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Optimising the Removal of Humic Substances from Peat Water by *Moringa oleifera* Seeds

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Widely used commercial coagulants such as alum and ferric chloride have been related to a variety of neurological and neurotoxic diseases due to the existence of their residuals. Therefore, the purpose of this work was to optimise the performance of *Moringa oleifera* seed, a natural coagulant, to remove humic substances from peat water, a potential source of drinking water. The coagulant was derived by drying the seeds for several days and then crushing them into a fine powder. Various instrumental techniques such as Scanning Electron Microscopy (SEM), Energy Dispersive X-ray analysis (EDX), Fourier Transform Infrared Spectroscopy (FTIR), and gas chromatography-mass spectrometry (GC-MS) were used to assess the morphology, functional groups and composition of the seeds. The SEM images revealed that the morphology of *Moringa* seeds is amorphous, with no fixed shape and numerous tiny pores. EDX statistics showed that carbon was the most abundant element, followed by oxygen. The FTIR spectrum revealed the coagulant's ionizable groups, which are hydroxyl, carboxyl, and amino groups. GC-MS results revealed that oleic acid is one of the primary components of the seeds, which mostly consist of fatty acids that aid in the coagulation process. In addition, the n-hexadecanoic acid composition in its methanolic extract demonstrated that the seeds possess antibacterial capabilities. Peat water samples were analysed for pH, dissolved oxygen (DO), turbidity, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solid (TSS), and humic substance ratio (E4/E6), with the water, was then classed into Class III of National Water Quality Standard (NWQS), requiring considerable treatment for water supply. Box-Behnken Design (BBD) and Response Surface Methodology (RSM) were used to optimise three independent factors that influenced the coagulation process (coagulant dosages, pH of peat water, and contact time) and a quadratic model was acquired data. The optimum conditions of 1.94 g/L of coagulant, pH 7.6, and contact time of 86 min resulted in the removal of 88.4% of humic substances, closer to the predicted removal of 91.1%. The largest inhibition zone was demonstrated by the ethanolic extracts against *S. aureus* (18.33 ± 0.58 mm), while the weakest activity was observed in hexanic extract against *E. coli* at 5.00 mm. These findings ascertain that *M. oleifera* possesses antibacterial properties against both gram-positive and gram-negative bacteria. In summary, this study explores the potential of *M. oleifera* seeds as a natural coagulant for treating peat water by characterising their properties, optimising their coagulation, and testing their antibacterial activity. The findings suggest that *M. oleifera* seeds have promising potential for use in water treatment and may provide a safer and more environmentally-friendly alternative to commercial coagulants.

Keywords: *Moringa oleifera*, coagulation-flocculation, response surface methodology, antibacterial

***Mengoptimumkan Penyingkiran Bahan Humik daripada Air Gambut oleh Biji* Moringa oleifera**

ABSTRAK

Koagulan komersial seperti alum dan ferik klorida telah dikaitkan dengan beberapa gangguan neurodegeneratif dan neurotoksik. Oleh itu, tujuan kajian ini adalah untuk mengoptimumkan prestasi biji Moringa oleifera, *penggumpal semulajadi, untuk menghilangkan bahan humik daripada air gambut, sumber air minuman yang berpotensi. Koagulan diperoleh melalui proses pengeringan biji benih selama beberapa hari yang kemudiannya melalui proses penghancuran sehingga menjadi serbuk halus. Pelbagai teknik analisa seperti Mikroskop Elektron Pengimbas (SEM), Sinar X Penyebaran Tenaga (EDX), Spektroskopi Inframerah Fourier Transformasi (FTIR), dan Gas Kromatografi-Jisim Spektroskopi (GC-MS) telah digunakan untuk mengkaji morfologi, kumpulan berfungsi dan komposisi benih. Imej SEM mendedahkan bahawa morfologi benih* Moringa *adalah amorfus, tanpa bentuk yang tetap dan mempunyai banyak liang yang kecil. Analisis EDX pula telah menunjukkan kehadiran karbon sebagai unsur yang paling banyak, diikuti oleh oksigen. Spektrum FTIR mendedahkan kumpulan terion koagulan, iaitu kumpulan hidroksil, karboksil, dan amino. Keputusan GC-MS telah menunjukkan bahawa asid oleik adalah salah satu komponen utama benih, yang kebanyakannya terdiri daripada asid lemak yang membantu dalam proses koagulasi. Di samping itu, komposisi asid n-heksadekanoik dalam ekstrak metanolnya menunjukkan bahawa benih mempunyai keupayaan antibakteria. Sampel air gambut telah dianalisis untuk pH, oksigen terlarut (DO), kekeruhan, permintaan oksigen kimia (COD), permintaan oksigen biokimia (BOD), jumlah pepejal terampai (TSS), dan nisbah bahan humik (E_4/E_6), dengan air kemudiannya dikelaskan ke dalam Kelas III Piawaian Kualiti Air Negara (NWQS), iaitu memerlukan rawatan yang agak banyak untuk* *dijadikan bekalan air. Reka Bentuk Kotak-Behnken (BBD) dan Metodologi Permukaan Respons (RSM) telah diaplikasikan untuk mengoptimumkan tiga faktor yang mempengaruhi proses koagulasi iaitu dos koagulan, pH air gambut, dan masa sentuhan dan model kuadratik telah digunakan dengan data yang diperoleh. Keadaan optimum iaitu 1.94 g/L koagulan, pH 7.6, dan tempoh sentuhan selama 86 minit telah menghasilkan penyingkiran bahan humik sebanyak 88.4%, hampir kepada penyingkiran yang diramalkan sebanyak 91.1% penyingkiran. Zon perencatan terbesar telah ditunjukkan oleh ekstrak etanol terhadap* S. aureus *(18.33 ± 0.58 mm), manakala aktiviti yang paling lemah didapati daripada ekstrak heksana terhadap* E. coli *pada 5.00 mm. Ini membuktikan bahawa* M. oleifera *mempunyai sifat antibakteria terhadap kedua-dua bakteria gram-positif dan gram-negatif. Secara ringkasnya, kajian ini meneroka potensi biji* M. oleifera *sebagai koagulan semula jadi untuk merawat air gambut dengan mencirikan sifatnya, mengoptimumkan pembekuannya, dan menguji aktiviti antibakterianya. Penemuan kajian ini menunjukkan bahawa biji* M. oleifera *mempunyai potensi yang baik untuk digunakan dalam rawatan air dan boleh dijadikan alternatif yang lebih selamat dan mesra alam kepada koagulan komersial.*

Kata kunci: Moringa oleifera, *koagulasi-flokulasi, Metodologi Permukaan Respons, antibakteria*

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOPs	Advance Oxidation Processes
BBD	Box-Behnken Design
BOD	Biochemical Oxygen Demand
CCD	Central Composite Design
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
EDX	Energy Dispersive X-ray Spectroscopy
FAS	Ferrous Ammonium Sulphate
FTIR	Fourier Transform Infra-Red Spectroscopy
GC-MS	Gas chromatography–mass spectrometry
H_2O	Water
HS	Humic Substance
IZ	Inhibition Zone
NWQS	National Water Quality Standard
RBI	4-(α-L-rhamnosyloxy)-benzyl isothiocyanate
RSM	Response Surface Methodology
SEM	Scanning Electron Microscope
TOC	Total Organic Carbon

TSS	Total Suspended Solid
UNIMAS	Universiti Malaysia Sarawak
UV-vis	Ultraviolet Visible

CHAPTER 1

INTRODUCTION

1.1 Study Background

Human well-being is a nebulous concept that incorporates all aspects from self-fulfilment needs to psychological needs and fundamental necessities (Aruma & Hanachor, 2017; Uysal et al., 2017). Thus, constant striving to improve Quality of Life (QOL) to achieve human well-being has been a long-term goal of individuals, communities and the government (Štreimikienė, 2015). To improve QOL, it is necessary to determine and fulfil human demands. Amongst various human demands, access to clean water is one of the most indispensable requirements in daily life.

Every living entity relies on water to sustain itself, making it the most crucial ingredient in life. Nowadays, the scarcity of drinkable water is one of the most important concerns faced by the world's population, with annual average scarcity rising from 30 to 40 % (van Vliet et al., 2021), prompting them to utilise polluted water as a substitute. Water pollution can result in a variety of illnesses, most notably diarrheal diseases such as cholera and dysentery (Davies et al., 2015). In 2016, nearly 0.9 million people around the world died because of unsafe drinking water, unsafe sanitation, and poor hygiene. This included more than 470 000 deaths of children under the age of 5 who died from diarrhoea (WHO, 2019). As a result, it is important to gain clean water access for consumption. Water resources such as groundwater, surface water, river, and collected rainwater are among the common sources for drinking water purposes (Aziz et al., 2014; Katsanou & Karapanagioti, 2017; Khayan et al., 2019). While most rural and distant populations have access to water resources such as rivers and lakes, as well as limited access to peatland, utilising these sources can have severe consequences owing to contamination and pollution, resulting in life-threatening sickness (Xu et al., 2018; Aikowe & Mazancová, 2021; Herawati et al., 2021).

Globally, peatlands occupy 4.23 million km^2, which is around 2.84% of land and store 10% of non-glacial freshwater (Xu et al., 2018). Many of Southeast Asia's coastal plains are covered in a thick layer of peat dating back to 30 000 years before the present (Page et al., 2004). While the contribution of peatlands to the world's drinking water supply is unknown, a study by Xu et al. (2018) demonstrated the usage of peat water in places where peatlands are a critical source of water, establishing that peat-rich catchments serve a population of 71.4 million people. Around 85% of all drinking water in the United Kingdom and Ireland is derived directly from peatlands, demonstrating that peatlands are vital to these countries' water security (Xu et al., 2018).

Many of Southeast Asia's coastal plains are covered in a thick layer of peat dating back to 30 000 years before the present(Page et al., 2004). Around 60% of peatland regions of the world are concentrated in Southeast Asia (Wall, 1989; Bell, 2014) with 82% in Indonesia, 8.8% in Papua New Guinea, and 8.3% in Malaysia. Smaller amounts of peatlands can be found in the Philippines (240 000 ha), Vietnam (183 000 ha), and Thailand (68 000 ha). Wosten et al. (2006) reported that these countries do use peatland as their water source in rural coastal areas when it is the only source of potable and irrigation water. Furthermore, knowledge of peat water treatment should be considered, as numerous watersheds may be reliant on shared peat domes.

Despite extensive water supplies, certain large areas in Malaysia, particularly Sarawak, rely heavily on peat water as their primary source of water (Eklip & Ling, 2018). With approximately 2.6 million hectares of peatland in Malaysia, more than 70% is located in Sarawak, a minute percentage in Sabah, and the remaining 20% is located in peninsular Malaysia (Irvine et al., 2012; Melling, 2016). Since peat water covers around 13% of Sarawak's total land area, this represents potential access to water resources provided investigation efforts are devoted to peat water treatment (Sa'don et al., 2014).

Numerous studies have attempted to treat peat water to produce clean water or drinking water. Ritson et al. *(*2016) conducted a study on the treatment of peatland vegetation in a chosen site in Southwest England. The treatment was done to obtain drinking water by adopting traditional water treatment methods, which include coagulation, flocculation, and sedimentation. Ferric sulphate was utilised as a coagulant in the study to replicate the removal of dissolved organic carbon (DOC) from the water. Peat water treatment is also widely performed in Indonesia, with the most common methods being flocculation-coagulation and membrane filtration (Ali et al., 2021). Indonesia also developed a technology called IPAG60, which has been effectively deployed to address the issue of clean water and drinking water scarcity in peat areas (Atmana Sutapa et al., 2020). The technology includes a coagulator, six flocculation tanks and a reservoir tank for the treatment to take place. Another investigation was conducted on the treatment of peat water through electrocoagulation by employing a pair of aluminium electrodes (Bow & Dewi, 2019). Apart from that, peat water treatment has been conducted using advanced oxidation processes (AOP), electrocoagulation, membrane technology, and reverse osmosis (Apriani et al., 2016; Daud et al., 2016; Turnip et al., 2017; Bow & Dewi, 2019).

As previously indicated, most of the processes used inorganic coagulants in their treatment, but researchers have discovered a few shortcomings arising from their utilisation. There are a few downsides to adopting these coagulants, including excessive sludge generation and the potentially toxic nature of the leftover metallic coagulants (Nogaro et al., 2013; Pontius, 2016). In addition, the usage of these coagulants is more expensive due to the necessary pH correction, and the high dosages required for water treatment (Sahu & Chaudhari, 2013). Interestingly, these coagulants for example alum and ferric chloride are suspected to cause neurological problems and cardiovascular diseases over time, which may be lethal to humans (Sieliechi et al., 2010; Bondy, 2016; Bondy & Campbell, 2017). As a result of these concerns, researchers all over the world have been attempting to replace commercial water coagulants with natural, and non-toxic water coagulants.

A green approach can be adopted in replacing hazardous coagulants with non-toxic coagulants. Green technology or approach frequently incorporates energy efficiency, recycling, safety and health considerations, and the use of renewable resources (Othmani et al., 2020; Bahrodin et al., 2021; Alazaiza et al., 2022). Thus, plant-based coagulants are included in green technology due to their renewable, non-hazardous, degradable, and potentially carbon-neutral properties and are gaining popularity as a replacement for conventional coagulants. Natural, water-soluble, organic, ionic, and non-ionic polymers of varying molecular weights generated from various plant components are referred to as plant-based coagulants (Othmani et al., 2020; Ahmad et al., 2021).

In colloid-free aqueous conditions, the plant-based coagulant molecules are randomly distributed in the liquid and do not interact with each other or with any other particles present (Ang et al., 2020). However, in solutions containing colloids, the plant-based coagulant molecules can adsorb onto the surface of these particles and form constrained, irreversible arrangements of loops, tails, and trains (Kurniawan et al., 2020). The adsorption process occurs due to the attractive forces between the charged functional groups on the coagulant molecule and the oppositely charged groups on the colloid surface (Katana et al., 2022). As more and more coagulant molecules adsorb onto the colloid particle, they can form bridges between multiple particles, leading to their aggregation and eventual settling.

Few researchers have used plant-based coagulants in their work, including *Cicer arietinum* (Gurumath & Suresh, 2019), *Plantago ovata* (Ramavandi, 2014), *Hibiscus sabdariffa* and *Jatropha Curcas* (Sibartie & Ismail, 2018), and *Strychnos potatorum* seeds (Kumar et al., 2016b). However, to the best of our knowledge, no study has been conducted on the use of plant-based coagulants for peat water treatment, and hence this study is proposed.

Moringa oleifera, widely known as the "multipurpose tree," was chosen for the study as its seeds are frequently employed in wastewater treatment (Azad & Hassan, 2020; Desta & Bote, 2021). This study attempted to apply seeds of *M. oleifera* specifically to remove humic substances (HS) that acidify the water and give it a brownish-blackish colour.

1.2 Problem Statement

Due to the shortage of local water resources, peat water is usually available as a reservoir of freshwater under natural conditions (Ritson et al., 2016; Xu et al., 2018). However, peat water needs to be treated as it is acidic and contains a high concentration of organic matter (Syafalni et al., 2013; Atmana Sutapa et al., 2020). Previously, peat water was treated using traditional wastewater treatment methods, with the addition of inorganic coagulants such as alum and ferric chloride. However, due to arising concerns about the residues left in the treated water, a growing number of studies have been conducted on the use of plant-based coagulants as a substitute for chemical-based coagulants since they are