percentage of MG removal can be increased to certain extend and the adsorption process become more efficient.

For better understanding of the surface characteristics of the spent tea leaves biochar, X-ray Diffraction (XRD) analysis and Brunauer-Emmett-Teller (BET) analysis can be applied to study the physical characterization of the biochar. Furthermore, adsorption isotherms namely Langmuir, Freundlich, Temkin and Redlich Peterson can be used to analyse the adsorption molecules distribution for the removal of MG dye from aqueous solution.

For the future study, there are several suggestions can be made in order to gain a better performance for the experimental work. One of the suggestions is by performing the adsorption process using other agricultural wastes to prevent the accumulation of plant biomass. Other than Malachite Green (MG) dye, investigation of various dyes removal using spent tea leaves biochar can be performed. Moreover, spent tea leaves biochar can be used to investigate the removal of heavy metal such as copper, lead, zinc and cadmium since this controversial issue created serious health impact towards the environment, animal and human health (Garba*et al.*, 2016).

Apart from CCD model, the optimisation studies using RSM can be carried out using other design model for instance Box-Behnken Design (BBD) model. Despite using RSM methods, optimisation process also can be generated using other method such as Artificial Neural Network (ANN). This will resulted in more verification points could be selected among the proposed levels of independent variables and uniform design tables with larger degree of freedom could be adopted if failure occurs when more variables were present (Li *et al*., 2016).

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APPENDIX A

Dilution for Experimental Run,

Formula used, $\mathbf{M_1V_1 = M_2V_2}$

M_1 = Concentration of Malachite Green (MG) stock solution prepared

V_1 = Volume of Malachite Green (MG) stock solution needed

M_2 = Concentration of Malachite Green (MG) solution needed

V_2 = Volume of Malachite Green (MG) solution needed

Preparation of 25.00 mg/L MG solution $M_1V_1 = M_2V_2$ (1000 mg/L) V_1 = (25.00 mg/L) (50mL) V_1 = 1.25 mL Hence, 1.25 mL of 1000 mg/L MG stock solution diluted to 50 mL to produce 25.00 mg/L MG solution.
Preparation of 62.50 mg/L MG solution $M_1V_1 = M_2V_2$ (1000 mg/L) V_1 = (62.50 mg/L) (50mL) V_1 = 3.125 mL Hence, 3.125 mL of 1000 mg/L MG stock solution diluted to 50 mL to produce 62.50 mg/L MG solution.
Preparation of 100.00 mg/L MG solution $M_1V_1 = M_2V_2$ (1000 mg/L) V_1 = (100.00 mg/L) (50mL) V_1 = 5.0 mL Hence, 5.0 mL of 1000 mg/L MG stock solution diluted to 50 mL to produce 100.00 mg/L MG solution.

Dilution for Calibration Curve,

Solution 1: Preparation of 0.5 mg/L MG solution $M_1V_1 = M_2V_2$ (1000 mg/L) V_1 = (0.5 mg/L) (50 mL) V_1 = 0.025 mL = 25 µL 25 µL of MG stock solution diluted to 50 mL to produce 0.5 mg/L MG solution.
Solution 2: Preparation of 10.0 mg/L MG solution $M_1V_1 = M_2V_2$ (1000 mg/L) V_1 = (10.0 mg/L) (50 mL) V_1 = 0.5 mL 0.5 mL of MG stock solution diluted to 50 mL to produce 10.0 mg/L MG solution.
Solution 3: Preparation of 20.0 mg/L MG solution $M_1V_1 = M_2V_2$ (1000 mg/L) V_1 = (20.0 mg/L) (50 mL) V_1 = 1.0 mL 1.0 mL of MG stock solution diluted to 50 mL to produce 20.0 mg/L MG solution.
Solution 4: Preparation of 30.0 mg/L MG solution $M_1V_1 = M_2V_2$ (1000 mg/L) V_1 = (30.0 mg/L) (50ml) V_1 = 1.5 mL 1.5 mL of MG stock solution diluted to 50 mL to produce 30.0 mg/L MG solution.
Solution 5: Preparation of 40.0 mg/L MG solution $M_1V_1 = M_2V_2$ (1000 mg/L) V_1 = (40.0 mg/L) (50 mL) V_1 = 2.0 mL 2.0 mL of MG stock solution diluted to 50 mL to produce 40.0 mg/L MG solution.
Solution 6: Preparation of 50.0 mg/L MG solution $M_1V_1 = M_2V_2$ (1000 mg/L) V_1 = (50.0 mg/L) (50 mL) V_1 = 2.5 mL 2.5 mL of MG stock solution diluted to 50 mL to produce 50.0 mg/L MG solution.

APPENDIX B

First Trial Data

Run	Average Absorbance reading	I. conc (mg/L)	F. conc (mg/L)	MG removal (%)
1	0.0227	62.50	7.556	87.91
2	0.0147	62.50	4.889	92.18
3	0.0137	100.00	4.556	95.44
4	0.0147	62.50	4.889	92.18
5	0.0077	25.00	2.556	89.78
6	0.0143	62.50	4.778	92.36
7	0.0273	100.00	9.111	90.89
8	0.0067	62.50	2.222	96.44
9	0.0073	62.50	2.444	97.56
10	0.0020	100.00	0.667	98.93
11	0.0057	25.00	1.889	92.44
12	0.0193	25.00	6.444	74.22
13	0.0137	62.50	4.556	92.71
14	0.0167	25.00	5.556	77.78
15	0.0887	100.00	29.556	70.44
16	0.0020	25.00	0.667	97.33
17	0.0147	62.50	4.889	92.18
18	0.0177	62.50	5.889	90.58
19	0.0293	62.50	9.778	84.36
20	0.0843	100.00	28.111	71.89

Second Trial Data

Run	Average Absorbance reading	I. conc (mg/L)	F. conc (mg/L)	MG removal (%)
1	0.0237	62.50	7.889	87.38
2	0.0147	62.50	4.889	92.18
3	0.0183	100.00	6.111	93.89
4	0.0150	62.50	5.000	92.00
5	0.0080	25.00	2.667	89.33
6	0.0147	62.50	4.889	92.18
7	0.0267	100.00	8.889	91.11
8	0.0067	62.50	2.222	96.44
9	0.0057	62.50	1.889	98.11
10	0.0027	100.00	0.889	98.58
11	0.0053	25.00	1.778	92.89
12	0.0217	25.00	7.222	71.11
13	0.0137	62.50	4.556	92.71
14	0.0167	25.00	5.556	77.78
15	0.1030	100.00	34.333	65.67
16	0.0017	25.00	0.556	97.78
17	0.0137	62.50	4.556	92.71
18	0.0157	62.50	5.222	91.64
19	0.0297	62.50	9.889	84.18
20	0.0840	100.00	28.000	72.00

Third Trial Data

Run	Average Absorbance reading	I. conc (mg/L)	F. conc (mg/L)	MG removal (%)
1	0.0230	62.50	7.667	87.73
2	0.0153	62.50	5.111	91.82
3	0.0160	100.00	5.333	94.67
4	0.0147	62.50	4.889	92.18
5	0.0073	25.00	2.444	90.22
6	0.0123	62.50	4.111	93.42
7	0.0260	100.00	8.667	91.33
8	0.0087	62.50	2.889	95.38
9	0.0047	62.50	1.556	98.44
10	0.0023	100.00	0.778	98.76
11	0.0023	25.00	0.778	96.89
12	0.0183	25.00	6.111	75.56
13	0.0147	62.50	4.889	92.18
14	0.0193	25.00	6.444	74.22
15	0.0843	100.00	28.111	71.89
16	0.0020	25.00	0.667	97.33
17	0.0147	62.50	4.889	92.18
18	0.0167	62.50	5.556	91.11
19	0.0307	62.50	10.222	83.64
20	0.0843	100.00	28.111	71.89

Percentage of MG Removal (%)

Run	First Trial %	Second Trial %	Third Trial %	Average %
1	87.91	87.38	87.73	87.67
2	92.18	82.18	91.82	92.06
3	95.44	93.89	94.67	94.67
4	92.18	92.00	92.18	92.12
5	89.78	89.33	90.22	89.78
6	92.36	92.18	93.42	92.65
7	90.89	91.11	91.33	91.11
8	96.44	96.44	95.38	96.09
9	97.56	98.11	98.44	98.04
10	98.93	98.58	98.76	98.76
11	92.44	92.89	96.89	94.07
12	74.22	71.11	75.56	73.63
13	92.71	92.71	92.18	92.53
14	77.78	77.78	74.22	76.60
15	70.44	65.67	71.89	69.33
16	97.33	97.78	97.33	97.48
17	92.18	92.71	92.18	92.36
18	90.58	91.64	91.11	91.11
19	84.36	84.18	83.64	84.06
20	71.89	72.00	71.89	71.93