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ADSORPTION AND BIODEGRADATION OF COMMERCIAL DYES USING CHEMICALLY-MODIFIED EMPTY FRUIT BUNCH BIOCHAR ASSISTED BY BACTERIA

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By

SARMILA A/P GUNASEKARAN

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June 2021

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The aim of this study was to investigate the adsorption capacity of activated empty fruit bunch biochar (EFBB) in removing commercial dyes (methylene blue and direct red 80) assisted by dye degrading bacteria. Empty fruit bunch (EFB) is one of the wastes that has been produced from oil palm industry and it can be utilised by converting them into biochar and use them as potential adsorbent of contaminants. Raw biochar usually possesses lower adsorption capacity. Therefore, activation of EFBB was required to increase its surface area, porosity and surface functional groups and consequently enhancing their adsorption capacity. In this study, EFBBs were modified chemically by treating them with sulphuric acid (H_2SO_4), potassium hydroxide (KOH) and by iron impregnation ($FeCl_3$). The acid coated biochar was known as A-EFBB, alkali coated biochar as B-EFBB and Iron coated biochar as Fe-EFBB. These adsorbents were used to remove cationic dye, methylene blue (MB) and anionic dye, direct red 80 (DR 80) at various initial concentration and their adsorption capacity was compared. The Langmuir model fitted the isotherm data better than the Freundlich's model which indicated that adsorption was homogeneous and monolayer. Results for MB showed higher adsorption capacity in the order of A-EFBB (125 mg/g) > B-EFBB (76.336 mg/g) > Fe-EFBB (10.130 mg/g) > EFBB (6.139 mg/g). While for Direct Red 80 results showed higher adsorption capacity in the order of B-EFBB (78.125 mg/g) > A-EFBB (40.160 mg/g) > Fe-EFBB (4.708 mg/g) > EFBB (1.150 mg/g). Apart from adsorption, biological treatments which are cost-effective and environmentally friendly techniques, have been also widely used for many years, but there are only a limited number of microorganisms that can degrade contaminants at high concentrations. Hence to make use of both adsorption and biological treatment techniques, a hybrid treatment which combined the two treatment processes was introduced in the removal of high concentration of dyes from wastewater. Pollutants can be removed efficiently and completely using this new strategy. Therefore, biodegradation was tested on MB which is being widely

used in dye industry at various initial concentrations and mixed bacterial culture was able to remove MB (100 mg/L) efficiently at 94.6% in 24 hours. The dominant bacterial orders present in the mixed culture were identified as *Sphingomonadales* followed by *Pseudomonadales, Betaproteobacteriales, Micrococcales, Clostridiales, Bacillales* and others. The highest decolourisation percentage was observed at 100 mg/L (94.60%), but the values were lower at higher concentrations. At the highest MB dye concentration (400 mg/L), the decolourisation percentage was the lowest (56.30%). The highly efficient combined treatment was able to remove MB at 99.2% within 24 hours. In nutshell, the highest percentage of MB dye decolourization was observed to be 76.5% by A-EFBB, 94.6% by mixed culture bacteria, and 99.2% by the mixed culture bacteria immobilized on A-EFBB at 100 ppm in 24 hours. The combined treatment has thus been proved to be an efficient way to remove dyes from wastewater compared to the individual adsorption and biodegradation process.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENJERAPAN DAN BIODEGRADASI PEWARNA KOMERSIAL MENGGUNAKAN BIOCAR TANDAN BUAH KOSONG YANG DIUBAHSUAI MENGGUNAKAN KAEDAH KIMIA DENGAN BANTUAN BAKTERIA

Oleh

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Tujuan kajian ini adalah untuk mengkaji keupayaan penjerapan biocar tandan buah kosong yang diaktifkan (EFBB) dalam menghilangkan pewarna komersial (*methylene blue and direct red 80*) dibantu oleh bakteria yang merosakkan pewarna. Tandan buah kosong (EFB) adalah salah satu bahan buangan yang dihasilkan dari industri kelapa sawit dan dapat dimanfaatkan dengan mengubahnya menjadi biocar dan menggunakannya sebagai penyerap berpotensi bahan pencemar. Biocar mentah biasanya mempunyai kapasiti penjerapan yang lebih rendah. Oleh itu, pengaktifan EFBB diperlukan untuk meningkatkan keluasan permukaan, keliangan dan kumpulan berfungsi permukaan dan seterusnya meningkatkan kapasiti penjerapan mereka. Dalam kajian ini, EFBB diubahsuai menggunakan kaedah kimia dengan mengaktifkan menggunakan asid sulfurik (H_2SO_4), kalium hidroksida (KOH) dan dengan impregnasi besi ($FeCl_3$). Biocar bersalut asid dikenali sebagai A-EFBB, biocar bersalut alkali sebagai B-EFBB dan biocar bersalut besi sebagai Fe-EFBB. Adsorben-adsorben ini digunakan untuk menghilangkan pewarna kationik, methylene blue (MB) dan pewarna anionik, direct red 80 (DR 80) pada pelbagai kepekatan awal dan kapasiti penjerapan mereka dibandingkan. Model Langmuir memuat data isoterm lebih baik daripada model Freundlich dan mengindikasikan penjerapan yang berlaku adalah homogen dan satu lapisan. Hasil untuk MB menunjukkan kapasiti penjerapan dalam urutan meningkat A-EFBB (125 mg/g) > B-EFBB (76.336 mg/g) > Fe-EFBB (10.130 mg/g) > EFBB (6.139 mg/g). Sementara untuk hasil Direct Red 80, hasil penjerapan menunjukkan kapasiti penjerapan dalam urutan meningkat, iaitu B-EFBB (78.125 mg/g) > A-EFBB (40.160 mg/g) > Fe-EFBB (4.708 mg/g) > EFBB (1.150 mg/g). Selain daripada penjerapan, rawatan biologi yang kos efektif dan mesra alam sekitar telah juga digunakan secara meluas selama bertahun-tahun,

tetapi hanya terdapat bilangan mikroorganisma yang terhad yang dapat degradasi bahan cemar pada kepekatan tinggi. Oleh itu, untuk memanfaatkan kedua-dua teknik penjerapan dan rawatan biologi, rawatan hibrid yang menggabungkan dua proses rawatan tersebut diperkenalkan dalam penyingkiran pewarna dengan kepekatan tinggi dari air sisa. Bahan pencemar dapat dikeluarkan dengan cekap dan sepenuhnya menggunakan strategi baru ini. Oleh itu, biodegradasi diuji pada MB yang penggunaanya agak luas dalam Industri pewarna pada pelbagai kepekatan awal dan kultur bakteria campuran yang diuji telah menghilangkan MB (100 mg/L) secara efisien pada 94.6% dalam 24 jam. Urutan bakteria dominan yang terdapat dalam kultur bakteria campuran dikenal pasti sebagai *Sphingomonadales* diikuti oleh *Pseudomonadales*, *Betaproteobacteriales, Micrococcales, Clostridiales, Bacillales* dan lain-lain. Peratusan dekolourisasi tertinggi diperhatikan pada 100 mg/L (94.60%), tetapi nilainya lebih rendah pada kepekatan yang lebih tinggi. Pada kepekatan pewarna MB tertinggi (400 mg/L), peratusan penyahwarnaan adalah yang paling rendah (56.30%). Rawatan gabungan yang sangat berkesan ini, dapat mengeluarkan MB pada 99.2% dalam masa 24 jam. Secara ringkasnya, peratusan tertinggi penyingkiran pewarna MB diperhatikan pada 76.5% oleh A-EFBB, 94.6% oleh kultur bakteria campuran, dan 99.2% oleh bakteria kultur campuran yang diimmobilisasi pada A-EFBB pada 100 ppm dalam 24 jam. Oleh itu, rawatan gabungan telah terbukti menjadi kaedah yang berkesan untuk menghilangkan pewarna dari air sisa berbanding dengan proses penjerapan dan biodegradasi individu.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

MB	Methlylene Blue
DR 80	Direct Red 80
AC	Activated Carbon
EFB	Empty Fruit Bunch
SAB	Simultaneous Adsorption and biodegradation
EFBB	Empty fruit bunch biochar
MPOB	Malaysian palm oil berhad
H_2SO_4	Sulphuric Acid
KOH	Potassium hydroxide
A-EFBB	Acid coated biochar
B-EFBB	Base coated biochar
Fe-EFBB	Iron coated biochar
EC	Electrical conductivity
BET	Specific surface area
SEM	Scanning electron microscopy
FTIR	Fourier transform infrared spectroscopy

CHAPTER 1

INTRODUCTION

1.1 Background

The world population in 2016 was 7.4 billion and it is expected to reach about 10 billion by 2050 (Population Reference Bureau, 2016). The continuously growing world population is putting a great strain on over 65% of the Earth's waters and causes serious global water contamination issue (Kay et al., 2016). The rising demand for water increases the amount of wastewater generated as most human activities produce wastewater (Afroz and Rahman, 2017) and when these wastewaters are directly released into the waterways, they become the source of water contamination. Globally, it is likely that over 80% of wastewater and 95% in several least developed countries are released to the environment without adequate treatment (WWAP, 2017).

Narrowing the issue, In Malaysia, water pollution is a serious problem as well where it has severe impacts on sustainability of water resources and also affects living plants and organisms, population health and the economy (Afroz et al., 2017). Point and non-point pollution sources are the major contributor for water pollution in Malaysia (Pang and Abdullah, 2013). Sewage treatment plants, manufacturing, agro-based industries, and animal farms are the examples of point sources of water pollution while agricultural activities and surface runoffs are the examples of non-point sources.

Textile industry is among the industries that branches out from manufacturing industries. In Malaysia, textile industry has a huge impact on the country's economy. In year 2017, foreign and domestic investors invested in our country's textile industry and this has earned RM 428.8 million as a total revenue for Malaysia (Sundarajoo & Maniyam, 2020). Hence, the textile industry utilises an enormous amount of water for its preparation and dyeing processes. Consequently, it can hike up to 3000m^3/day for its total water consumption and it accounts for 22% of the total volume of industrial wastewater produced in Malaysia (Idris et al., 2007; Pang & Abdullah, 2013).

As a developing country, industries involving dyes are rapidly growing in Malaysia. Synthetic dyes are widely used in the textile, paper, leather, pharmaceutical, and printing industries (Sajab et al., 2011; Liu et al., 2012; Zheng et al., 2017) and these industries release these dye effluents directly into the environment and cause water pollution (Pang and Abdullah, 2013). Smallest concentration of dye in waters can become unsuitable for human consumption. Not only humans, dyes can affect aquatic plants because they reduce sunlight penetration and inhibit the growth of aquatic plants. Naturally photosynthesis process also is disrupted followed by ecosystem of aqua organisms. Besides, dyes may transmit toxicity to aquatic life and may be mutagenic, carcinogenic and cause severe damage to human beings such as dysfunction of the kidneys, reproductive system, liver, brain and central nervous system (Corso and Almeida, 2009; Salleh et al., 2011; Sajab et al., 2013). Thus, such wastewater needs to be properly treated before it can be released into the environment.

A variety of methods have been developed for treatment of dye-bearing wastewater such as coagulation, flocculation, oxidation, biological treatments, membrane filtration and adsorption. Yet some of these techniques have major drawbacks such as very expensive and having a long duration process (Salleh et al., 2011; Pathania et al., 2013). Therefore, most of these techniques are not practical on large scale. Amidst the above strategies, adsorption shows a number of essential benefits such as flexibility and simplicity of technique, ease of operation and insensitivity to toxic substances. Furthermore, there is no need for extra pre-treatment steps for adsorption techniques (Santhi et al., 2010). Thus, adsorption is preferable over the above-mentioned processes and is being widely used because of its low cost and efficient performance.

In recent times, the study of adsorbents prepared from agricultural by-products has been intensely investigated due to its cost effectiveness and high adsorption capacities (Bharathi and Ramesh, 2013). Such example is biochar, which is a carbonaceous material obtained from the pyrolysis of biomass under zero or limited supply of oxygen and at relatively low temperature, usually below 700 ^{0}C. It has been recognized as a good sorbent for different kinds of organic and inorganic pollutants (Chen et al., 2011; Nartey and Zhao, 2014). Raw biochar usually possesses lower adsorption capacity compared to modified ones. Therefore, activation of biochar is required to increase its surface area, porosity and surface functional groups and consequently enhancing their adsorption capacity (Rajapaksha et al., 2016; Tan et al., 2016). Malaysia is one of the world largest palm oil exporters. Solid agricultural wastes produced from palm oil industry such as empty fruit bunches (EFB) hence can be further utilized by converting them into biochar to be used as an adsorbent for removal of dyes from aqueous solutions (Sajab et al., 2013).

Meanwhile, biological treatments, which are cost-effective and environmentally friendly technique, have been also widely used for many years. However, this technique has some drawbacks too which includes there are only a limited number of microorganisms that can biodegrade contaminants at high concentrations (Zheng et al., 2017). Therefore, in this study adsorption was combined with biodegradation techniques in efforts to utilise both the techniques' advantages and convert the drawbacks to advantages to completely remove dyes from wastewater. Several studies found out that concurrent adsorption and biodegradation methods, which involve adsorption followed by biological treatment, have the ability to completely remove regardless of pollutants (Zheng et al., 2017; Li et al., 2019; Zhao et al., 2020). This is because the adsorption of the target pollutants onto solid surface of the adsorbent provides more contact time with the cells and speeds up the biodegradation process and consequently removes the target pollutants completely (Li et al., 2019).

In nutshell, this small-scale study would be an essential step among many to combat water pollution in a wider scale. Ability of agricultural waste that turned into biochar and its coexistence with microbiological community will provide more information on this hybrid technique effort. Hence, in this study, dye removal capacity was evaluated by combining bacteria and chemically modified empty fruit bunch biochar.

1.2 Aim of Study

The aim of this study was to explore the adsorption capacity of activated empty fruit bunch biochar (EFBB) in removing commercial dyes assisted by bacteria isolated from the sludge sample collected from Sri Serdang Lake.

1.3 Objectives of Study

The specific objectives of the study were:

1. To assess and study the adsorption capacity and physicochemical properties of chemically modified empty fruit bunch biochars (EFBB) in removing anionic dye (Direct Red 80) and cationic dye (Methylene Blue) in aqueous solutions compared to non-modified biochar and activated carbon (AC).

2. To assess the decolourisation of cationic dye (Methylene Blue) in aqueous solution by using bacteria isolated from the sludge sample collected from Sri Serdang Lake and to identify the degrading bacteria.

3. To assess the dissipation of cationic dye (Methylene Blue) by using simultaneous adsorption and biodegradation method utilising modified EFBB that has proven to have high adsorption capacity for methylene blue assisted by bacteria isolated from the sludge sample collected from Sri Serdang Lake.

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