



**Faculty of Resource Science and Technology**

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

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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* ( $18.33 \pm 0.58$  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 ( $18.33 \pm 0.58$  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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|                  |                                                     |
|------------------|-----------------------------------------------------|
| 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 <sub>2</sub> O | Water                                               |
| HS               | Humic Substance                                     |
| IZ               | Inhibition Zone                                     |
| NWQS             | National Water Quality Standard                     |
| RBI              | 4-( $\alpha$ -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<sup>2</sup>, 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 (Ekli & 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

renewable, non-hazardous, biodegradable, and potentially carbon-neutral (Hamad et al., 2016; Saleem & Bachmann, 2019). *M. oleifera* seeds are considered one of the most favourable coagulants in improving water quality owing to their natural cationic peptides of molecular weight ranging from 6 to 16kDa that can function for water clarification (Ndabigengesere et al., 1995; Raj et al., 2011; Oladoja, 2015). Besides the increasing study on replacing chemical-based coagulants in water treatment with *M. oleifera*, there is a paucity of research on its characterisation and chemical properties. In response to this problem, this study investigated the characterisation of *M. oleifera* seeds to ascertain their efficacy as a clarifying agent and also determined the effectiveness of *M. oleifera* as a natural water coagulant in treating peat water samples. HS are important components of the natural organic matter (NOM) in soil, water, and geological organic deposits such as lake sediments, brown coals, and peats. In peat water, it imparts a brownish-blackish hue that affects the water's odour, flavour, and pH. The removal of this substance will diminish the water's colour, hence increasing the water's acceptability for human consumption.

### **1.3 Hypothesis**

- i. *M. oleifera* seeds can effectively remove HS from peat water.
- ii. An optimum condition is required to maximise the removal of HS from peat water
- iii. *M. oleifera* has antibacterial properties against common microbes found in water namely, *Escherichia coli* and *Staphylococcus aureus*.

### **1.4 Research Questions**

- i. Can *M. oleifera* seeds effectively remove HS from peat water?

- ii. What is the optimum condition required for the application of *M. oleifera* in peat water treatment?
- iii. Does *M. oleifera* have antibacterial properties against common microbes found in water namely, *E. coli* and *S. aureus*?

## 1.5 Objectives

This study aims to evaluate the feasibility of *M. oleifera* seeds in degrading HS in peat water to reduce colour. This can be achieved through the following set of objectives:

- i. To characterise the chemical and physical properties of *M. oleifera* seeds and the physicochemical parameters of peat water samples,
- ii. To optimise the coagulation of HS with *M. oleifera* seed powder via Response Surface Methodology (RSM), and
- iii. To compare the antibacterial activity of *M. oleifera* against common microbes that contaminate water sources.

## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **2.1 Peat Soil and Peat water**

Malaysia relies heavily on river water for its water supplies. Out of 189 river basins in Malaysia, only 22 of them are located in Sarawak (Gasim et al., 2014). Due to the shortage of local water sources, peat lands which cover most of the land in Sarawak are usually available as a reservoir of freshwater under natural conditions (Ritson et al. 2016; Xu et al. 2018). Sarawak, Malaysia's largest state, features one of the largest peatlands reserves (Figure 2.1). Coastal and riverine areas comprise around 19% of the state's land area. This area has both freshwater peatland (1.698 million hectares) and mangrove forest (154,000 hectares). Peat soils are located mostly inside the delta and along the riverine systems of the Samarahan-Sadong, Lupar-Saribas, Rajang, Baram, and Limbang rivers. However, peat water needs to be treated as it is acidic and contains a high concentration of organic matter (Syafalni et al., 2013).

Peats are frequently referred to be partially degraded biomass and organic matter that has accumulated from plants during periods of waterlogging and oxygen deprivation (Page & Baird, 2016; Rein, 2016). To understand the formation of peat swamps, it is necessary to understand how organic deposits occur and the conditions that favour their development. Organic materials are created through biochemical processes, although their accumulation is mostly determined by climatic conditions and the habitats in which the peat forms (Page & Baird, 2016). Peat is generated when organic molecules aggregate under specific conditions, particularly when the production of biomass exceeds its chemical breakdown, resulting in the formation of peat (Andriessse, 1988; Zulkifley et al., 2013).



**Figure 2.1:** Location of peat swamps in Malaysia (Source: Melling, 2016)

Peat formation is typically a quick biological process. It is theorised to occur as a result of aerobic bacteria that are capable of entirely decomposing organic matter in deposit surface layers during periods of low subsurface water (Andriesse, 1974; Szafranek-Nakonieczna & Stêpniewska, 2014). Differing from aerobic conditions, no single type of anaerobic bacteria seems to be able to completely mineralize organic matter in peat soils. Due to this, peat that is made in the peat-producing layer stays the same and undergoes minor changes over time. (Zulkifley et al., 2013; Szafranek-Nakonieczna & Stêpniewska, 2014). Anaerobic marshy conditions are caused by the accumulation of partially digested biomass or organic matter in the form of peat, which is caused by specific hydrotopography (Zulkifley et al., 2013; Melling, 2016). Climate, landform, local geology, and hydrology are only a few of the factors that influence these characteristics. This circumstance inhibits microbial activity, which is necessary for the chemical breakdown of organic molecules (Andriesse, 1974).

Therefore, the existence of aerobic or anaerobic conditions determines whether or not biomass accumulates and how it occurs. This observation can be verified by examining the peat composition at various types of sites (Dai et al., 2020). Table 2.1 shows the compositions of swamp and forest peat as dry organic matter. There is a distinction drawn between forest peat which is more aerated than peats generated under swampy conditions that are very anaerobic. It appears that lignin and carbohydrates have been completely broken down in forest peat. This implied low levels of these organic compounds in forest peat. In swamps, on the other hand, peats have high levels of unaltered lignin and cellulose (Zulkifley et al., 2013).

**Table 2.1:** Compositions of swamp and forest peat (in the percentage of dry organic matter) (adapted from Andriesse 1988; Kurbatov 1968)

| Fraction       | Swamp Peat                      |                                | Forest Peat                   |
|----------------|---------------------------------|--------------------------------|-------------------------------|
|                | Carex Swamp<br>(30% Decomposed) | Reed Swamp<br>(40% Decomposed) | Birchwood<br>(55% Decomposed) |
| Bitumen        | 3.3                             | 1.1                            | 8.8                           |
| Humic acids    | 32.2                            | 33.6                           | 52.2                          |
| Hemicellulose  | 15                              | 8.6                            | 1                             |
| Cellulose      | 3.5                             | 3.7                            | 0                             |
| Lignins        | 12.9                            | 18.6                           | 0                             |
| Cutin          | 11.9                            | 5.2                            | 16                            |
| Not determined | 21.2                            | 29.2                           | 22                            |

The term “peat” also refers to the net accumulation of pure organic matter, and the distinction between soil and organic or vegetative accumulation varies (Sina et al., 2011; Teong et al., 2016). This is most likely due to differing definitions in the various academic

fields concerned with the practical study of peat and its properties. Although there are numerous definitions of peat, the Soil Division of Sarawak (Malaysia) has adopted a profile partition-based definition for peat, defining it as soil that contains 50 cm or more organic soil matter within a 100-cm profile or soil that contains more than twice the mineral content of the overlying bedrock within 50 cm (Mohamed et al., 2002).

Peat water comes from peatlands (Yallop et al., 2010). In Malaysia, peatlands have grown near the coast, behind mangrove coastlines. Sulphides in the mud and water of mangroves inhibit bacteria, causing organic waste to build up as peat (Jon et al., 2010). Peat dome commonly occurred between two rivers (Koh et al., 2013). In their floodplain stage, rivers have natural levees that recede, leaving a thin strip of alluvium behind. These mineral-rich soils foster the growth of freshwater swamp forests. Peat formation is prevented by riverbank inundation, which declines with time. This distinguishes 'topogeneous' peats, which receive nutrient inputs from river water, from 'ombrogenous' peats or bogs, which receive solely precipitation as water input (Jon et al., 2010). Between the two rivers, a dome-shaped expanse of peat will form (Figure 2.2). Typically, topogeneous peat occupied the base and edges of peat domes, as well as the lower bank of streams draining peat swamps, whereas ombrogenous peat occupied the central area of peat domes (Mohamed et al., 2002; Ahamad, 2005). In relatively young domes, the centre bog plain is quite small, whereas in older domes, it is enormous.

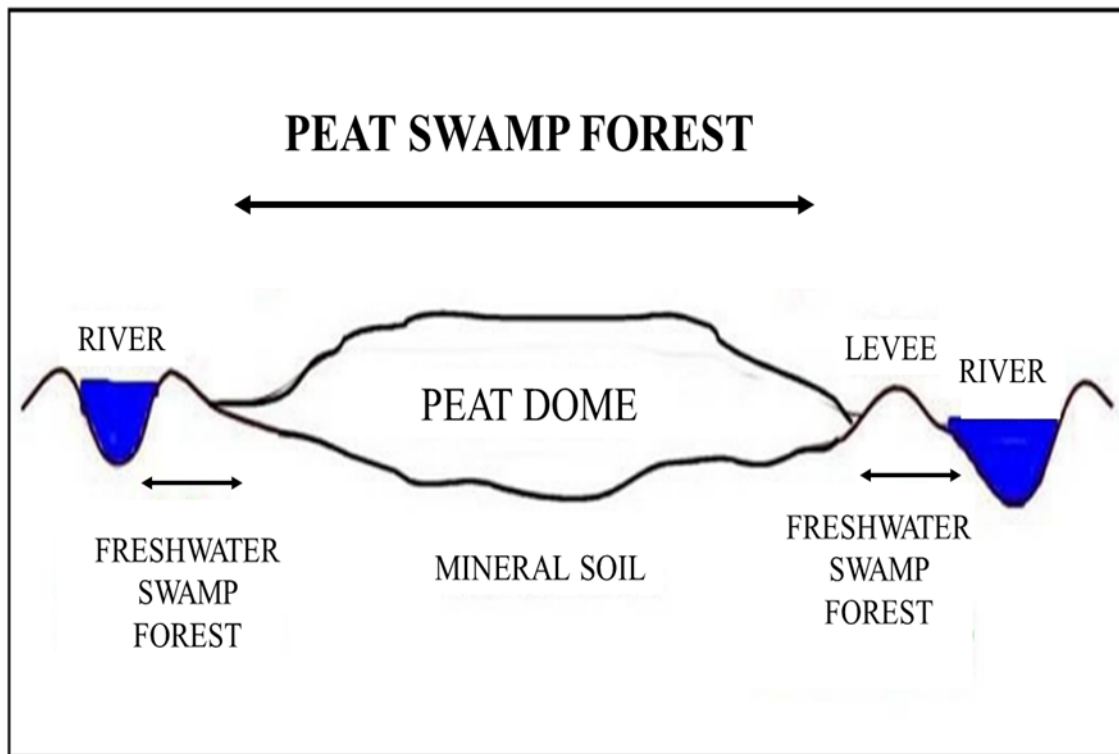


**Figure 2.2:** Diagrammatic representation of a more highly developed inland peat dome.  
Adapted from Jon et al. (2010)

### 2.1.1 pH

In Sarawak, peat soil has relatively acidic or low pH ranging from 3.2 to 4.0 (Sime Darby Services, 1999). The acidity of peat soil increases as hydrogen ions ( $H^+$ ) accumulate in the solution and hydroxyl ions ( $OH^-$ ) decrease. It is a by-product of microbial degradation processes, cation exchange, and acid intake from the atmosphere in peatlands.

Variations or changes in the pH of the soil would affect the acidity of peat water. In the first case, bacteria and fungi decompose dead plant and animal matter, releasing acid into the surrounding environment during the decay process. Also, the abundance of *sphagnum* in peat water acts as a cation exchanger, acidifying the surrounding environment (Tveit et al., 2020). It adsorbs base cations from the soil water, such as calcium (Ca) and magnesium (Mg), and exchanges or releases hydrogen ions in their place (Priest & Davis, 2012). Thus,

as *sphagnum* moss develops and populates the peatland habitat, it increases the number of hydrogen ions in the ecosystem, acidifying the wetland. Besides, the differences in pH might occur due to the mineral soil admixtures that generally raise the pH or by specific sites within the peat swamp (Rezanezhad et al., 2016). Different areas of ombrogenous peat swamps have the thickest section's surface layer having a lower pH than the shallower organic soils towards the edge.

### **2.1.2 Humic Substance**

Peat water can also be classed based on the amount of HS present, as the pH of peat water is proportional to the number of HS present. HS is a family of yellow to black organic compounds formed from the decayed plant, animal and microbial matter that occurs in the environment extensively (Sen et al., 2020; Gautam et al., 2021). HS are an identifiable part of the organic matter in soil and they constitute the biggest reservoir of inert organic carbon in the terrestrial environment (Kelleher & Simpson, 2006; Zavarzina et al., 2021). HS can be classified based on its UV-vis absorbance ratio of  $E_4/E_6$ , molecular weights, acid-base content, elemental atomic ratio and total hydrolysable carbohydrates. HS can be classified into three types namely fulvic acid (FA), humic acid (HA), and humin (Pham et al., 2021). Table 2.2 shows the characteristics of HS from the topogeneous organic soils in Sarawak.

**Table 2.2:** Chemical characteristics of humic substances from the topogeneous organic soils in Sarawak (Source: Fong, 2000)

| Parameter                              | Unit  | Humic Acids | Fulvic Acids | Humin |
|----------------------------------------|-------|-------------|--------------|-------|
| UV-Vis, E <sub>4</sub> /E <sub>6</sub> | -     | 7.7 – 10.2  | 10.5 – 18.2  | 3.3   |
| Apparent molecular weight, Mw          | Da    | 5003 – 5488 | 4233 – 4910  | -     |
| Acid base content                      |       |             |              |       |
| COOH                                   | meq/g | 3.3 – 5.4   | 3.3 – 9.0    | 0.6   |
| Phenolic OH                            | meq/g | 2.5 – 4.4   | 0.7 – 5.9    | 1.7   |
| Elemental atomic ratio                 |       |             |              |       |
| H/C                                    |       | 1.13 – 1.19 | 1.14         | -     |
| O/C                                    |       | 0.61 – 0.76 | 0.77         | -     |
| N/C                                    |       | 0.03 – 0.04 | 0.01         | -     |
| Total hydrolysable carbohydrates       | mg/g  | 19.6 – 33.8 | 10.8 – 27.9  | 2.3   |

Note: - : not reported

HA, the most prevalent constituent of HS, is insoluble in acidic water below pH 2, but soluble at higher pH values (Baglieri et al., 2014). Primarily, they are derived from biochemically active environments, such as soil, rivers, and leonardite (Kulikova et al., 2016; Jin et al., 2018). In general, the term HA refers to a component of humus that is brown to black, polymeric, and alkali-soluble, and is found in certain fungus (Mahler et al., 2021; Vikram et al., 2022).

Fulvic acid is produced via biological and geochemical processes such as the decomposition of food scraps in the compost. It can also be derived from manure, soil, composting materials, or materials that can be processed into a supplement (Mahler et al., 2021). The compound is soluble in water within the whole pH range.

Another fraction of HS known as humin, however, is insoluble in an aqueous solution within the whole pH range (Li et al., 2015). Creon and apocreon acids were identified as subgroups of the fulvic acids fraction; hymatomelanic acid is considered as the initial product in the biosynthesis of humic acids; meanwhile, ulmic acids and ulmins - substances belong to both humic acid and humin fractions (Kosobucki & Buszewski, 2014).

### **2.1.3 Dissolved Organic Carbon (DOC)**

It is rather unusual to examine the carbon content in peat water though it is known that the wetland contains a high concentration of dissolved organic carbon (DOC). Generally, the organic carbon content of the surface is greater than that of the subsoil. A study by Salome et al. (2010) showed that the carbon content in the topsoil is higher than that of the subsoil which is 12.56 mg/g soil and 3.39 mg/g soil, respectively. Another study by George et al. (2013) reported that the organic carbon content of peat soil ranges from 50% to 60%, whereas subsoil has only contained 20% organic carbon.

The DOC levels, however, change according to the peats' surroundings (Kalbitz et al., 2000). A study by Nuriman et al. (2016) measured the DOC values of peat water from different sites of land usage. The chosen sites were secondary forests, open areas, bush, agriculture, young rubber plantation and oil palm plantation. DOC values were found to be greater in new rubber plantations and secondary woodland compared to the other locations. Intensive land agriculture causes DOC concentrations to decrease. Due to oxic conditions and microbial activity, freshly disturbed peat will release the most DOC into the surroundings. This is consistent with another study conducted by Gandois et al. (2013) whereby the DOC of peat water in deforested peatland was found to be higher than the pristine site with values of  $79.9 \pm 5.5$  mg/L and  $62.2 \pm 2.2$  mg/L, respectively.

In addition, the DOC concentration also varies according to the degree of peat decomposition. A study was undertaken to determine the DOC content in peat water at three different depths, namely surface, middle, and bottom, and the findings revealed that the DOC concentration decreased from surface to bottom (Nuriman et al., 2016). A decline in the DOC content at the bottom layer indicated that the rate of decomposition is faster at the surface of peat water.

## **2.2 Water Treatment**

To satisfy the required drinking water quality standard, the water industry is working extremely hard to provide high-quality drinking water at a cheaper cost. The water treatment process encompasses physical, chemical, and biological changes that transform untreated water into potable water. Typically, a traditional water treatment procedure involves coagulation, flocculation, sedimentation, filtration, pH control, and disinfection.

Figure 2.3 illustrates the processes in a sequential water treatment plant. The coagulation process, which facilitates the removal of dirt and colloidal particles, might be regarded as the most essential stage of the treatment plant's activities as is critical for producing acceptable water quality and maintaining plant profitability. However, poor control of the coagulation process will result in chemical waste, poor water quality, and the failure of sedimentation and filtration operations.

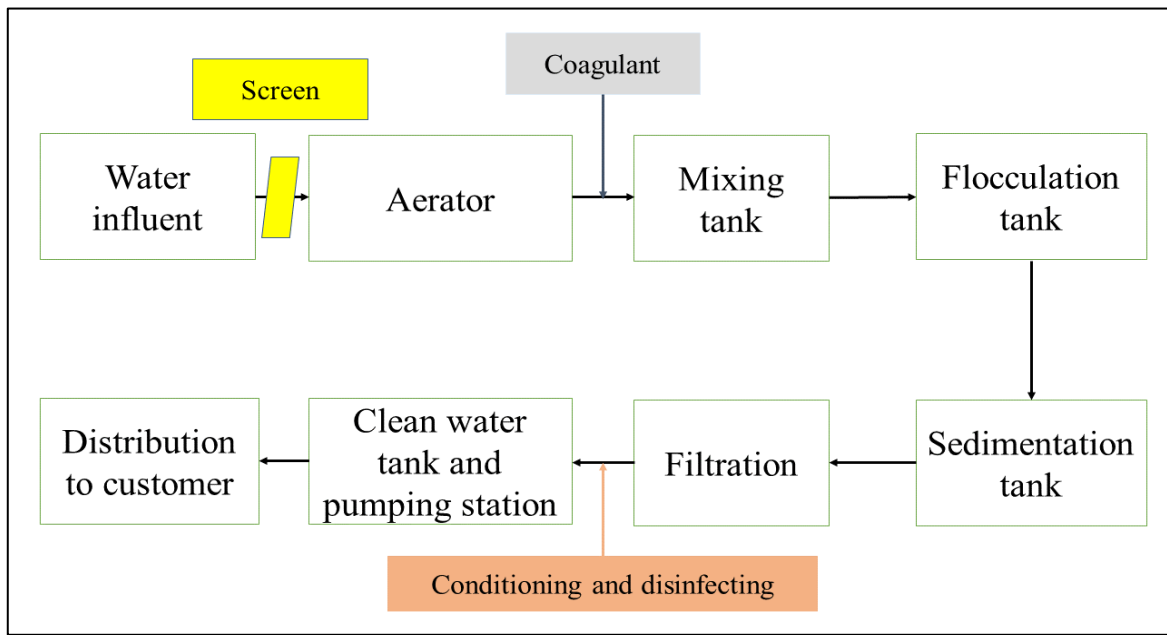


**Figure 2.3:** Sequence of procedures involved in the water treatment

Numerous technologies have been applied for water treatment; membrane filtration (Domany et al., 2002; Lowe & Hossain, 2008; Prisciandaro et al., 2008; Katsoufidou et al., 2010; Hakami et al., 2019; Subagyo & Isworo, 2020), adsorption (Duan et al., 2003; Gupta et al., 2009; Zulfikar et al., 2014; Liu et al., 2015; Derakhshani & Naghizadeh, 2018), biological method (Zouboulis et al., 2004), oxidation (Sanly et al., 2007; Uyguner et al., 2007; Jin et al., 2013; Salvestrini et al., 2020) and coagulation-flocculation (Zhang et al., 1990; Yu et al., 2003; Bratskaya et al., 2004). These techniques, however, have their pros and cons (Table 2.3). However, only a few of them had been used in the treatment of peat water, with a subset of those applied exclusively for the removal of humic compounds, the primary component of peat water in its original or commercial form.

Filtration using a membrane system is an excellent way for purifying peat water. Multiple layers of membranes are required in the filtration process to eliminate dissolved

organic contaminants and to produce drinking water. To ease the processes that occur in each layer of the membrane, the flow pattern, velocity, and pressure at each layer level must be optimised (Jørgensen et al., 2020). Numerous filtration technologies have been studied including ceramic membrane microfiltration (Saifuddin et al., 2020), filtration using xylem plants (Boutilier et al., 2014), filtration method using self-cleaning materials (Mo et al., 2016; Sun et al., 2018), and multi soil layering (MSL) filtration (Zein et al., 2016). In addition, hybrid filtration methods were also investigated for peat water treatment by a combination of two difference techniques, such as hybrid slow sand filtration-coagulant treatment (Muhammad, 2020) and hybrid coagulation-ultrafiltration method (Arhin et al., 2018).

**Table 2.3:** The advantages and disadvantages of water treatment methods

| Methods             | Advantages                                                                                                                                                                                                                                  | Disadvantages                                                                                                                                                                                                                                                                                                                                                        | Reference                                                                                                                                                         |
|---------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Membrane filtration | <ul style="list-style-type: none"> <li>- Remove humic acid in macromolecules</li> <li>- Does not require additional chemical substances</li> <li>- Very minimum energy requirement</li> </ul>                                               | <ul style="list-style-type: none"> <li>- Membrane fouling</li> <li>- Act as a very specific filter</li> </ul>                                                                                                                                                                                                                                                        | Domany et al. (2002);<br>Lowe and Hossain (2008);<br>Prisciandaro et al. (2008);<br>Katsoufidou et al. (2010);<br>Hakami et al. (2019);<br>Subagyo et al. (2020); |
| Adsorption          | <ul style="list-style-type: none"> <li>- Able to remove various substances in water with high efficiency</li> <li>- Relatively low energy usage</li> <li>- Simple design</li> <li>- Cost-effective (land-used and initial costs)</li> </ul> | <ul style="list-style-type: none"> <li>- Require synthesis for more advanced adsorbent which increases the cost</li> <li>- The need for the regeneration process and loss of activated carbon after each regeneration cycle</li> </ul>                                                                                                                               | Duan et al. (2003); Gupta et al. (2009); Muzek et al. (2013); Liu et al. (2015);<br>Derakhshani and Naghizadeh (2018); Utama et al. (2020); Sukmana et al. (2021) |
| Biological method   | <ul style="list-style-type: none"> <li>- Relatively low costs</li> <li>- No pH adjustment is needed</li> <li>- Low dosage needed</li> </ul>                                                                                                 | <ul style="list-style-type: none"> <li>- Significant amount of organic, non-biodegradable substances may remain</li> <li>- Complete colour removal is not possible</li> <li>- Require a large land area, diurnal, and greater time to function</li> <li>- Little flexibility in design and operation</li> <li>- Yet to be applied to peat water treatment</li> </ul> | Zouboulis et al. (2004);<br>Pell and Wörman (2009);<br>Roy and Saha, (2021)                                                                                       |

**Table 2.3** continued

|                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                       |
|-------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Advanced Oxidation Processes (AOPs) | <ul style="list-style-type: none"><li>- Modular construction and small footprint</li><li>- Excellent disinfection and oxidation qualities</li><li>- Reduce a broad spectrum of chemical n biological contaminants</li><li>- The stability of the design</li><li>- High removal efficiency</li><li>- Environmental compatibility</li></ul>                                                                                                                                       | <ul style="list-style-type: none"><li>- Adjustment of pH needed after treatment to stop oxidation.</li><li>-Relatively high capital/operating and maintenance cost</li><li>- Has specific chemistry for specific contaminants</li></ul>                                                                            | Sanly et al. (2007); Uyguner et al. (2007); Chelme-Ayala et al. (2009); Jin et al. (2013); Hutagulung et al. (2014); Apriani et al. (2016); Salvestrini et al. (2020) |
| Coagulation-flocculation            | <ul style="list-style-type: none"><li>- Reduce membrane fouling</li><li>- Occurred in a wider concentration range which reduces the dosage of coagulant-flocculant</li><li>- Simplicity of its technology</li><li>- Cost-effective (use abundant chemicals and low-cost chemicals)</li><li>- Inexpensive capital cost</li><li>- Good sludge settling and dewatering characteristics</li><li>- Rapid and efficient for insoluble contaminants (pigments, etc.) removal</li></ul> | <ul style="list-style-type: none"><li>- Possibility of secondary contamination which may be hazardous to human health</li><li>- High sensitivity of inorganic coagulants to pH</li><li>- Formation of sludge has a limited potential for recycling due to the non-biodegradability of synthetic polymers</li></ul> | Zhang et al. (1990); Yu et al. (2003); Bratskaya et al. (2004); Ardiansyah et al. (2013)                                                                              |

Membrane filtration functions as a very selective filter, which is one of the key downsides of this approach. They can only filter contaminants that are compatible with their pores (Subagyo & Isworo, 2020). The other drawbacks of membrane technology include membrane fouling, lowering flux and short membrane life duration (Pal, 2015). Such approaches, on the other hand, do not require any additional chemical compounds, eliminating the risk of residual contaminants in treated water while also consuming very little energy.

Adsorption is commonly regarded as a reliable and cost-effective technology for water treatment based on startup costs and land needs (Rashed, 2013). Several studies have explored its potential for peat water treatment. Adsorption is a mass transfer process that involves the transfer of a solute or removable species from a liquid phase to the surface of a solid phase (Barakat, 2011; Rashid et al., 2021). Through physiochemical interactions, pollutants that have been adsorbed stick to a solid surface, which has high efficiency and requires little energy to remove a variety of compounds from water (Barakat, 2011; Rashid et al., 2021). The process consists of three stages: (1) migration of the adsorbate to the adsorbent's border shell, (2) intraparticle diffusion into pores, and (3) solute adsorption and desorption (Plazinski & Rudzinski, 2009; An, 2020); which is governed by the adsorbate, adsorbent, and matrix properties (Rashid et al., 2021). Ideally, an adsorbent should include sufficient binding sites to perform effective adsorption of targeted contaminants. Activated carbon, a common treatment adsorbent, has the disadvantage of becoming more expensive to manufacture and requiring regeneration after each treatment cycle. (Derakhshani & Naghizadeh, 2018). More sophisticated adsorbents, such as nanostructured metal oxides, carbon nanotubes, porous boron nitride nanosheets, and porous graphene, are now available

as a result of technical advancements, albeit these advanced adsorbents necessitate the production of intricate molecules or materials (Rashed, 2013).

Biological treatment is an extensively employed and well-accepted for water treatment; which involves the treatment of biosorbents including microorganisms (eg: *Sphingomonas paucimobilis*, *Staphylococcus hominis*), fungi (e.g. *Phanerochaete chrysosporium*), yeasts (eg: *Candida tropicalis*), and algae (eg: *Chlorella vulgaris*) (Aksu & Tezer, 2005; Charumathi & Das, 2010; Ayed et al., 2011; Rani et al., 2014; Singh et al., 2014). Biological treatment is a natural process in which the organisms contribute to water treatment simply by carrying out their life-sustaining functions in the presence of oxygen (aerobic treatment), or in the absence of oxygen (anaerobic treatment) or the combination of both (Pell & Wörman, 2009; Jung & Pauly, 2011; Roy & Saha, 2021). This technique is known to be reasonably inexpensive due to the minimal dosage required and the absence of pH adjustment (Zouboulis et al., 2004). This strategy, however, has a few drawbacks, including a significant quantity of organic, non-biodegradable substances that may remain in the treated water. Biological treatment also offers less design and operation flexibility and necessitates a large land area, diurnal operation, and a longer operating duration (Roy & Saha, 2021). Additionally, it has not yet been used to remediate peat water.

Advanced oxidation processes (AOPs) are a group of chemical treatment technologies that are exceptionally successful at removing a wide variety of pollutants, both chemical and biological, as well as being an excellent disinfectant for water treatment (Giannakis et al., 2015; Badmus et al., 2018). AOPs are based on the oxidative powder of in-situ generated reactive radicals, for example, hydroxyl radicals ( $\text{HO}\bullet$ ) and sulphate radicals ( $\text{SO}_4^{\bullet-}$ ) (Glaze et al., 1987; Uyguner et al., 2007; Deng & Zhao, 2015; Cuerda-

Correa et al., 2020). However, AOPs, in general, are regarded as high cost as a result of the usage of costly reagents (for example,  $H_2O_2$ ) and high energy consumption (generation of  $O_3$  or UV radiation) (Cuerda-Correa et al., 2020). Fenton, ozonation, photocatalysis, sonolysis, ultraviolet (UV) photolysis, or wet air oxidation reactions have their own merits and flaws (Mishra et al., 2017; Tufail et al., 2020). Fenton process and ozone-based AOPS were applied to peat water for the removal of colour, control of taste and odour, turbidity, oxidation of trace synthetic organic compounds, and many other parameters (Hutagalung et al., 2014; Apriani et al., 2016).

### **2.3 Coagulation-flocculation**

Coagulation-flocculation appears to be the most efficient and frequently used method in water and wastewater treatment due to its simplicity and cost-effective process. Due to its simplicity of design, it is the most frequently used chemical treatment method for both drinking water and wastewater. Studies have reported that coagulation-flocculation successfully reduced turbidity, suspended particles, organic matter, colour, oil, and COD in water (Crini and Lichtfouse, 2019; Owodunni and Ismail, 2021). This technology frequently involves the use of coagulants to alter the physical state of dissolved and suspended solids, hence facilitating their removal via a subsequent sedimentation process (Iwuozor, 2019; Kurniawan et al., 2020).

Coagulation is a type of chemical water treatment that involves the modification of electrostatic charges of suspended particles in water to remove solids. As such, it is important to add coagulants to water that contain small, highly charged molecules to disrupt the charges on suspended particles, colloids, and greasy components present in the water. Coagulants may be either metallic salts (such as alum) or polymers; organic substances which can be

synthesised in the laboratory consisting of a lengthy chain of smaller molecules (Sahu and Chaudhari, 2013).

The agitation process, commonly known as flocculation, subsequently follows. Three distinct phases comprise the conventional flocculation unit process (Figure 2.4). The first one is rapid or flash mixing in which the appropriate coagulants are applied to a stream of wastewater that is agitated and intensely mixed at high speed. Rapid mixing is fundamental to ensure that the added coagulant is distributed or dispersed evenly and thoroughly in the water medium. This is then followed by slow mixing where the wastewater is only moderately churned to generate huge flocs that can be easily sorted out. The last phase is sedimentation. The floc formed during flocculation is allowed to settle out and is separated from the effluent stream.

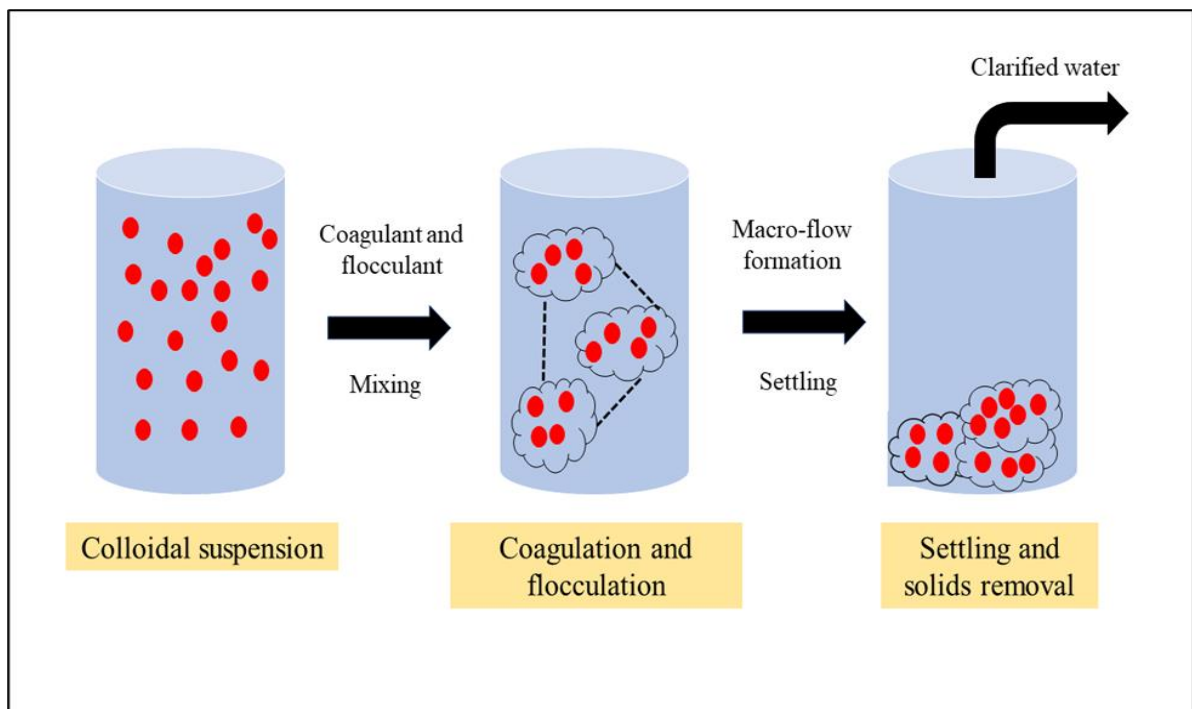


**Figure 2.4:** Coagulation-flocculation process. Adapted from Wilson (2014)

## 2.4 Types of Coagulants

Coagulants are used to remove various hazardous materials suspended in water and wastewater including organic pollutants and pathogens (e.g. bacteria and viruses) (Barlovská & Ilavsky, 2012; Mangale Sapana et al., 2012; Ramavandi, 2014; Wang et al., 2019) and, as well as other contaminants such as phosphorous, and arsenic to meet certain water quality criteria or standard (Araújo et al., 2013; Polepalli & Rao, 2018; de la Fuente et al., 2019). There are various forms of coagulants used in water and wastewater treatment. These coagulants may be chemical, synthetic, or natural (Tetteh & Rathilal, 2019).

### 2.4.1 Inorganic Coagulants

Aluminium-based salts and iron-based salts are the most common inorganic coagulants that are frequently applied in water and wastewater treatment as it is widely available (Niquette et al., 2004). Inorganic coagulants are frequently used because they are easy to handle and can be applied for the removal of turbidity (Zand & Hoveidi, 2015; Khan et al., 2016; Kalavathy et al., 2017; Malik, 2018), chemical oxygen demand (COD) (Guida et al., 2007; Di Bella et al., 2014; Prajapati et al., 2015; Yateh et al., 2020), and dye (Ashtekar et al., 2014; Naghan et al., 2015; Gu et al., 2019). When these coagulants are added to wastewater, they would react with hydroxyl ions ( $\text{OH}^-$ ) which are dissociated from water molecules, forming monomeric and polynuclear species (Verma et al., 2012; Tetteh & Rathilal, 2019). This would results in the dissolution of metal salts, releasing their trivalent ions to form complex water molecules of  $[\text{Al}(\text{H}_2\text{O})_6]^{3+}$  and  $[\text{Fe}(\text{H}_2\text{O})_6]^{3+}$  for aluminium and iron, respectively (Gupta et al., 2012). Typically, under acidic conditions, the substitution of  $\text{OH}^-$  for water molecules ( $\text{H}_2\text{O}$ ), forms soluble  $\text{Al}(\text{OH})^{2+}$  and  $\text{Fe}(\text{OH})^{2+}$ , which improves coagulation efficiency. This is due to the trivalent ions,  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$  being strongly adsorbed onto the colloidal negative surface (Tetteh & Rathilal, 2019).

The efficiency of most inorganic coagulants to remove impurities such as turbidity, COD, BOD and others is dependent on the pH of the water. The optimal pH range of aluminium-based coagulants is within pH of 6.5 to 7.5 whilst iron-based coagulants have a significantly greater range of pH from 4 to 11 (Khan et al., 2016; Kalavathy et al., 2017).

Table 2.4 the commonly applied inorganic coagulants along with their advantages and disadvantages. Despite being widely applied due to their low cost and availability; metal-salts coagulants also have their limitations. Its application generates a massive amount of sludge. When no harmful substances are present, this sludge can be reused as fertiliser for agriculture; nevertheless, when toxic sludge is present, the solid waste must be treated or disposed of properly which increases the cost of water treatment (Yonge, 2012). As previously stated, most inorganic coagulants have a pH range to work efficiently. Thus, pH adjustment is required in wastewater treatment to boost the treatment efficiency. pH adjustment is not only required during the water treatment process but also thereafter to ensure the pH of the drinking water is within the permitted range from 6.5 to 8.5 as specified by the World Health Organization (WHO) (Tang et al., 2014; Jagaba et al., 2018; Malik, 2018). Another disadvantage of utilising metal-based coagulants is their noxious and corrosive nature. Ferric-based coagulants are extremely caustic, leaving prominent rust stains or a reddish tinge associated with chemical spills and leaks, detracting from their aesthetic look and imparting a metallic flavour to the water (Sharma & Bhattacharya, 2017).

**Table 2.4:** Advantages and disadvantages of inorganic coagulants

| Coagulants                                                                                   | Advantages                                                                                                                                                                                                                                                                                                                                | Disadvantages                                                                                                                                                                                                                                                                                                             |
|----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Aluminium sulphate ( $\text{Al}_2(\text{SO}_4)_3$ ), aluminium chloride ( $\text{AlCl}_3$ )  | Inexpensive, stable, easy to handle and apply, available in both solid and solution forms, effective in soft water, can be stored almost indefinitely without any loss of effectiveness, optimum pH (6.5 – 7.5)                                                                                                                           | Increase dissolved solids (salts) to water, only effective over a limited pH range (6.5-7.5), pH adjustment is needed, high dosages are often required, may result in various health issues, high alkalinity consumption; the production of large volumes of post-treatment sludge, high post-treatment aluminium residue |
| Sodium aluminate ( $\text{Na}_2\text{Al}_2\text{O}_4$ )                                      | Effective in hard waters, only a small dosage is usually needed, no requirement for the addition of lime and hydroxides, and minimal chemical sludge is produced                                                                                                                                                                          | Often act as coagulant aid and paired with alum, high cost, ineffective in soft water                                                                                                                                                                                                                                     |
| Polyaluminium Chloride (PAC)<br>$\text{Al}_{13}(\text{OH})_{20}(\text{SO})_4.\text{Cl}_{15}$ | Floc formed is denser and faster settling than alum in some applications, produces less sludge, and causes a minimum drop of pH which is avoided/minimized. The need for pH adjustment, limits aluminium residuals, has a broader optimum pH than other aluminium-based metals coagulant (5 – 8); available both in solid and liquid form | PAC liquid storage conditions require a maximum temperature of 40°C; resulting in high turbidity and colour levels in the treated water if the addition of coagulant exceeds the limit; sludge has a high content of nitrogen, phosphorous, potassium and other metal elements                                            |

**Table 2.4** continued

|                                                                                                                                                                                                |                                                                                                                                                                |                                                                                                                                                                                                                                                                                                           |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Ferric Sulphate<br>( $\text{Fe}_2(\text{SO}_4)_3$ ),<br>Ferric Chloride<br>( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ),<br>Ferrous Sulphate<br>( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) | Has a broader optimum pH (4-11), produces a denser floc that can be easily removed; easy to handle and apply, not sensitive to temperature changes unlike alum | Add dissolved solids (salts) to water, high alkalinity consumption, corrosive, require greater chemical addition, very corrosive, residual iron affects the chlorination process in treated water, the coloration of water and the metallic taste of water, causes health issue; produces a lot of sludge |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

However, none of the disadvantages outweighs its impact on human health (Kumar et al., 2017; Krupińska, 2020). The excess levels of aluminium and iron in the water from water treatment or plumbing systems might result in intestinal constipation, abdominal colic, and spasms (Tetteh & Rathilal, 2019). Additionally, elevated iron levels in the blood caused by the consumption of residual metals in water could damage the gastrointestinal tract (GIT), impairing its ability to regulate absorption (Sieliechi et al., 2010). Individuals with a higher intake of aluminium and iron residues have a higher risk of developing neurodegenerative disorders over time, including Parkinson's disease and other nervous and cardiovascular diseases, which may eventually result in death from this condition known as Amyotrophic Lateral Sclerosis (Sieliechi et al., 2010; Bondy, 2016). Long-term exposure to these residues may also generate dialysis encephalopathy, a degenerative neurological condition characterised by renal failure and Alzheimer's disease (Kvech & Edwards, 2001; Sieliechi et al., 2010; Knowles et al., 2015; Bondy, 2016). Aluminium residues found in the brains of Alzheimer's disease patients have been shown to impair neurotransmission by directly blocking enzymes or membrane components (WHO, 2003; Sieliechi et al., 2010).

#### **2.4.2 Organic Coagulants**

Organic coagulants have both synthetic and biological origins. They are typically employed to separate particles from liquids and produce sludge. Organic coagulants are classified according to the covalent charges and linkages of their polymeric molecules. They have a range of charges and are grouped as anionic, non-ionic, or cationic. Typically, molecular weights (MWs) of organic coagulants range from 3,000,000 to 20,000,000 g/mol and the higher the MWs, the more effective they are due to their lengthy chain (Ghernaout & Arabia, 2020). This is due to the fact that they have fibres and threads that bind the small

particles together into substantial agglomerates, making the long chain the most advantageous (Irki et al., 2018; Ghernaout et al., 2019).

Synthetic polymers, however, are the most often used organic coagulants. Polyamines, polydiallyldimethylammonium chloride (poly-DADMACS), dicyandiamide resins, and melamine-formaldehyde resins are a few examples of organic coagulants. The most well-known cationic coagulants are polyacrylamides and poly-DADMACs.

Chitosan originated from the shells of crabs and shrimps. It is used as an effective biological coagulant in the potable water sector due to its multiple benefits. They operate across a wider pH range without altering the original pH of the treated water and no residuals of aluminium are left behind. However, the price is still a major concern. Organic polymers are significantly more expensive than inorganic coagulants, and biopolymers are even more expensive than manufactured organic polymers (Tetteh & Rathilal, 2019; Jiang et al., 2021).

The second category of organic wastewater chemicals is melamine formaldehydes and tannins which are used in the coagulation of colloidal particles in water. Due to their high capacity for absorbing organic contaminants such as oil and grease, these chemicals are particularly well-suited for hazardous sludge treatment (Abdullah & Abidin, 2018; Rosa et al., 2020). They behave similarly to inorganic coagulant products whereby they not only coagulate colloidal particles in the water but also precipitate their floc. These organic chemicals can be more cost-effective than inorganic coagulants when sludge removal and disposal costs are considered (Ibrahim et al., 2021; Tomasi et al., 2022).

## 2.5 Types of Polymeric Coagulants

Due to their unique properties, hydrophobic polymeric coagulants developed from inorganic coagulants have attracted much attention (Kango et al., 2013). Organic coagulant polymers can be categorised into two types: natural and synthetic. Natural polymers are molecules that are harmless to people, abundant, and environmentally beneficial. However, many natural polymers may not be useful in all wastewater treatment applications. This could be owing to their chemical structure (e.g. tannin, starches, gelatine galactomannans). Organic polymers can be easily tweaked and adjusted during the manufacturing process to expand their applications. Straight, branched, or cross-linked polymer chains are used to produce a variety of modified versions. Figure 2.5 displays the chemical structures of an anionic synthetic polymer, poly(diallyldimethylammonium chloride) (pDADMAC), cationic polyacrylamides (CPAMs) as well as chitosan, a cationic natural polymer (Tetteh & Rathilal, 2019; Maliki et al., 2022)

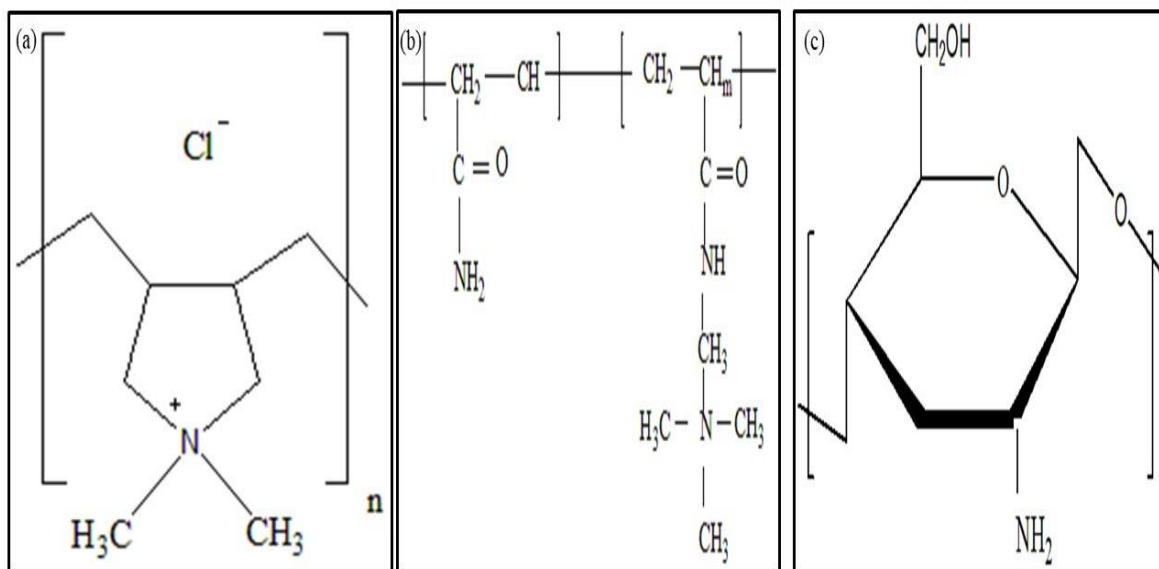


**Figure 2.5:** The chemical structures of (a) poly(diallyldimethylammonium chloride) (pDADMAC) (b) cationic polyacrylamides (CPAMs) and (c) chitosan (Tetteh & Rathilal, 2019; Maliki et al., 2022)

Organic polymers are frequently used with inorganic coagulants to increase the coagulation-flocculation effectiveness, however, occasionally, the synthesised organic polymers can be harmful to people (Kango et al., 2013; Sillanpää et al., 2018). Table 2.5 shows examples of different types of polymeric coagulants (Shewa & Dagnew, 2020).

**Table 2.5:** Types of polymeric coagulants/flocculants and their examples (Source: Shewa and Dagnew, 2020)

| Polymer Type | Examples                                                                                                                                                                                                                                                                                                                                                                                                         |
|--------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cationic     | <ul style="list-style-type: none"> <li>• Poly(diallyldimethyl ammonium chloride)</li> <li>• Epichlorohydrin/dimethylamine polymers</li> <li>• Cationic polyacrylamides</li> <li>• Poly(alkylamines) [poly(ethyleneimine), poly(vinylamine)]</li> <li>• Poly(styrene) derivatives</li> <li>• Ionenex</li> <li>• Sulphonium polymers</li> <li>• Natural cationic polymers (chitosan, cationic starches)</li> </ul> |
| Anionic      | <ul style="list-style-type: none"> <li>• Anionic polyacrylamides</li> <li>• Carboxylic acid polymers</li> <li>• Phosphonic acid polymers</li> <li>• Sulphonic acid polymers</li> <li>• Natural anionic polymers (sulphated polysaccharides, modified lignin sulphonates)</li> <li>• Polyacrylamide (PAM)</li> </ul>                                                                                              |
| Nonionic     | <ul style="list-style-type: none"> <li>• Natural non-ionic polymers (starch, cellulose derivatives)</li> </ul>                                                                                                                                                                                                                                                                                                   |

### 2.5.1 Anionic Polymers

Anionic polymers lose their positive charge when they are dissolved in a medium. Their polymerisation is highly sensitive. The process involves changes in molecular weight,

density, charge groups and structure (Wilhelm et al., 2020). Adding bases like hydroxide or an acid like hydrochloric acid causes hydrolysis. As shown in Figure 2.6 the polyacrylamide is hydrolysed at basic pH. Through the process, an anionic polymer is formed (Figure 2.6).

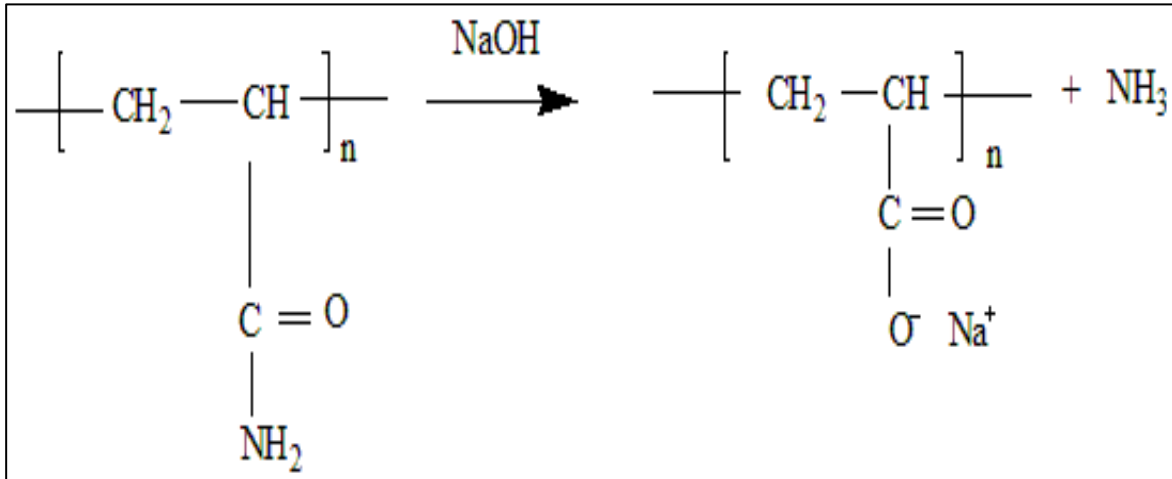


**Figure 2.6:** Hydrolysis of the polyscrylamide under basic pH conditions

### 2.5.2 Cationic Polymers

Cationic polymers are positively charged coagulants derived from natural or synthetic sources. For example, poly(diallyldimethylammonium chloride) (pDADMAC), epichlorohydrin/dimethylamine polymers (ECH/DMA), and cationic polyacrylamides (CPAMs) are all synthetic cationic polymers, whereas chitosan is a naturally occurring cationic polymer. They are powerful electrolytes regardless of their pH fluctuation (Tetteh and Rathilal, 2019). Both charge density and pH affect the protonated amine groups. The increased charge density indicates that the polymer has a larger loop to facilitate interparticle bridging and act as an effective destabilisation medium (Makhtar et al., 2020; Moud, 2022).

### 2.5.3 Natural or Non-ionic Polymers

Numerous naturally occurring polymers possess intrinsic cationic characteristics that can be changed to create a cationic polyelectrolyte that is suitable for use as flocculants in

solid-liquid separations (Dao et al., 2016; Jiang et al., 2021). Non-ionic polymers exhibit a wide range of structural, molecular weight, and degradability characteristics. Polyacrylamides (PAMs), chitosan, starch without substitutions, cellulose derivatives, and glues are a few examples. Chitosan, like most natural polymers, is non-toxic, making it generally safe for human consumption. This polymer has been used in water purification applications such as decolorizing dye house effluents, treating food processing wastes, removing metal ions, and conditioning sludge (No & Meyers, 2000; Ong et al., 2011; Vithanage et al., 2015; Labidi et al., 2019).

Additionally, natural polymers are abundant in plants and minerals. Several examples are Nirmali seeds, *M. oleifera* seeds, eggplant seeds, and radish seeds, all of which are locally available as vegetables for water treatment. These coagulants are non-toxic, renewable, reduce sludge production, biodegradable, and inexpensive. Also, natural coagulants have a broad dosage range and do not alter the pH value of treated water (Owodunni & Ismail, 2021; Alazaiza et al., 2022; Koul et al., 2022).

## **2.6 *M. oleifera* and its Active Components**

*M. oleifera* which belongs to the Moringaceae family is an Indian native plant that grows in tropical and subtropical climates (El Sohaimy et al., 2015; Gopalakrishnan et al., 2016). Cationic proteins in *M. oleifera* seeds with a net positive charge serve as a clarifying agent that destabilises the negatively charged impurities in water (Adesina et al., 2019). It can produce flocs, which are then removed by flocculation, filtration, or sedimentation, making the treated water appear clearer and less cloudy. The mechanisms involved are adsorption and charge neutralisation (Beltrán-Heredia et al., 2009; El-Kammah et al., 2022; Hadadi et al., 2022). The technique involves direct filtration, employing lower dosages of

coagulant and coagulation pHs, which are required for the creation of stronger microflocs, resulting in enhanced filtering conditions (Yin, 2010; Choy et al., 2014; Cruz et al., 2020).

The seeds are also rich in the sources of hemagglutinating proteins called lectins; a carbohydrate-binding protein that mediates attachment and binding on its intended target (Saleem & Bachmann, 2019). The active agents in *M. oleifera* seeds namely carbohydrates and proteins are the reasons behind its purification effect (Choy et al., 2014). The coagulation properties demonstrated by the proteins in *M. oleifera* seeds are used to improve the physicochemical properties of water such as turbidity (Chaudhuri & Khairuldin, 2009; Ali et al., 2010; Sotheeswaran et al., 2011; Egbuikwem & Sangodoyin, 2013; Varkey, 2020), colour (Chaudhuri & Khairuldin, 2009; Padmapriya et al., 2014; Kavitha & Kalaicelvi, 2016), electrical conductivity, temperature (Tunggolou & Payus, 2017b) and total hardness (Sotheeswaran et al., 2011; Padmapriya et al., 2014).

It is known that the pH of water is not significantly altered by the addition of *M. oleifera* seeds compared to alum. A study demonstrated the addition of *M. oleifera* seeds only affects the final pH of water slightly by reducing its pH from 6.70 to 6.43 contrary to the addition of alum which caused a marked reduction from pH 6.70 to pH 3.76 (Delelegn et al. 2018). The seeds of *M. oleifera* also exhibited a higher turbidity removal than alum in a study conducted by Tunggolou and Payus (2017). At the dosage of 15mg/L of *M. oleifera* seeds and 55 mg/L of alum, the turbidity can be reduced to  $3.17 \pm 0.7$  NTU and  $7.26 \pm 2.13$  NTU, respectively. The treated water sample by using the seeds of *M. oleifera* was found to meet the WHO's drinking water turbidity requirement of less than 5 NTU, demonstrating its ability to be employed as a water coagulant (WHO, 1993).

Studies on the active components of *M. oleifera* have been documented in a few studies (Madrona et al., 2010; Shunmugapriya et al., 2017; Okuda et al., 1999). Flocculating protein was extracted from the seeds of *M. oleifera* using phosphate buffer extraction followed by cation exchange chromatography. The protein has a molecular mass of roughly 6.5 kDa, according to an older study (Gassenschmidt et al., 1995; Ndabigengesere et al., 1995), although more recent research indicates that its molecular mass can range from 6.5 kDa to 14 kDa with an isoelectric point of 10 to 11 (Sánchez-Martín et al., 2010; Camacho et al., 2017; Formentini-Schmitt et al., 2019; Vunain et al., 2019).

The amino acid analysis and sequencing revealed significant glutamine, arginine, and proline content, as well as a total of 60 residues. The cationic protein of *M. oleifera* was then compared to a synthetic cationic polymer, demonstrating the natural product can operate as a water coagulant equivalent to those synthetic polymers (Gassenschmidt et al., 1995).

Besides that, water-soluble cationic proteins are found in *M. oleifera* seed extracts with a low molecular weight that aid in coagulant activity (Sengupta et al., 2012; Choudhary & Neogi, 2017; Narender et al., 2019). The active ingredients are present on the seeds as well as on the kernel, or embryo of the seeds. Nonetheless, the seed themselves yield higher turbidity removal and thus should be considered a better source coagulant than its coat (Gaikwad & Munavalli, 2019).

## **2.7 Mechanism of Coagulation**

Various mechanisms have been proposed to elucidate how *M. oleifera* proteins could function as a water coagulant (Kwaambwa et al., 2010; Santos et al., 2012; Garde et al., 2017; Polepalli & Rao, 2018). In general, the positively charged proteins of *M. oleifera* can adhere to negatively charged suspended particles. Through colloidal charged neutralization,

the positively charged *M. oleifera* proteins bind to the negatively charged suspended particles by coulomb forces neutralizing the surface charge (Sengupta et al., 2012). Through the adsorption mechanism, the bounded opposite charges lead to the formation of different charged particles' surface flocs due to particle collision and interparticulate saturation (Gassenschmidt et al., 1995; Ndabigengesere et al., 1995; Ali et al., 2016; Hoa & Hue, 2018; Gaikwad & Munavalli, 2019). The efficiency of the adsorption mechanism depends on the adsorbent's surface area, surface morphology, pore size distribution, polarity and functional groups attached to the adsorbent surface (Gaikwad and Munavalli (2019). Naturally dried seed kernel extract showed higher removal of turbidity, suggesting that it contains more active ingredients for adsorption and inter-particulate bridging.

*M. oleifera* seed powder can be used to treat wastewater by adsorbing and neutralizing charges. The seed powder contains water-soluble cations made up of various proteins, which can receive a proton from water and release a hydroxyl group due to the presence of basic amino acids in the seeds (Muthuraman et a., 2013; Vunain et al., 2019). This release of hydroxyl ions increases the pH of the solution, destabilizing particles. This process combines destabilized particle adsorption and charge neutralization or adsorption and particle bridging, resulting in particle agglomeration and floc formation. The flocs can then be decanted from the solution after sedimentation.

Another study reported chemisorption as the main mechanism of *M. oleifera* for the removal of arsenic (Sumathi & Alagumuthu, 2014). It is a type of adsorption characterised by a chemical reaction between the surface and the adsorbate. Amongst various isotherms models that were tested (Langmuir, Freundlich, Temkin, Dubinin Radushkevich (D-R), and

Flory-Huggins (F-H)), the Temkin isotherm model showed a linear plot with a correlation coefficient,  $R^2$  of 0.9976.

## 2.8 Preparation of *M. oleifera* as a Coagulant

There are a few methods involved in the preparation of *M. oleifera* as a water coagulant. According to Kansal and Kumari (2014), there are three main stages in preparing the coagulants namely primary, secondary, and tertiary (Figure 2.7).

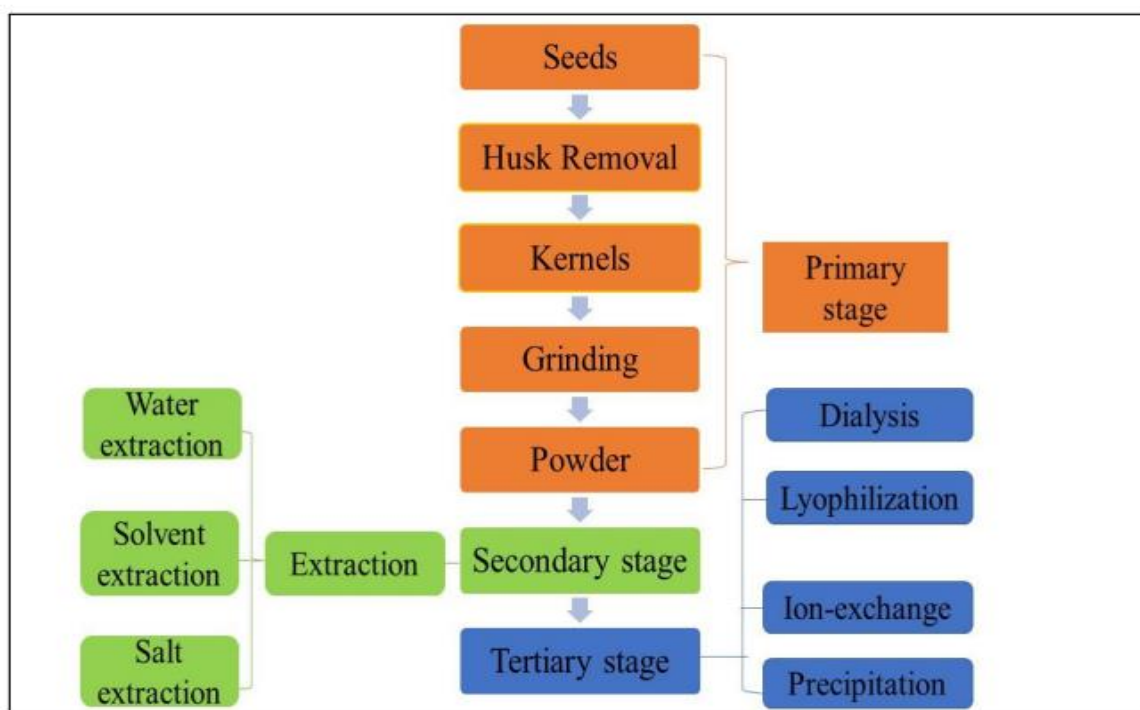


**Figure 2.7:** Preparation of coagulant from seeds via the primary, secondary and tertiary stages

The seeds are removed from their husk, washed with distilled water and left to dry. The drying process can be done in two ways: naturally by air or sun, or by using an oven. (Gaikwad & Munavalli, 2019). After drying, the seeds will be ground and sieved through a mesh strainer to obtain fine powders. These typical steps have been applied in several studies to prepare *M. oleifera* coagulant as it is a facile method (Hamza et al., 2013; Yuliasri et al., 2016; Muruganandam et al., 2017; Delelegn et al., 2018; Varkey, 2020). These steps are, however, suspected of causing the rising increase of organic load in treated water as plant tissues are still present in the seeds (Kansal & Kumari, 2014). Also, the presence of oil in *Moringa* seeds powder might affect its effectiveness as a water coagulant (Hoa & Hue, 2018). Thus, further steps are needed to overcome these limitations.

The subsequent stage, which is the secondary process, entails extracting the powdered *M. oleifera* seeds using either distilled water or salt solutions (e.g. sodium chloride (NaCl), and potassium chloride (KCl) (Chaudhuri and Khairuldin, 2009; Madrona et al., 2010; Sotheeswaran et al., 2011). Several studies have reported the use of water specifically distilled water as a solvent for seed extraction (Sengupta et al., 2012; Awad et al., 2013; Garde et al., 2017; Shan et al., 2017; Abiyu et al., 2018; Hoa and Hue, 2018; Adesina et al., 2019; Gaikwad and Munavalli, 2019). This is due to the abundance of water, its cost-effectiveness and the active agents of *M. oleifera* are water-soluble (Yin, 2010; Kansal and Kumari, 2014; Saleem et al., 2020).

Nevertheless, better coagulation activity using *M. oleifera* product via salt solution extract has been also reported. Oladoja (2015) compared the performance of *M. oleifera* seeds extracted using distilled water and a variety of salt solutions against the turbidity of synthetic turbid water. Compared to the extraction of distilled water, the 1 M NaCl product

had the lowest residual turbidity at 1.6 NTU, demonstrating that the ionic presence aids in the elimination of turbidity. Other studies have also investigated salt solution as a favourable solvent for seed extraction purposes (Barrado-Moreno et al., 2016; Carvalho et al., 2016; Teixeira et al., 2017; Formentini-Schmitt et al., 2019; Megersa et al., 2019). Madrona et al. (2012) extracted the protein in *M. oleifera* seeds using distilled water and NaCl solution (0.01 M, 0.1 M and 1 M). The amount of protein extracted using the aforementioned salt solution is much greater than that of the one extracted using distilled water at higher molarity. The protein in water, 0.01 M NaCl, 0.1 M NaCl, and 1 M NaCl were 1832.60 mg/L, 1290.86 mg/L, 4388.01 mg/L, and 4499.19 mg/L, respectively (Madrona et al., 2012). The coagulation activity is aided by an increase in ionic strength due to the presence of salts. According to studies, *M. oleifera* contains a cationic dimeric protein with a high molecular weight that can destabilize particles in water through neutralization and adsorption, leading to the flocculation of colloids and subsequent sedimentation (Madrona et al., 2012; Camacho et al., 2017).

However, other studies have found that applying the extracted *M. oleifera* protein (secondary stage) increased the organic load in treated water, which has been associated with a variety of additional adverse effects (Awad et al., 2013; Kansal & Kumari, 2014; Camacho et al., 2017; Villaseñor-Basulto et al., 2018). The presence of DOC also led to the increased level of COD, colour, taste, odour and overall water quality. Besides that, DOC could also lead to a higher level of disinfection by-products in the chlorination process (Pandit & Kumar, 2015). The increasing levels of organic nitrate and phosphate (Kansal & Kumari, 2014) and the formation of microbial re-growth (Notodarmojo et al., 2017) has been linked to the presence of DOC in the treated water.

At the tertiary stage, the aim is to isolate and purify the bio-active components in the aqueous extract. This can be achieved by the application of precipitation, dialyzing and lyophilization methods (Choudhary & Neogi, 2017), cross-flow filtration method (Ali et al., 2010; Kwaambwa et al., 2010), and purification by ion exchange (Sánchez-Martín et al., 2010). The extracted protein from the isolation and purification stage used as water coagulants showed a remarkable effect; almost complete removal of turbidity was recorded with no increase of organic load found in the treated synthetic sample (Choudhary & Neogi, 2017). Trivedi et al. (2011) employed 95 mg/L of *M. oleifera* seeds as a coagulant and reported that almost 99% of turbidity was reduced from a model turbid water sample. The amount of coagulant utilised was approximately 25% less than the amount of alum used, which was 125 mg/L, indicating requires only a little amount of extract to reduce turbidity effectively.

Despite its effectiveness, the tertiary stage of coagulant synthesis is not commonly used by researchers for water treatment due to its inconvenience, requirement for expensive equipment, and longer processing time, which make it less cost-effective (Sánchez-Martín et al., 2010; Sánchez-Martín et al., 2012; Choudhary & Neogi, 2017; Hoa & Hue, 2018). Hence, it is more convenient and cost-effective for researchers to synthesise their coagulant up to the first or second stage.

## **2.9 Response Surface Methodology**

RSM was introduced by George E. P. Box and K. B. Wilson back in 1951 (Olayiwola et al., 2011). It is a collection of mathematical and statistical methodologies used to enhance, improve, and optimise the parameter in question (Aydar, 2018; Behera et al., 2018). RSM aims to achieve the best possible outcome for a response variable that is affected by multiple

independent variables. To determine the factors that cause changes in the output response, the experiment involves a series of tests called runs, where the input variables are modified.

The response can be graphically depicted, either in three-dimensional space or as contour plots that aid in visualising the form of the response surface at each level of a component and in combination with other factors (Jahan et al., 2016). A graphical representation may depict a response surface that is above the two variables which simplifies the optimisation process. Thus, by inspecting the plot, one can determine the settings of the variables at the targeted response, usually the maximum or the minimum.

Simultaneously, RSM allows one to study several factors and responses by examining their possible relationships through regression analysis while saving time and cost as compared to the conventional universal approach (Behera et al., 2018). RSM is a critical tool for identifying the smallest number of tests required to address the experiment objectives when more than two independent variables are involved (Boyacı, 2005; Aydar, 2018). In circumstances when a huge number of factors have an impact on a response, this application has proven to be highly effective (Behera et al., 2018; Rajewski & Dobrzyńska-Inger, 2021).

The experimental data are analysed to determine the best fit of an empirical model (linear, quadratic, cubic, or two-factor interaction). The coefficients of the model express the linear interaction, and quadratic effects. The correlation coefficient ( $R^2$ ), adjusted determination coefficient ( $\text{Adj-}R^2$ ), and adequate precision are used to determine the model's adequacy; the model is considered adequate when the  $p\text{-value} < 0.05$ , lack of fit  $p\text{-value} > 0.05$ ,  $R^2 > 0.9$  and Adeq Precision  $> 4$  (Ganapathy et al., 2011; Ramakrishnan et al., 2018).

There are two main experimental designs used in RSM namely the Central Composite Design (CCD) and Box-Behnken Design (BBD).

### 2.9.1 Central Composite Design

Central Composite Design (CCD) is the most popular design for fitting the second-order model which is composed of three components: (1) a factorial (cube) portion of at least resolution V, (2) the star (axial) portion at distance  $\alpha$ , from the centre of the design along each axis, and (3) a central point which located at the centre of the design space (Figure 2.8) (Chigbu & Ukaegbu, 2017; Das & Dewanjee, 2018). The model yields numerical values for the major variables and interaction effects by measuring the curvature of the response surface.

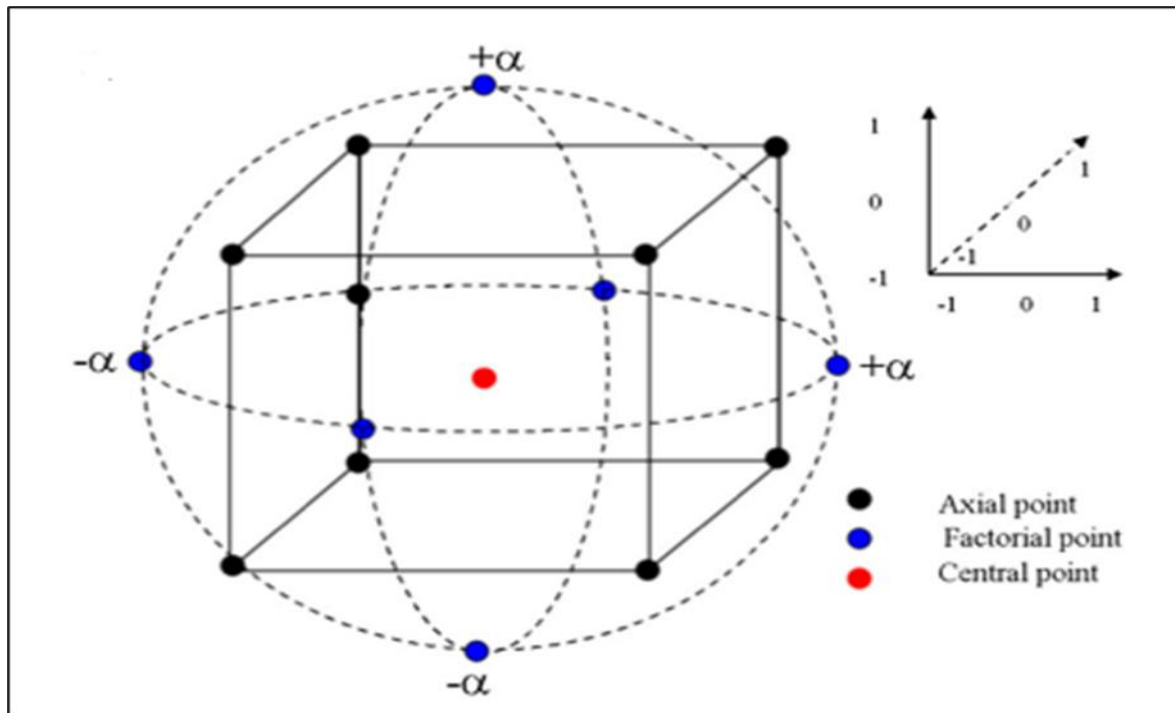


**Figure 2.8:** Schematic diagram of central composite design (CCD) (Source : Kumar and Prasad (2019))

For full uniformly rotatable CCD, it requires an experiment number based on  $N = k^2 + 2k + cp$ , where  $k$  is the factor number and  $cp$  is the replicate number of the central point. It also requires  $\alpha$ -values that are proportional to the number of variables and can be determined using the formula  $\alpha = 2^{(k-p)/4}$ . On top of that, all components evaluated in CCD are studied into five levels  $(-\alpha, -1, 0, +1, +\alpha)$  (Ranade & Thiagarajan, 2017).

CCD has been widely used in different types of wastewater treatments such as paper mill wastewater, saline wastewater, textile wastewater, and tannery wastewater. Table 2.6 highlights various examples of CCD application in wastewater treatment. The results indicate that their experimental data were in agreement with the predicted value generated by the fitted model, hence indicating that this design is a viable tool for optimising wastewater treatment processes (Körbahti & Tanyolaç, 2008; Ali et al., 2012; Darvishmotevalli et al., 2019; Genawi et al., 2020; Pandey & Thakur, 2020).

This design, on the other hand, necessitates a high number of experiments, with a minimum of 20 for three factors, rather than 15 for three factors as with BBD (Genawi et al., 2020). Since BBD requires fewer tests than CCD, more researchers are inclined to use this tool in their studies.

**Table 2.6:** Examples of studies on the optimisation of wastewater treatment using CCD

| Wastewater type                                  | Treatment technique               | Independent variables (X <sub>1</sub> , X <sub>2</sub> , .....)                                                               | Response(s)                                             | Optimum conditions and final result                                                                                                                                                                     | Reference                      |
|--------------------------------------------------|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|
| Synthetic water (kaolin suspension), river water | Coagulation-flocculation          | Agitation speed (X <sub>1</sub> ), Mixing time (X <sub>2</sub> ), Dosage (X <sub>3</sub> )                                    | Final turbidity                                         | X <sub>1</sub> = 40 rpm; X <sub>2</sub> = 41 min; X <sub>3</sub> = 0.75 mg/L<br><br><u>Final turbidity:</u><br><br>Synthetic water = 4.51 NTU<br><br>River water = 1.60 NTU                             | Ali et al. (2012)              |
| Papermill wastewater                             | Electrocoagulation                | pH (X <sub>1</sub> ), conductivity (X <sub>2</sub> ), electrode distance (X <sub>3</sub> ), current density (X <sub>4</sub> ) | Removal of COD, colour, and total dissolved solid (TDS) | X <sub>1</sub> = 7.02; X <sub>2</sub> = 5.67 mS/cm; X <sub>3</sub> = 1.57 cm; X <sub>4</sub> = 15.14 mA/cm <sup>2</sup><br><br>Percentage removal:<br><br>COD = 89.71%; Color = 94.91%;<br>TDS = 82.75% | Pandey and Thakur (2020)       |
| Saline wastewater                                | Electrochemical oxidation process | pH (X <sub>1</sub> ); reaction time (X <sub>2</sub> ); salt concentration (X <sub>3</sub> ); voltage (X <sub>4</sub> )        | Removal of COD and TOC                                  | X <sub>1</sub> = 7.69; X <sub>2</sub> = 30.71 min; X <sub>3</sub> = 30.94 g/L; X <sub>4</sub> = 7.41 V<br><br>Percentage removal:<br><br>COD = 91.78%; TOC = 68.49%                                     | Darvishmotevalli et al. (2019) |

**Table 2.6** continued

|                    |                           |                                                                                                                            |                                       |                                                                                                                                             |                             |
|--------------------|---------------------------|----------------------------------------------------------------------------------------------------------------------------|---------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| Textile wastewater | Electrochemical treatment | Pollution load (X1); applied potential (X2); electrolyte concentration (X3); reaction temperature (X4); reaction time (X5) | Removal of COD, colour, and turbidity | X1 = 100%, X2 = 8 V ; X3 = 25 g/L; X4 = 30°C X5 = 183 min<br><br>Percentage removal:<br><br>COD = 61.6%; colour = 99.6%; turbidity = 66.4%, | Körbahti and Tanyola (2008) |
| Tannery wastewater | Electrocoagulation        | Current density (cd) (X1); pH (X2); concentration (X3)                                                                     | Removal of chromium                   | X1 = 13 mA/cm <sup>2</sup> ; X2 = 7; X3 = 750 ppm<br><br>Percentage removal of chromium = 100%                                              | Genawi et al. (2020)        |

### 2.9.2 Box-Behnken Design (BBD)

Box and Behnken proposed BBD in 1960. They devised a three-level incomplete factorial design as a substitute for full factorial design and a much more efficient design for estimating second-order surfaces (Zhang et al., 2018; Alam et al., 2021). Differing from CCD, BBD contains no embedded factorial or fractional factorial points. The BBD design is rotatable and requires three levels for each factor where the parameters are assigned a value between -1 and 1, with -1 representing a low level, 0 representing a medium level, and 1 representing a high level (Ye et al. 2020). BBD could be considered for experiments with more than two variables with coded values ( $X_1, X_2, X_3 \dots X_k$ ) as shown in Table 2.7. Each variable has three levels and the centre point will be executed in triplicate. The optimum is expected to be located around the middle of the factor ranges.

**Table 2.7:** Example of coded matrix for the BBD of a three-variable system (Rajewski and Dobrzyńska-Inger, 2021)

| Experiment | $X_1$ | $X_2$ | $X_3$ |
|------------|-------|-------|-------|
| 1          | 1     | -1    | 0     |
| 2          | -1    | -1    | 0     |
| 3          | 0     | 1     | 1     |
| 4          | 0     | -1    | 1     |
| 5          | 1     | 0     | -1    |
| 6          | -1    | 1     | 0     |
| 7          | -1    | 0     | 1     |
| 8          | 0     | 1     | -1    |
| 9          | 0     | -1    | -1    |
| 10         | 0     | 0     | 0     |
| 11         | 0     | 0     | 0     |

**Table 2.7** continued

|    |    |   |    |
|----|----|---|----|
| 12 | -1 | 0 | -1 |
| 13 | 0  | 0 | 0  |
| 14 | 0  | 0 | 0  |
| 15 | 0  | 0 | 0  |
| 16 | 1  | 1 | 0  |
| 17 | 1  | 0 | 1  |

Figure 2.9 illustrates the geometry of BBD with three factors. The design points are chosen along the edges and central points rather than at the corners, as the corners indicate extreme situations. This is to avoid extreme combinations of all input parameters occurring at the same time. The primary characteristic of this design is it requires an experiment number equal to  $N = 2k(k-1) + C_p$ , where  $k$  is the number of factors and ( $C_p$ ) is the number of central points (Bezerra et al., 2008). To account for experimental or simulation errors, the central design point is repeated.

In comparison to CCD, BBD is significantly more labour-efficient in terms of experimental design as it allows a significant reduction in the number of experimental sets without jeopardizing optimisation accuracy (Qiu et al., 2014; Bo et al., 2015; Ye et al., 2020). Recent studies using BBD have been undertaken to optimise various types of wastewater treatment using various forms of coagulants in Table 2.8. The studies demonstrate that the experimental outcomes are consistent with the predicted values, indicating that this design is capable of optimisation study (Siti et al., 2014; Okolo et al., 2017). Adesina et al. (2016) applied BBD in the colour removal experiment of peat water by optimising simultaneously the coagulant dosage (g/L), pH of peat water, and contact time (min).

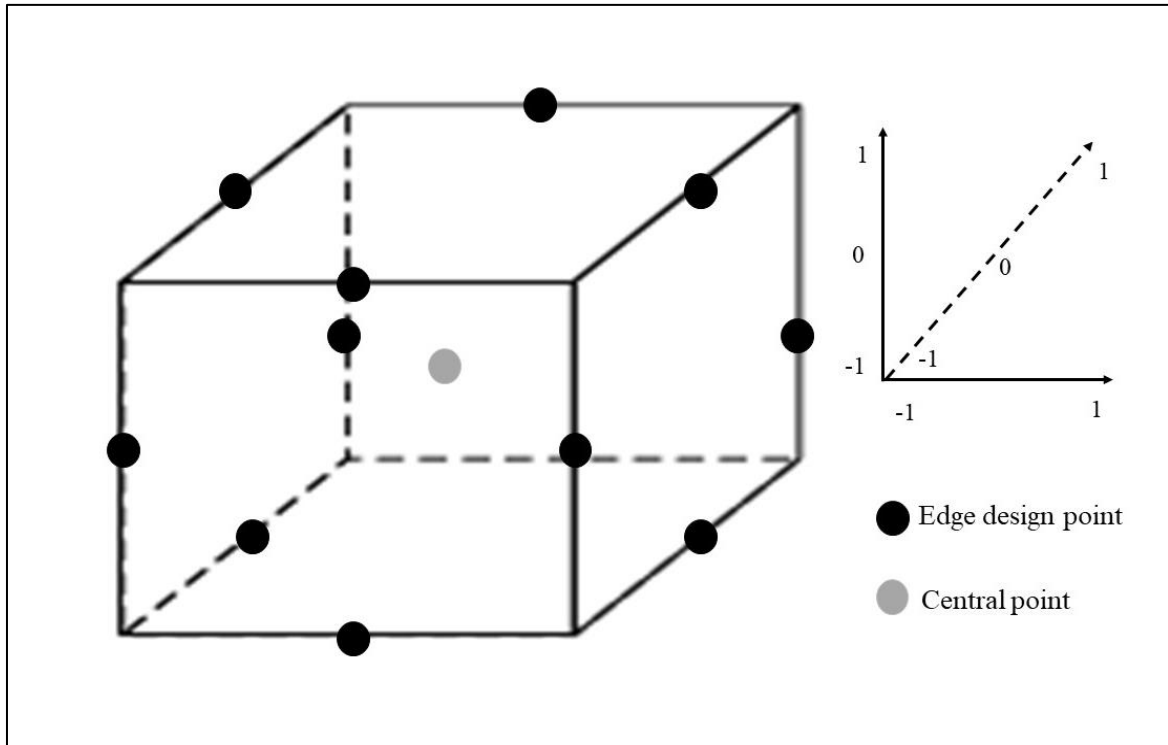


**Figure 2.9:** Schematic diagram of BBD (Source: Kumar et al. 2013)

**Table 2.8:** Examples of studies on the optimisation of wastewater treatment using BBD

| Type of wastewater         | Independent variables                                                                                                       | Response(s)                             | Optimum conditions and the final result                                                                                      | References              |
|----------------------------|-----------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|------------------------------------------------------------------------------------------------------------------------------|-------------------------|
| Surface water              | Settling time ( $X_1$ ), agitation time ( $X_2$ ), agitation speed ( $X_3$ ) and <i>M. oleifera</i> concentration ( $X_4$ ) | Residual turbidity                      | $X_1 = 120$ min; $X_2 = 10$ min; $X_3 = 100$ rpm; $X_4 = 3$ g/L<br><br>Final turbidity: Residual turbidity = 5.51 NTU        | Adesina et al. (2019)   |
| Dye wastewater (Congo red) | pH ( $X_1$ ), dosage ( $X_2$ ), and dye concentration ( $X_3$ )                                                             | Removal of colour                       | $X_1 = 2$ ; $X_2 = 190$ mg/L; $X_3 = 400$ ppm<br><br>Percentage removal:<br><br>Dye removal = 91.2%                          | Yong et al. (2016)      |
| Peat Water                 | Dosage ( $X_1$ ), and pH ( $X_2$ )                                                                                          | Removal of colour, turbidity, iron, COD | $X_1 = 800$ mg/L; $X_2 = 4.80$<br><br>Percentage removal : Colour = 78%; turbidity = 89%; iron = 90%; COD = 67%              | Siti et al. (2014)      |
| Dye wastewater (Congo red) | pH ( $X_1$ ), initial dye concentration ( $X_2$ ), coagulant dosage ( $X_3$ ), and adsorbent dosage ( $X_4$ )               | Removal of dye                          | $X_1 = 2$ ; $X_2 = 385$ ppm; $X_3 = 209$ mg/L; $X_4 = 150$ mg/L<br><br>Percentage removal:<br><br>Congo red removal = 96.67% | Hoong and Ismail (2018) |

**Table 2.8** continued

|                  |                                                                   |                                |                                                                                                                                                                                                                                                                       |                     |
|------------------|-------------------------------------------------------------------|--------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| Brewery effluent | Coagulant dosage ( $X_1$ ), pH ( $X_2$ ), stirring time ( $X_3$ ) | Removal of solid particle (SP) | Detarium Microcarpum seed powder:<br>$X_1 = 100$ mg/L; $X_2 = 2.0$ ; $X_3 = 25$ min<br>Percentage removal:<br>Removal of SP = 90.42%<br>Oyster-dried shell powder:<br>$X_1 = 100$ mg/L; $X_2 = 4.0$ ; $X_3 = 30$ min<br>Percentage removal:<br>Removal of SP = 92.73% | Okolo et al. (2017) |
|------------------|-------------------------------------------------------------------|--------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|

## 2.10 Antibacterial Properties of *M. oleifera*

Infections caused by bacteria and fungi possess serious hazards to human health. Despite the widespread use of antibiotics and vaccinations, infectious diseases continue to be a primary cause of sickness and death globally, with bacterial infections being the most common cause. Antimicrobial agents are also abundant in medicinal plants, and in this case, we will focus on *M. oleifera*.

Delelegn et al. (2018) used a variety of methods to test the efficacy of *M. oleifera* seeds extract against bacterial growth. These methods include agar well diffusion, determination of the minimum inhibitory concentration (MIC), and determination of the minimum bactericidal concentration (MBC). Through agar well diffusion test, *M. oleifera* revealed promising effects with zone inhibition ranging from 7.66 mm to 19 mm. The aqueous extract of *M. oleifera* was shown to have the smallest zone of inhibition against clinical isolates of *Shigella dysenteriae* and *Salmonella typhi*, whereas the acetone extract of *M. oleifera* had the largest zone of inhibition against *S. typhi* (clinical isolate). This finding corroborates a prior study that water or aqueous extracts of plants are often inadequate or indicate a low level of efficiency in combating microorganisms (Merawie et al., 2013). This could be because the antibacterial active compounds contained in plant products, such as tannins and polyphenols, are insoluble in water, resulting in a decrease in antimicrobial efficacy.

In addition, the efficacy of *M. oleifera*'s extract was tested using MIC and MBC, with findings ranging from 6.25 mg/mL to 25 mg/mL (Moyo et al., 2012). While there is no standard concentration for determining the antibacterial activity of plant materials, Moyo et

al. (2012) found that their maximum concentration against *E. coli* was 5.00 mg/mL for *M. oleifera* acetone extract.

Another study by Dasgupta et al. (2016) found that *M. oleifera* seed extracts are effective antibacterial agents due to the presence of a short, cationic protein within the seeds. The extracted protein inhibits bacterial growth and higher concentrations (w/v) allow greater bactericidal activity, which was demonstrated via the inhibition test on agar and in a nutrient medium (lauryl tryptose broth). Plates impregnated with 5% of the concentrated extract (w/v) inhibited *E.coli* growth the greatest, with only 10% of the initial germs regrowing. Meanwhile, the lowest concentration of seed extract (1%) could only inhibit the growth of 14% of the plated bacteria. *M. oleifera*'s active proteins; causing cell death by coagulation-flocculation and flushing (Ndabigengesere et al., 1995; Dasgupta et al., 2016). A 10 mL seed extract was able to kill up to 93.2% of *E. coli* and 96.2% of *B. subtilis*. Lysozyme is present in *M. oleifera*'s cationic protein. This compound significantly increases the aggregation rate of gram-positive bacteria, hence rising its removal rate (Shebek et al., 2015).

*M. oleifera*'s antibacterial properties were also been assessed using the pour plate method, which measured the reduction of bacteria in wastewater samples following the addition of *M. oleifera* seed before the jar test (Shan et al., 2017). The results showed that *M. oleifera* seed cakes can reduce the growth of microorganisms in the water samples though the microbes were not fully removed. The concentration of bacteria was reduced to  $7.50 \times 10^4$  from  $1.70 \times 10^5$  CFU/mL and  $8.90 \times 10^4$  from  $1.06 \times 10^6$  CFU/mL for the “Sungai Baluk” river and wastewater samples respectively. This work concurs with Vunain et al. (2019) findings, who observed a reduction in microbial load from 209 to consistent CFU; 7 CFU and coliform bacteria reduced from 238 CFU to 94 CFU following coagulation-

flocculation using the jar test on water samples. Physical floc removal during the jar test possesses the additional benefit of substantially lowering the microbial population in the water matrix. This is because bacteria are inextricably linked to both organic and inorganic particles, both of which contribute to the turbidity that is eliminated during the coagulation process (Vunain et al., 2019).

## CHAPTER 3

### MATERIALS AND METHODS

#### 3.1 Materials and Reagents

Seeds of *M. oleifera* were purchased from a local vendor in Kota Samarahan, Sarawak, who planted the tree using seeds supplied from India. Potassium dichromate ( $K_2Cr_2O_7$ ), mercury (II) sulphate ( $HgSO_4$ ), ferrous ammonium sulphate (hexahydrate) ( $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ ) were obtained from R & M. Methanol ( $CH_3OH$ , laboratory reagent  $\geq 99.6\%$ ) and calcium chloride dehydrate, ( $CaCl_2$ , 99%) were both procured from Sigma Aldrich. Ferroin indicator solution and magnesium sulphate hydrated ( $MgSO_4$ ) were obtained from Fluka and Univar, respectively. Sodium chloride ( $NaCl$ ) and sodium carbonate anhydrous ( $Na_2CO_3$ ) were acquired from UniChem whilst silver sulphate ( $Ag_2SO_4$ ) and iron chloride 6-hydrate were provided by Bendosen. Sulphuric acid ( $H_2SO_4$ ) and sodium hydroxide ( $NaOH$ ) pellets were procured from Merck whereas hexane was purchased from HmbG Chemicals. Distilled water ( $dH_2O$ ) was obtained from the Environmental Sustainability Laboratory, Faculty of Resource Science and Technology, UNIMAS.

#### 3.2 Sampling of Peat Water

The peat water sampling was performed between June 2019 and June 2020 at Asajaya river ( $1^\circ 30' 42.4''N$ ,  $110^\circ 41' 38.9''E$ ) (Figure 3.1). All water samples were collected at the sub-surface between 9 cm and 11 cm. The gallons used for water sample collection were pre-rinsed with peat water to avoid other contaminants that could alter the properties of the samples (Guettaf et al., 2017). The water samples were immediately brought to the

laboratory and stored in a refrigerator at a temperature of -6°C to keep them frozen until additional analysis could be performed (Madrid & Zayas, 2007; Fellman et al., 2008).

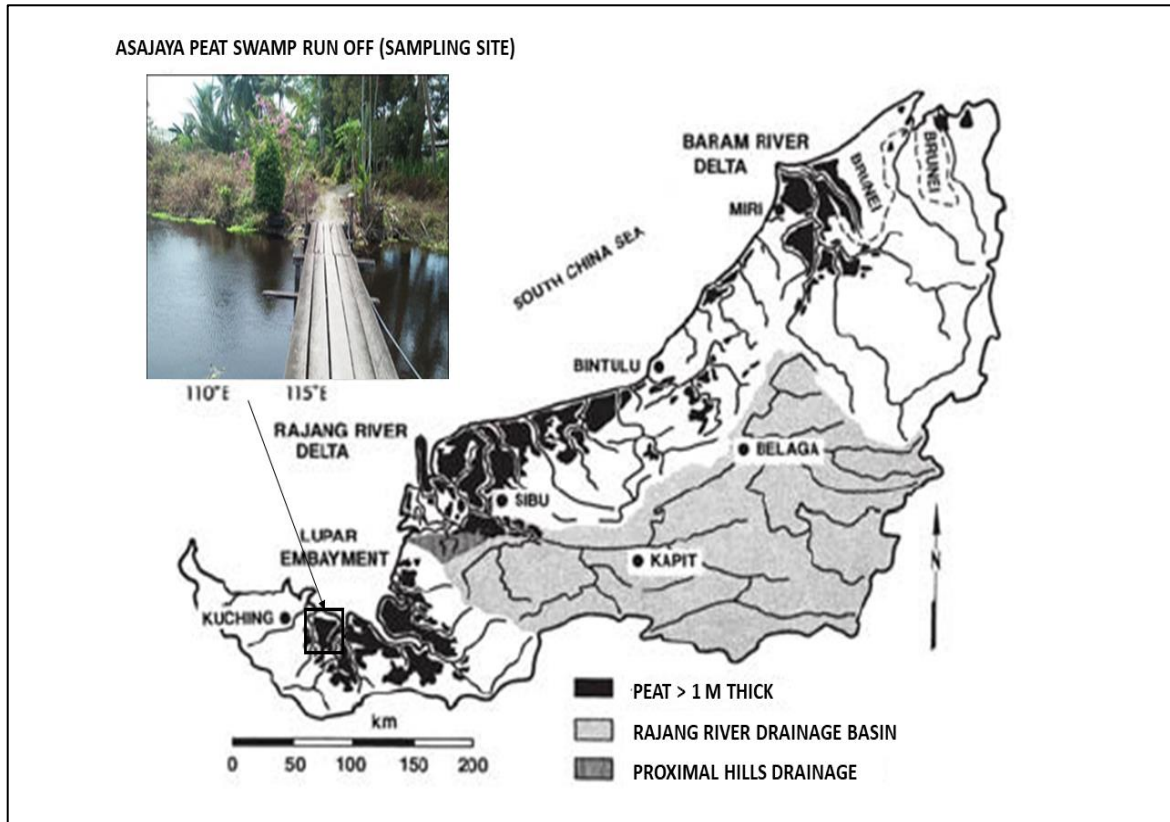


**Figure 3.1:** Sampling site of Asajaya peat swamp runoff. Adapted from Omorinoye et al. (2019))

### 3.3 Characterisation of Peat Water Sample

Peat water (Figure 3.2) was subjected to both in-situ and ex-situ analyses. An in-situ analysis is defined as the analysis that is done on-site while an ex-situ analysis is typically done after transporting samples to another site (Azubuike et al., 2016). To obtain greater validity, all water quality measurements were made in triplicate.

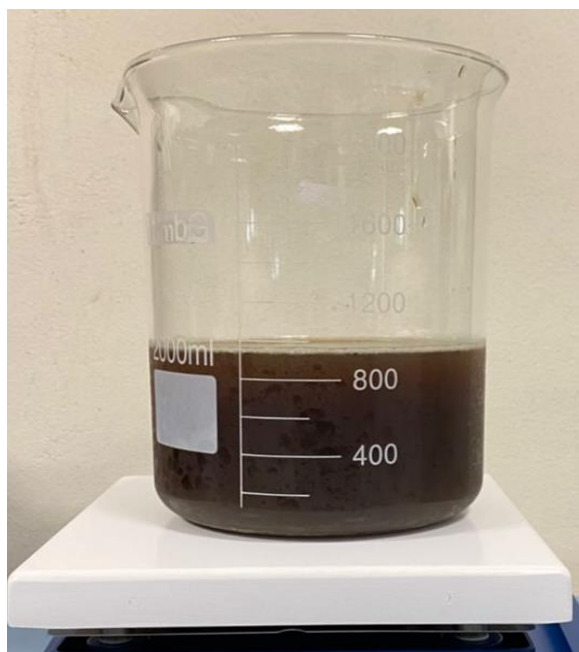


**Figure 3.2:** Peat water sample

### **3.3.1 *In-situ* Analysis**

Both the pH and temperature of peat water samples were taken by using a pH/temperature meter (Model Milwaukee pH 55) that was previously calibrated using buffer solutions pH 4.00, 7.00, and 10.00. The dissolved oxygen (DO) concentration was determined using a portable dissolved oxygen metre (Model Milwaukee MW600), and the turbidity concentration was determined using a turbidity metre that had been initially calibrated with 0 NTU, 10 NTU, and 500 NTU standard solutions (Model Martini Instruments Mi415). The characterisations were done based on the Standard Methods for Water and Wastewater Examination (APHA, 1999).

### **3.3.2 *Ex-situ* Analysis**

Total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), and humic substances were all determined in the laboratory due to the

requirement for specific instruments. The analyses were carried out within 24 h following sample collection. The TSS analysis was done by filtering 200 mL of well-mixed peat water sample with a 0.45 µm membrane filter. The membrane filter with the water residual was later dried in an oven for an hour at 105°C before the final mass was taken (APHA, 1999). The final TSS value was calculated by using Equation 3.1:

$$TSS (mg/L) = (A - B)/V \quad \text{Equation 3.1}$$

where, A is the mass of filter + dried residue (in mg); B is the mass of filter (in mg); V is the volume of sample filtered (in L)

To prepare dilution water, 2 mL of each of the following solutions were added to a 2 L volumetric flask: ferric chloride (FeCl<sub>3</sub>) solution, calcium chloride (CaCl<sub>2</sub>) solution, and phosphate buffer solution. The volumetric flask was then filled with distilled water, up to the mark. The solution was transferred to a beaker and permitted to aerate for 24 h prior to BOD analysis. A 30 mL of peat water sample was transferred into the BOD bottle (300 mL) followed by the addition of water to the brim. The initial DO level in the solution was measured using a DO meter. The BOD bottles then were left to incubate in a dark place for 5 days before their final DO was measured. The BOD<sub>5</sub> value was calculated by using Equation 3.2:

$$BOD_5 (mg/L) = (DO_i - DO_f)/P \quad \text{Equation 3.2}$$

where, DO<sub>i</sub> is the initial dissolved oxygen; DO<sub>f</sub> is the final dissolved oxygen; P is the volumetric fraction of wastewater.

COD was determined following the Standard Method 5220 C (APHA, 1999). A 2.5 mL peat water was added to a COD tube, followed by 1.5 mL K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> digestion solution

and 3.5 mL concentrated  $\text{Ag}_2\text{SO}_4\text{H}_2\text{SO}_4$ . After heating the solution mixture for 2 h at  $150^\circ\text{C}$  in a COD digester, it was cooled to room temperature. The solution was transferred into a conical flask and titrated with 0.0125 N ferrous ammonium sulphate (FAS) solution. Two drops of ferroin indicator were added to the solution prior to titration to indicate the solution's colour change to reddish-brown. The volume of titrated FAS solution was determined, and COD was calculated using the following formula (Equation 3.3):

$$\text{COD (mg/L)} = ((A - B) \times (N \times 8000))/V \quad \text{Equation 3.3}$$

where, A is the FAS volume for blank (mL); B is the FAS volume for sample (mL); N is the normality of FAS (N); V is the volume of sample (mL)

HS in the peat water was measured as colour removal using a UV-visible spectrophotometer (Model Cary 500) (Kvinnesland & Ødegaard, 2004). The absorbance was recorded at 465 nm and 665 nm or known as E4/E6 ratio to study the humic acid fraction (Notodarmojo et al., 2017). The samples were pre-filtered with a  $0.45 \mu\text{m}$  membrane using a filtration apparatus before analysis using a UV-vis spectrophotometer to remove particulates that might interfere with the absorbance (Albrektienė et al., 2012; Notodarmojo et al., 2017).

### 3.4 Preparation of *M. oleifera*-based Coagulant

The seeds of *M. oleifera* (Figure 3.3) were retrieved from their pods, washed with distilled water, and left to air dry for 3 days (Azwanida, 2015; Tunggolou & Payus, 2017a). There were no indications of browning, softness, or significant desiccation in the seeds used.



**Figure 3.3:** *M. oleifera* (a) with its husk and (b) without its husk

To improve the recovery of the seed's active ingredients, the dried seeds were ground with a laboratory grinder and sieved (500  $\mu\text{m}$ ) to obtain a fine powder (Salem et al., 2021) (Figure 3.4). The powder was stored in a clean bottle in a dark location at room temperature, as any exposure to the environment could cause the active components in the seeds to degrade (Katayon et al., 2006; Alo et al., 2012).

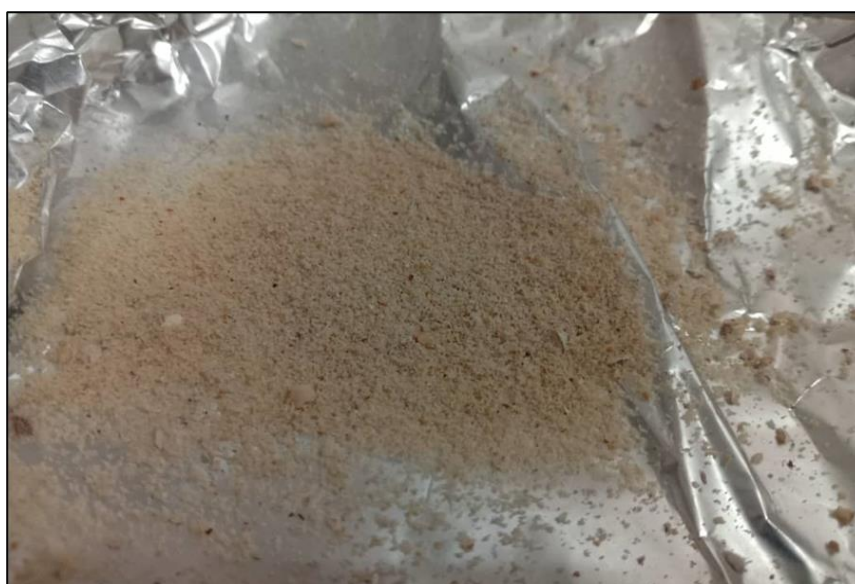


**Figure 3.4:** *M. oleifera* seeds powder

### 3.5 Characterisation of *M. oleifera* Seeds

The pH was analysed by adding 1 g of powdered sample into 100 mL of distilled water and left stirred for 10 min before its pH was taken using a pocket-size pH/temperature meter (Model Milwaukee pH 55) (Alo et al., 2012).

Fourier-transform infrared (FTIR) spectroscopy (Model Thermo Nicolet iS10) was used to determine the functional groups that facilitate the coagulation activity (Bouaouine et al. 2018) with KBr pellet method. The analysis was performed in the wave number range of 4000-500 cm<sup>-1</sup>.

The surface morphology of the powdered sample was examined using a scanning electron microscope (SEM) (Model JOEL JSM-6390LA) to analyse the pores on their surface before and after treatment. A sticky carbon disc was used to place the sample on a metal stub, and the excess powder was removed. The performance was carried out under vacuum at a 10 kV accelerating voltage. According to Adesina et al. (2019), a good coagulant exhibits pores that facilitate coagulation. The elemental composition was performed by using Energy dispersive X-ray (EDX) (Model JOEL JSM-6390LA) which is coupled to the SEM.

The crude methanolic extract was also analysed for its chemical composition present. About 50 g of the seed powder was soaked in 250 mL methanol and left overnight on an orbital shaker (Delelegn et al., 2018). After being filtered, the filtrate was evaporated using a rotary evaporator system (Model BUCHI) and reconstituted in hexane and for gas chromatography-mass spectrophotometer (GC-MS, QP2010plus, Shimadzu, Japan). The temperature was held at 50°C for 1 min and increased to 220°C at 5°C/min and hold for 10 min. The injector and MS temperature were set at 250°C. Identification of compounds was performed using a BPX5 (Restek) 30 m × 0.25 um × 0.25 mm column. This involved several

steps, such as preparing the sample by extracting the compounds of interest and injecting the extracted sample into the GC-MS system. Additionally, the sample components were separated using the BPX5 (Restek) column based on their properties, and the components were detected using a mass spectrometer to produce a mass spectrum. Finally, the components were identified by comparing their mass spectra and retention times to those of known compounds.

### 3.6 Coagulant Test

#### 3.6.1 Preliminary Test

The optimisation of the HS removal performance of the powdered seed sample was performed by employing RSM. RSM, however, requires a reasonable range for each of the investigated variables. Thus, a preliminary study was carried out to screen the appropriate range for the chosen independent variables (coagulant dosage, pH value of peat water and contact time) with colour removal of 465 nm. The steps that were performed are based on a modified jar test (Figure 3.5).

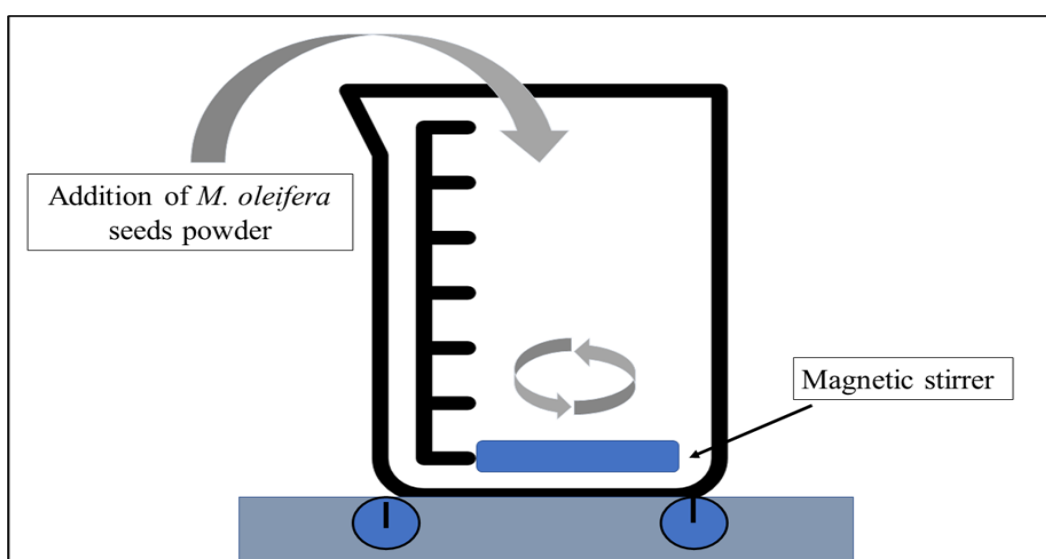


**Figure 3.5:** Modified jar test diagram (Natumanya & Okut-Okumu, 2015)

The first stage was to investigate the optimum contact time. A 1 g/L of *M. oleifera* seeds was added to 1 L of unaltered pH peat water, mixed, and left undisturbed for a period ranging from 0 min to 240 min before the optimal contact time was determined.

The second stage was to determine the optimum dosage of coagulant for treatment. Different coagulant dosages ranging from 0.25 g/L to 4 g/L were added into different beakers containing 1 L of unaltered pH of peat water, stirred, and left at the previously selected optimum contact time. The optimum coagulant dosage was selected for further analysis.

The final preliminary test was to observe the optimum pH of peat water for treatment. At this stage, the selected coagulant dosage and contact time were applied for the treatment of peat water with varying pH values ranging from pH 5 to pH 12. The pH of peat water was adjusted by the addition of 0.1 M HCl or 0.1 M NaOH. The acidification was performed to adjust the pH of the peat water to the desired range for the coagulation process. The coagulant dosage and contact time were previously determined, but the pH of the peat water needed to be controlled and maintained within a specific range for effective coagulation. This is important because the optimal pH range for coagulation can vary depending on the specific coagulant used and the composition of the water being treated. After the treatment, the optimum pH was selected for further analysis.

After the treatment procedure was completed, the water was collected at the water's surface and centrifuged to remove any solid particles. The absorbance at 465 nm was chosen as it was the most suggested for the measurement of colour of water caused by HS (Sim et al., 2014). The final HS removal (%) was then calculated through Equation 3.4:

$$HS\ removal\ (\%) = ((HS_i - HS_f) / HS_i) \times 100 \quad \text{Equation 3.4}$$

where HS is humic substances,  $HS_i$  is the initial HS removal and  $HS_f$  is the final HS removal.

### 3.7 Optimisation Studies of Coagulation Process by Response Surface Methodology

Based on the preliminary study (Section 3.6.1), an appropriate factor domain was selected and applied in RSM via BBD. BBD was chosen because it is well-suited for fitting a quadratic surface and enables the chosen parameters to be optimised with a small number of runs, while also allowing for the analysis of parameter interactions (Wu et al., 2012). The chosen factor domain includes coagulant dosages (0.5 g/L to 2 g/L), pH value (pH 6 to pH 10) and contact time (30 min to 120 min). The experiment was conducted as shown in Figure 3.6.

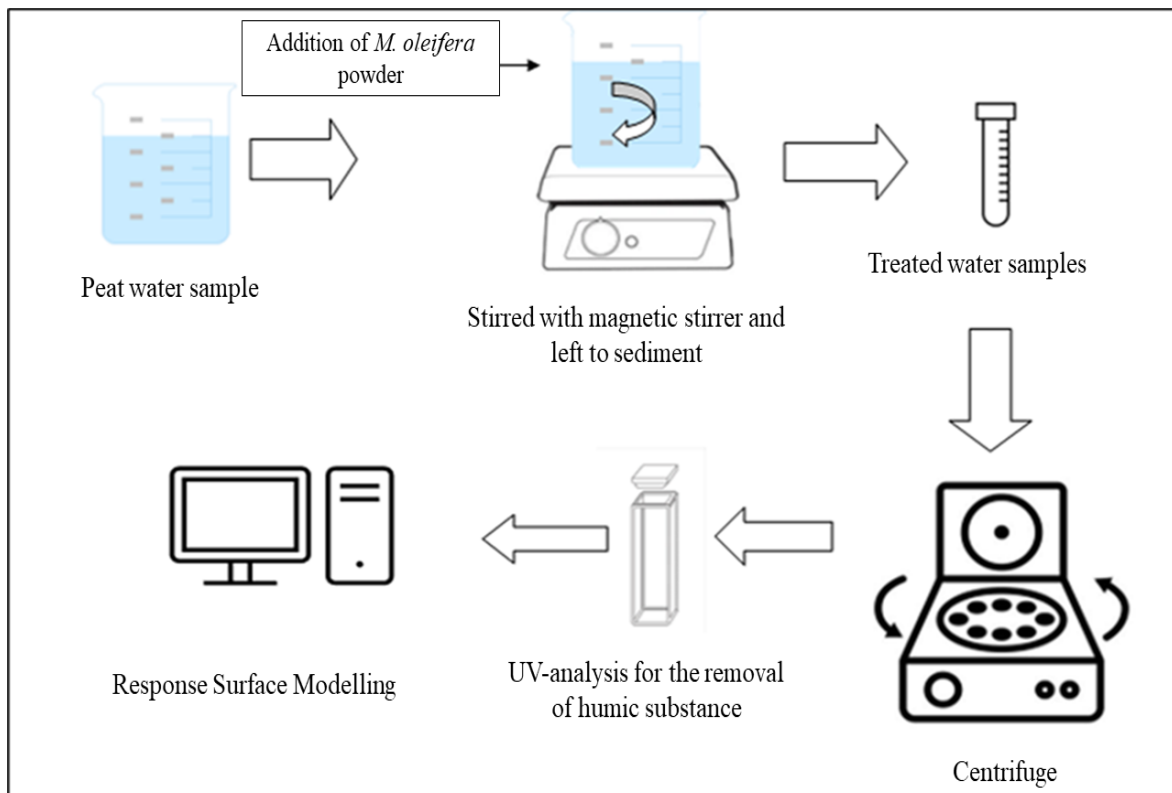


**Figure 3.6:** Schematic diagram of humic substances analysis

Table 3.1 summarises the factor domain in this experiment; where factorial levels were coded as -1 (low), 0 (medium) and +1 (high).

**Table 3.1:** Factor domain

| Variables          | Coded | Range and levels |      |     |
|--------------------|-------|------------------|------|-----|
|                    |       | -1               | 0    | +1  |
| Dosage (g/L)       | A     | 0.5              | 1.25 | 2   |
| pH value           | B     | 6                | 8    | 10  |
| Contact time (min) | C     | 30               | 75   | 120 |

A total of 15 sets of experiments were created with Design Expert Version 7.1.6. Each experiment was divided into three blocks, where two factors were changed at each of the four points, and one central point was included. Table 3.2 summarises the designed experiments conducted.

**Table 3.2:** RSM for the three independent variables in coded units

| Run | Independent variable |    |                       | Coded variables |    |   |
|-----|----------------------|----|-----------------------|-----------------|----|---|
|     | Dosage<br>(g/l)      | pH | Contact time<br>(min) | A               | B  | C |
| 1   | 0.50                 | 6  | 75                    | -1              | -1 | 0 |
| 2   | 2.00                 | 6  | 75                    | +1              | -1 | 0 |
| 3   | 0.50                 | 10 | 75                    | -1              | +1 | 0 |
| 4   | 2.00                 | 10 | 75                    | +1              | +1 | 0 |

**Table 3.2** continued

|    |      |    |     |    |    |    |
|----|------|----|-----|----|----|----|
| 5  | 0.50 | 8  | 30  | -1 | 0  | -1 |
| 6  | 2.00 | 8  | 30  | +1 | 0  | -1 |
| 7  | 0.50 | 8  | 120 | -1 | 0  | +1 |
| 8  | 2.00 | 8  | 120 | +1 | 0  | +1 |
| 9  | 1.25 | 6  | 30  | 0  | -1 | -1 |
| 10 | 1.25 | 10 | 30  | 0  | +1 | -1 |
| 11 | 1.25 | 6  | 120 | 0  | -1 | +1 |
| 12 | 1.25 | 10 | 120 | 0  | +1 | +1 |
| 13 | 1.25 | 8  | 75  | 0  | 0  | 0  |
| 14 | 1.25 | 8  | 75  | 0  | 0  | 0  |
| 15 | 1.25 | 8  | 75  | 0  | 0  | 0  |

The collected data was fitted with linear and quadratic models and the best model was selected based on its Lack of Fit and coefficient of determination ( $R^2$ ). A non-significant "Lack of Fit" value with a p-value ( $p > 0.05$ ) means the model is accepted. A higher value of  $R^2$  or closer value to 1 shows that the model well explained the experimental result (Siti et al., 2014; Yong and Ismail, 2016). Equation 3.5 is a mathematical representation of the model (Ram Talib et al., 2019). The data was then further analysed with analysis of variance (ANOVA) to determine the regression coefficients' statistical significance (Equation 3.5).

$$Y = b_0 + \sum_{i=1}^k b_i X_i + \sum_{i=1}^k b_{ii} X_i^2 + \sum_{i < j}^k b_{ij} X_i X_j + e \quad \text{Equation 3.5}$$

where Y represents the dependent variables (the removal percentage of HS); k is the number of factors;  $b_0$  is the offset or free term called intercept term,  $X_1, X_2, \dots, X_k$  are the coded independent variables,  $b_i$  is the first-order (linear) main effect,  $b_{ii}$  is the quadratic

(square) effect,  $b_{ij}$  is the interaction effect, and  $e$  designates the random error between experimental and predicted values (Sangeetha et al., 2014; Adesina et al., 2019).

Once the data is fitted to a suitable model, a response plot, contour plot, and 3D surface plot are created using the resulting data. The objective of the analysis is to maximize the desired response, which in this case is the HS removal. The independent variables, such as dosage, pH, and contact time, are kept within a certain range to ensure the effectiveness of the analysis, as described by Adesina et al. (2019).

### **3.8 Antibacterial Study**

The disc diffusion method was used to determine the sensitivity of two selected microorganisms, *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923), to the *M. oleifera* seed extract (MOSE). These two microbes were selected because they are commonly found and contaminate water sources (Pandey et al., 2014). The standard cultures were obtained from the Molecular Microbiology Laboratory, Faculty of Resource Science and Technology, UNIMAS. They were chosen to demonstrate MOSE's antibacterial effectiveness against both gram-negative and gram-positive bacteria. MOSE was made in three different concentrations: 2 g/L, 1.25 g/L, and 0.5 g/L. This was done using three different solvent extraction methods: ethanol (EMOS), methanol (MMOS), and hexane (HMOS) so that the antibacterial activity of MOSE could be tested on test organisms. 2–3 colonies of the various strains to be examined were suspended in 1 mL of normal saline (0.85%). The bacterial suspension was then compared to the standards set by 0.5 McFarland tubes. The test strain was placed onto the Mueller Hinton-Agar plate using a sterile swab to produce a lawn culture, followed by 40 min pre-diffusion on the bench. 100  $\mu$ L of MOSE, 20  $\mu$ L of positive control (amoxicillin) at a concentration of 100 mg/mL, and 10  $\mu$ L of negative control

(distilled water) were impregnated aseptically onto the disc and left air-dried before being placed onto the inoculated MHA plate. After 24 h of incubation at 37 °C, the diameter of the inhibitory zone was determined and interpreted as susceptible or resistance categories. The diameter of the clear zone for each antibiotic disc was interpreted as susceptible or resistant categories according to the guideline recommended by the Clinical and Laboratory Standard Institute (2016). Triplicate analyses of each sample were performed.

## CHAPTER 4

### RESULTS AND DISCUSSION

#### 4.1 Peat Water Characterisation

Eight parameters were determined to characterise peat water samples (Table 4.1).

**Table 4.1:** Peat water characterisation

| Parameters (unit)           | Range        | Mean $\pm$ SD   | NWQS<br>(Class I) |
|-----------------------------|--------------|-----------------|-------------------|
| pH                          | 5.20 - 6.80  | 5.7 $\pm$ 0.7   | 6.5 -8.5          |
| Temperature (°C)            | 25.00        | 25.00           | -                 |
| Turbidity (NTU)             | 29.71 - 34.5 | 32.3 $\pm$ 1.8  | 5                 |
| DO (mg/L)                   | 1.20 – 1.60  | 1.4 $\pm$ 0.1   | 7                 |
| TSS (mg/L)                  | 20-30        | 26.1 $\pm$ 3.3  | 25                |
| COD (mg/L)                  | 32- 80       | 53.3 $\pm$ 19.6 | 10                |
| BOD (mg/L)                  | 3.00 - 14.00 | 8.6 $\pm$ 3.4   | 1                 |
| Humic substances<br>(E4/E6) | 6.24 - 6.55  | 6.4 $\pm$ 0.2   | -                 |

The acidic nature of the peat water was confirmed with a mean pH of  $5.70 \pm 0.68$ . The pH of water may be changed by the activities in the surrounding area; therefore, the pH value of peat water may fluctuate. However, it will remain acidic due to the nature of the adjacent peat soil, which is comprised of decomposing organic matter including acidic HS (Amri and Azis, 2018). Ai'dah et al. (2018) also reported the acidic pH of peat water ranging from 4 to 5.7 for samples collected from both housing and forest area. Likewise, acidic conditions were also observed in peat water collected from a forest area (pH 3.03 – 3.84)

and a village area (pH 4.06-5.13) (Shuhaimi-Othman et al., 2009; Rosli et al., 2010). The pH of peat water is determined by the carboxylic acid (-COOH) content of dissolved organic matter emanating from the peat, and to a lesser extent by ion exchange with living plants, causing the water to be acidic (Sjörs and Gunnarsson, 2002).

The turbidity of the peat water exceeded the standard permissible turbidity of water which is 5 NTU (Suratman et al., 2015; Mohiyaden et al., 2019). The turbidity may be caused by algae that feed on nutrients that enter the stream as a result of leaf breakdown or other natural decomposition processes (Apriani et al., 2016). As turbidity is caused by particles in water that are suspended, it is known that turbidity is linked to the TSS value (Serajuddin et al., 2019). The TSS level recorded in the peat water was  $26 \pm 3$  mg/L.

COD level is inversely related to DO as a higher level of COD will lead to a lower amount of DO. From the study, COD for peat water ranged from 32 mg/L to 80 mg/L (moderate level) while DO levels are considered low ranging from 1.2 mg/L to 1.6 mg/L. The low DO level may result from the increased organic materials in the water, whose decomposition depletes DO levels (Koekemoer, 2019). A reduction in DO can lead to anaerobic conditions, which are detrimental to higher aquatic life forms. Additionally, a low DO level led to a high BOD level. This is supported by the recorded BOD level of the water sample which was  $8.56 \pm 3.36$  mg/L. Unpolluted waters typically have BOD values of 2 mg/L or less, indicating that the water samples are considered polluted (Ratnayaka et al., 2009). The results of the high BOD levels observed from the water samples might be due to the disposal of waste from the nearby community area surrounding the peat water.

HS compounds in the peat water were determined through the wavelength ratio of E4/E6. The ratio is widely used for the characterisation of HS through optical absorbance.

HS in peat water contributes to the high organic content which consists of a variety of oxygen-containing functional groups and fractions (humic acid, fulvic acids, and humin) with varying molecular weights that cause colour in water (Syafalni et al., 2013; Yule et al., 2016; Notodarmojo et al., 2017; Li et al., 2019). According to Table 4.1, the E4/E6 ratio for the study was comparable to a study conducted (Sim & Mohamed, 2007). The study reported that the E4/E6 ratio ranged from 7.22 to 10.2, which is close to the value obtained in the present study ( $6.34 \pm 0.23$ ).

All parameters exceeded the standard Class I except for temperature and HS (E4/E6), which are not included in the National Water Quality Standards (NWQS) standard (Hee et al., 2019). Class I requires no treatment prior to use, and the water are suitable for fisheries farming of especially delicate aquatic species (Suratman et al., 2015). By comparing the data to the NWQS standard, the sample water falls into Class III, which requires extensive treatment for water supply but can be used for livestock drinking and farming-tolerant species of fish (Mohiyaden et al., 2019).

#### **4.2 *M. oleifera* Seeds Powder Characterisation**

The functional groups of the powdered *M. oleifera* seeds were identified using FTIR ranging from 500–4000  $\text{cm}^{-1}$ . Figure 4.1 shows the seeds before and after being used in the water treatment. The FTIR spectra of coagulants include multiple prominent peaks corresponding to amino ( $-\text{NH}$ ), hydroxyl ( $\text{OH}$ ), and carbonyl ( $\text{C}=\text{O}$ ) functional groups. The bandwidth centred at the 3301.19  $\text{cm}^{-1}$  peak possibly attributes to the existence of macromolecules such as proteins, carbohydrates (such as starch and cellulose), and lipids (such as fats and oils) (Reddy et al., 2012; George et al., 2016).

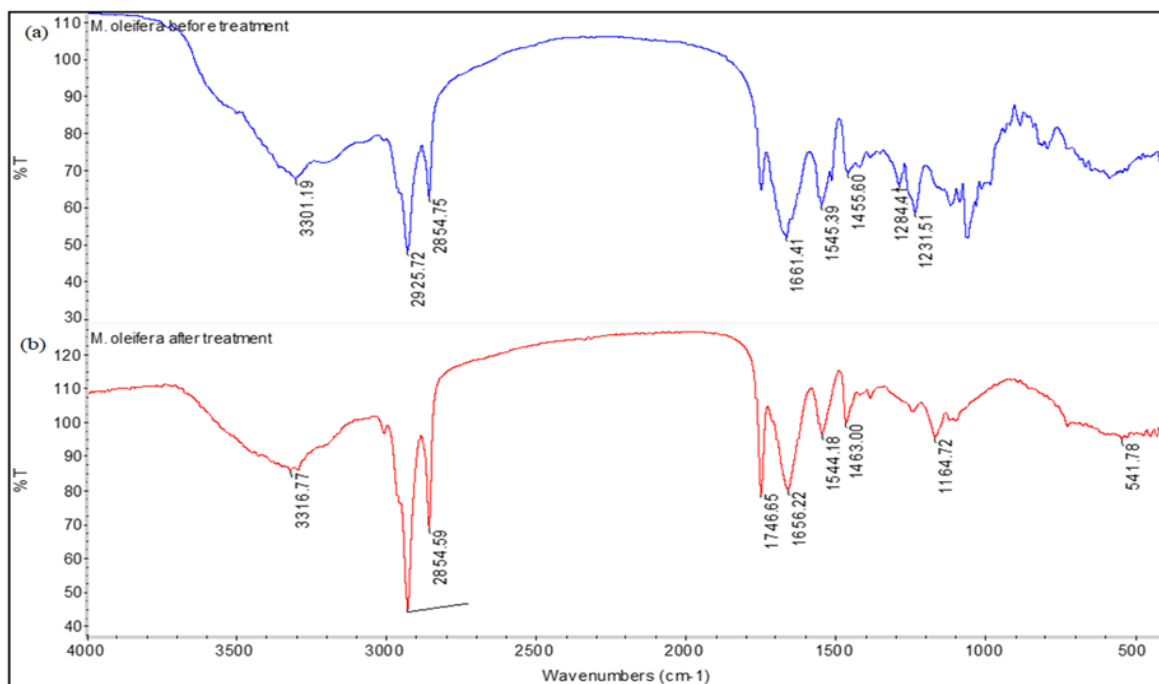


**Figure 4.1:** FTIR analysis of *M. oleifera* seeds powder: (a) before, and (b) after treatment

Two peaks that present at  $2925.72\text{ cm}^{-1}$  and  $2854.75\text{ cm}^{-1}$ , respectively, correspond to asymmetric and symmetric stretching of aliphatic chains of the  $\text{CH}_2$  group could well from other sources (Araújo et al., 2013; Dalvand et al., 2016). The peak at  $1746.39\text{ cm}^{-1}$  can be assigned to the  $\text{C=O}$  group that originates from fatty acids of the lipid or carbonyl group of the protein portion due to its heterogeneous nature (Araújo et al., 2013). The bandwidth at  $1661.41\text{ cm}^{-1}$  shows the existence of amide bond from the stretching of  $\text{O=C-NH}$  (Kumar et al., 2016a). Small peaks at  $1545.39\text{ cm}^{-1}$  to  $1455.60\text{ cm}^{-1}$  may be attributed to the presence of aromatic ring stretch,  $\text{C=C-C}$  (Kumar et al., 2016a; Choudhary & Neogi, 2017). The FTIR spectra confirm the presence of ionizable groups in the coagulant which are hydroxyl, carboxyl and amino groups (Bouaouine et al., 2018). These are the groups that played an active role in floc creation during the coagulation-flocculation process (Saritha et al., 2019).

In addition, the presence of carboxyl groups in the coagulant demonstrated that the material of the coagulant is a natural biosorbent that is ideal for the treatment of peat water (Sim et al., 2014). The changes in the peaks shifts after treatment;  $3301.19\text{ cm}^{-1}$  to  $3316.77\text{ cm}^{-1}$ ,  $1661.41\text{ cm}^{-1}$  to  $1656.22\text{ cm}^{-1}$ ,  $1455.60\text{ cm}^{-1}$  to  $1463.00\text{ cm}^{-1}$  and  $1231.51\text{ cm}^{-1}$  to  $1164.72\text{ cm}^{-1}$ , (Figure 4.1) indicate the reduction of functional groups which are responsible for the treatment.

The morphology of powdered seeds of *M. oleifera* before and after treatment was acquired using SEM. The surface of the coagulants can be observed to be rough and amorphous with no regular shape. The scattered available small pores on the surface indicate the ability to adsorb pollutants by the coagulant (Figure 4.2).

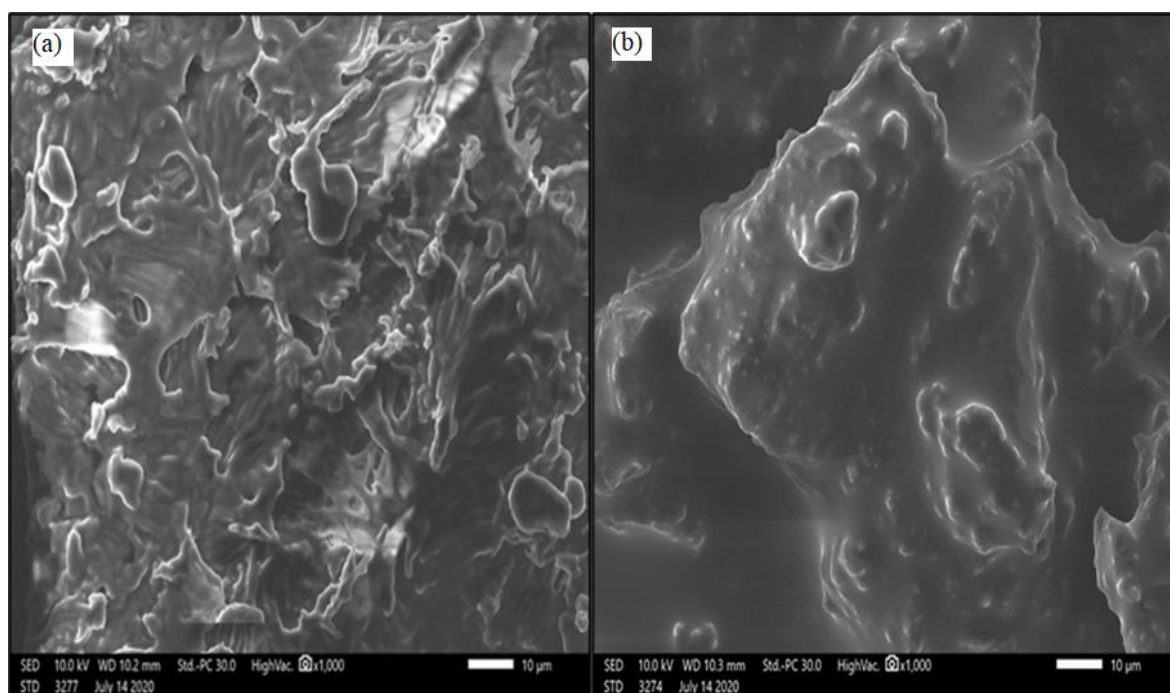


**Figure 4.2:** SEM analysis of *M. oleifera* seeds powder (a) before, and (b) after peat water treatment (1000× magnification)

This finding corroborates other studies that observed interstices on the coagulant surface which are known to be associated with adsorption properties (Araújo et al., 2010; Araújo et al., 2013; Kumar et al., 2016a). However, no pores were observed on the SEM image after treatment Figure 4.2(b). The presence of some beads on the coagulant surface implies that colloids in the water had been adhering to the coagulant. The rough surface structure with entrapped small colloidal particles suggests the sweep flocculation mechanism for the removal of colloidal particles in the peat water mixture (Ramavandi, 2014; Abdullah et al., 2017).

EDX analysis was performed on *M. oleifera* seeds powder to determine the elemental composition of the material. Based on Figure 4.3, the results show major elements of carbon and oxygen, and the minor elements of sulphur, aluminium, and phosphorus.

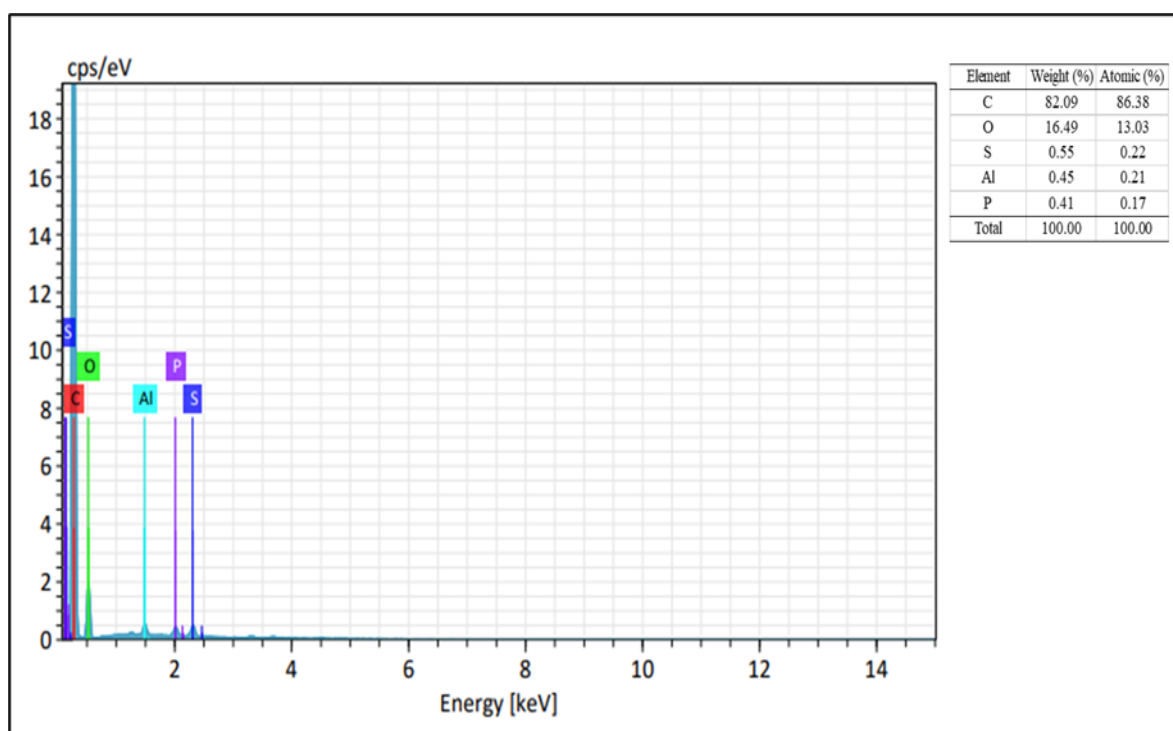


**Figure 4.3:** EDX analysis of *M. oleifera* seeds powder

Similarly, Belbali et al. (2021) found that carbon (45%) and oxygen (39%) were the most abundant elements in *M. oleifera* seeds. Jayan et al. (2021) found that the major elements in *Moringa oleifera* leaves were carbon (80.9%) and oxygen (13.9%), which is consistent with the present study. Another study also reported similar results for *M. oleifera* leaves, with carbon and oxygen being the major elements at 60.45% and 29.28%, respectively, while potassium and calcium were minor elements at 5.83% and 3.74%, respectively (Bello et al., 2017). It has been suggested that a coagulant with high carbon content and low oxygen content can be used as an adsorbent for the removal of dyes, heavy metals, and other organic pollutants from aqueous solutions (Xiong et al., 2013). The highest amount of carbon by weight at 82.09% and oxygen by weight at 16.49%; proposed that the seeds of *M. oleifera* can act as a good coagulant.

GCMS is an analytical technique used to identify and quantify organic compounds in a sample. Based on Table 4.2, 25 peaks were identified with their respective retention times, area percentages, names, molecular formulas, molecular weights, and structure index (SI).

**Table 4.2:** GC-MS analysis of *M. oleifera* seeds extracted with methanol

| Peak number | Retention time (min) | Area % | Name                    | Molecular formula               | Molecular weight (g/mol) | SI |
|-------------|----------------------|--------|-------------------------|---------------------------------|--------------------------|----|
| 1           | 10.137               | 0.27   | Hexane, 3,3-dimethyl-   | C <sub>8</sub> H <sub>18</sub>  | 114                      | 92 |
| 2           | 11.483               | 0.05   | Nonane, 4,5-dimethyl-   | C <sub>11</sub> H <sub>24</sub> | 156                      | 91 |
| 3           | 16.45                | 0.17   | Dodecane, 4,6-dimethyl- | C <sub>14</sub> H <sub>30</sub> | 198                      | 92 |
| 4           | 16.694               | 0.14   | Hexane, 3,3-dimethyl-   | C <sub>8</sub> H <sub>18</sub>  | 114                      | 92 |

**Table 4.2** continued

|    |        |       |                                         |                                                |     |    |
|----|--------|-------|-----------------------------------------|------------------------------------------------|-----|----|
| 5  | 22.037 | 0.13  | Dodecane, 4,6-dimethyl-                 | C <sub>14</sub> H <sub>30</sub>                | 198 | 92 |
| 6  | 22.284 | 0.06  | Hexane, 3,3-dimethyl-                   | C <sub>8</sub> H <sub>18</sub>                 | 114 | 91 |
| 7  | 26.979 | 0.09  | Tridecanol, 2-ethyl-2-methyl-           | C <sub>16</sub> H <sub>34</sub> O              | 242 | 88 |
| 8  | 27.185 | 0.01  | Nonane, 1-iodo-                         | C <sub>9</sub> H <sub>19</sub> I               | 254 | 89 |
| 9  | 27.971 | 0.04  | Hexane, 3,3-dimethyl-                   | C <sub>8</sub> H <sub>18</sub>                 | 114 | 90 |
| 10 | 31.396 | 0.07  | Dodecane, 4,6-dimethyl-                 | C <sub>14</sub> H <sub>30</sub>                | 198 | 91 |
| 11 | 31.701 | 0.03  | 9-Octadecenoic acid (Z)-, methyl ester  | C <sub>19</sub> H <sub>36</sub> O <sub>2</sub> | 296 | 95 |
| 12 | 32.105 | 0.24  | Hexadecanoic acid, methyl ester         | C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> | 270 | 95 |
| 13 | 32.27  | 0.04  | Hexane, 3,3-dimethyl-                   | C <sub>8</sub> H <sub>18</sub>                 | 114 | 89 |
| 14 | 32.742 | 0.78  | Palmitoleic acid                        | C <sub>16</sub> H <sub>30</sub> O <sub>2</sub> | 254 | 97 |
| 15 | 33.223 | 6.99  | n-Hexadecanoic acid                     | C <sub>16</sub> H <sub>30</sub> O <sub>2</sub> | 256 | 95 |
| 16 | 35.394 | 0.08  | 2-Methyltetracosane                     | C <sub>25</sub> H <sub>52</sub>                | 353 | 84 |
| 17 | 35.484 | 3.03  | 9-Octadecenoic acid, methyl ester, (E)- | C <sub>19</sub> H <sub>36</sub> O <sub>2</sub> | 296 | 83 |
| 18 | 35.588 | 0.27  | 11-Octadecenoic acid, methyl ester      | C <sub>19</sub> H <sub>36</sub> O <sub>2</sub> | 296 | 94 |
| 19 | 35.985 | 0.23  | Methyl stearate                         | C <sub>19</sub> H <sub>38</sub> O <sub>2</sub> | 298 | 95 |
| 20 | 36.281 | 0.04  | Dodecane, 2,6,11-trimethyl-             | C <sub>15</sub> H <sub>32</sub>                | 212 | 91 |
| 21 | 37.109 | 63.2  | Oleic Acid                              | C <sub>18</sub> H <sub>34</sub> O <sub>2</sub> | 282 | 97 |
| 22 | 37.381 | 23.47 | Octadecanoic acid                       | C <sub>18</sub> H <sub>36</sub> O <sub>2</sub> | 284 | 94 |

**Table 4.2** continued

|        |        |      |                               |                                                |     |    |
|--------|--------|------|-------------------------------|------------------------------------------------|-----|----|
| 23     | 39.37  | 0.34 | Phenacyl 11-octadecenoate     | C <sub>36</sub> H <sub>40</sub> O <sub>3</sub> | 400 | 61 |
| 24     | 40.608 | 0.13 | Oleic Acid                    | C <sub>18</sub> H <sub>34</sub> O <sub>2</sub> | 282 | 93 |
| 25     | 41.479 | 0.13 | Methyl 18-methylnonadecanoate | C <sub>12</sub> H <sub>42</sub> O <sub>2</sub> | 326 | 92 |
| <hr/>  |        |      |                               |                                                |     |    |
| 100.03 |        |      |                               |                                                |     |    |
| <hr/>  |        |      |                               |                                                |     |    |

By referring to Table 4.2, some of the similar compounds were found at different retention times. It can be attributed to a combination of factors, including the properties of the compounds, the instrument parameters, and the sample matrix effects (Silvestro et al., 2013; Falaki, 2019).

Based on the GCMS peak areas, fatty acids were the major constituent particularly oleic acid (63.2%). This is in agreement with other studies which had observed oleic acid as a major component of *M. oleifera* seeds (Rashid et al., 2008; da Silva et al., 2010). This compound aids in the coagulation process due to its ionisable functional group. The other fatty acids were octadecanoic acid (23.47%) and n-Hexadecanoic acid (6.99%). These compounds are anticipated to impart antibacterial activity to *M. oleifera* (Seidel & Taylor, 2004; Ojinnaka et al., 2015).

Water coagulation is the process of destabilizing and removing suspended particles in water to produce clear water. Coagulation is achieved by adding a coagulant to the water, which neutralizes the charges on the suspended particles, causing them to clump together and settle out of the water.

In this context, the GCMS analysis can be related to water coagulant properties by identifying the organic compounds present in the coagulant that could aid in coagulation. For example, the presence of fatty acids, such as oleic acid (63.2%), palmitoleic acid (0.76%), and hexadecanoic acid (6.99%), in the coagulant could aid in destabilizing suspended particles in water by reducing the surface tension and increasing the charge neutrality of the particles (Rashid et al., 2008; da Silva et al., 2010)

Additionally, the presence of alkane compounds, such as 3,3-dimethylhexane (0.27%) and 4,6-dimethyldodecane (0.17%); though low, could also aid in coagulation by forming a hydrophobic layer around the suspended particles, causing them to clump together and settle out of the water (Aziz et al., 2021; Varsani et al., 2022).

Furthermore, the presence of methyl esters, such as methyl stearate (0.23%) and in the coagulant could also aid in coagulation by reducing the interfacial tension between the suspended particles and water, causing them to agglomerate and settle out of the water more easily (Rashid et al., 2008).

Besides that, several compounds identified based on GCMS analysis are also known to possess antibacterial properties. For example, hexadecanoic acid (0.24%) and n-hexadecanoic acid (6.99%) are both known to have antibacterial properties against a range of bacteria, including *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* (Sanabria-Ríos et al., 2020; Casillas-Vargas et al., 2021). Similarly, palmitoleic acid (0.78%) has been shown to possess antimicrobial activity against both gram-positive and gram-negative bacteria (Karimi et al., 2015).

Oleic acid (63.2%, 0.13%) is another compound that has been shown to have antibacterial properties, particularly against *S. aureus* (Yoon et al., 2018; Casillas-Vargas et

al., 2021). This fatty acid is also known to demonstrate anti-inflammatory and antioxidant properties, making it a potentially useful compound for the treatment of various diseases.

Overall, the GCMS analysis provides valuable information about the organic compounds present in the coagulant, which could aid in understanding its coagulation properties and optimising its performance in water treatment processes.

### 4.3 Preliminary Experiments

The purpose of this preliminary experiment was to determine the range of variables particularly contact time, dosage and pH to be fixed in the subsequent optimisation study. HS infuses a brown-yellow or blackish-brown tint to peat water, and the efficacy of *M. oleifera* to remove HS can be visually observed through the colour changes of the treated water. When *M. oleifera* was added to the peat water, it turned clear, indicating that the HS was removed by the adsorption process (Figure 4.4).

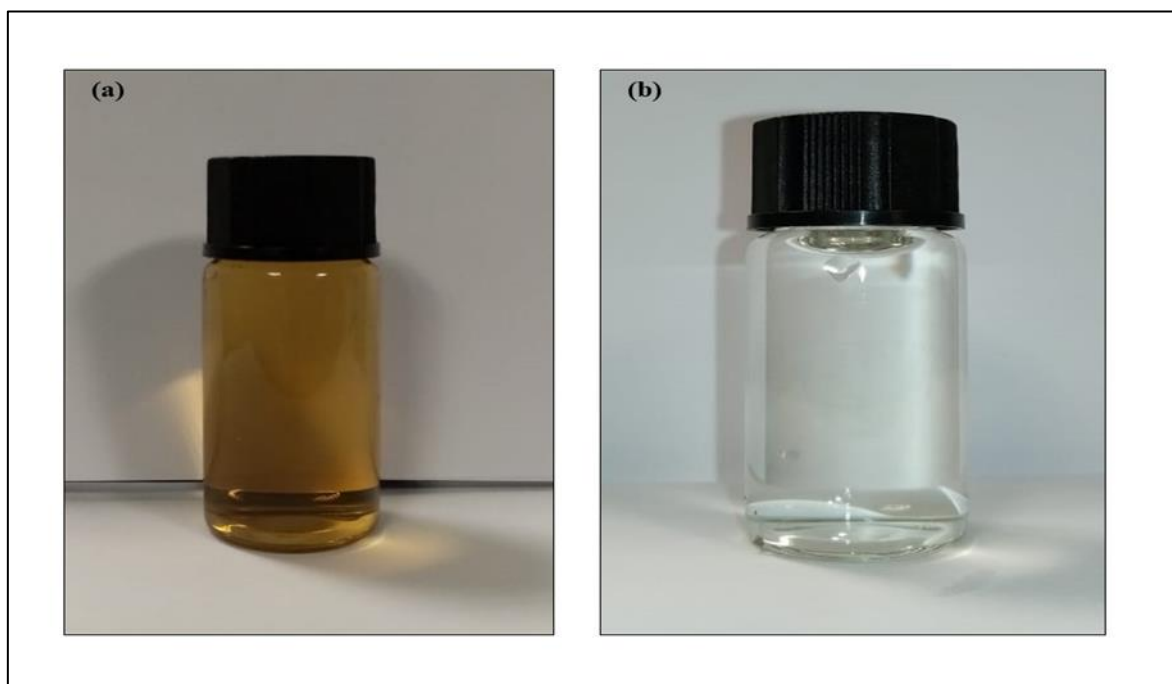


**Figure 4.4:** Peat water (a) before and (b) after treatment

According to Figure 4.5, HS reduction rose exponentially from a treatment time of 0 min to 60 min, with the highest elimination of 74.3% at t60 and a minor decline at t120, which is 72.5%.

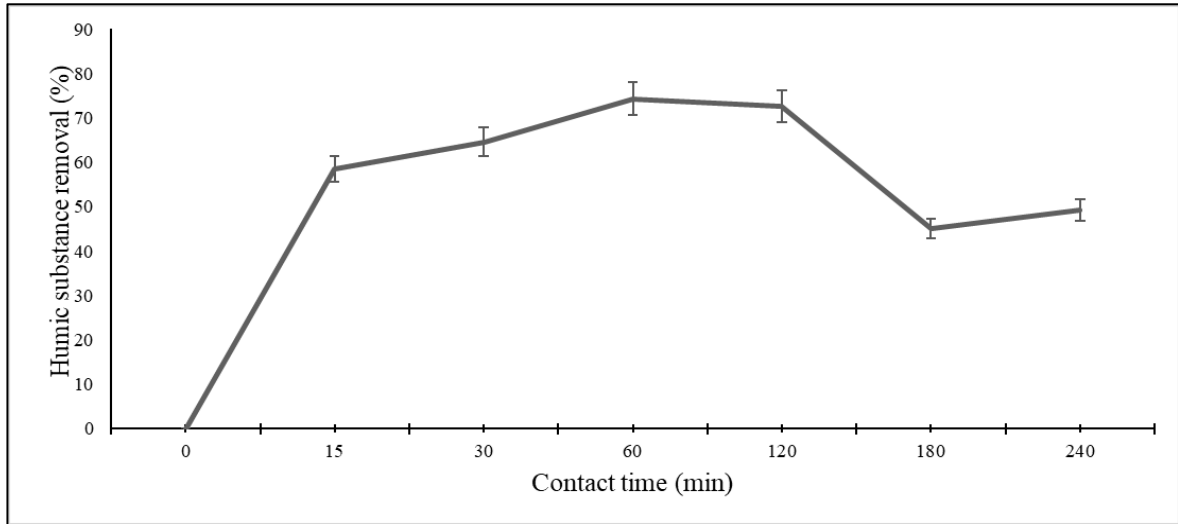


**Figure 4.5:** The removal of humic substance (%) against contact time (min) with constant coagulant dosage (1 g/L) and pH of peat water (pH 5.7)

This finding is lower than the one reported by Suryawan et al. (2020), where the highest level of colour removal was up to 90% at 120 min by ozone treatment. The difference between these studies could be primarily attributable to the treatment methods applied (Suryawan et al., 2020). However, a study by Robinson et al. (2001) found that plant-based coagulants require a longer retention period for colour removal due to their hardness, particularly in their trial using wood chips as adsorbents, making them less effective than other coagulants. In addition, HS removal decreased significantly at 180 min (45.1%), demonstrating that contact time is a crucial factor in colour removal. It is possible that at a certain time, the effectiveness of the HS removal process decreased because most of the suspended particles in the peat water had already been coagulated and removed by the coagulant. At this point, the remaining coloured particles may be more difficult to be

removed because they are either smaller in size or more resistant to coagulation. In addition, it is also possible that the seeds from *M. oleifera* itself started to break down or degrade after prolonged exposure to the peat water, which could also reduce its effectiveness in removing colour from the water.

Figure 4.6 depicts the preliminary study of the effect of dosage on HS removal at a fixed treatment time and pH.

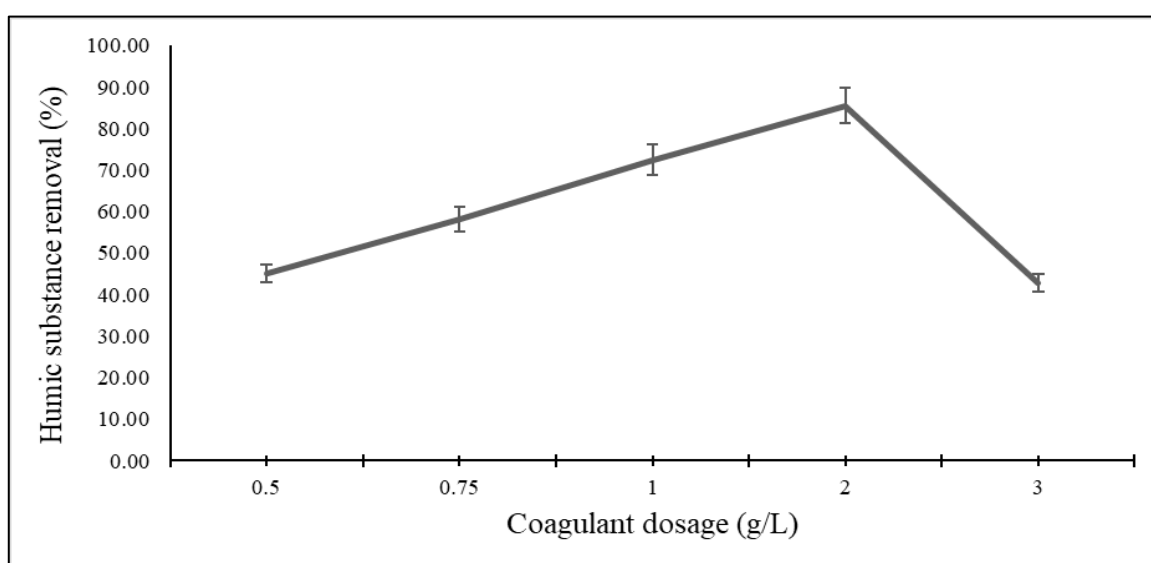


**Figure 4.6:** The removal of humic substance (%) against coagulant dosage (g/L) with constant contact time (120 min) and pH of peat water (pH 5.7)

A gradual decline in HS was seen, with the highest removal occurring at 2 g/L of dosage, or 85.6%. However, when the dosage was increased to 3 g/L, the HS removal was reduced significantly. The development of a steady-state situation may be the cause of the lower HS elimination at the higher coagulant dosage. Specifically, more *M. oleifera* might have been used than the amount required to remove HS, producing extra coagulant that could have interfered with the coagulation process. More coagulants may not always result in additional advancements in HS elimination beyond a specific dosage level. This is due to the

establishment of a steady-state condition, where the extra coagulant can disrupt the coagulation process and even re-suspend coagulated particles. It is important to note that the development of a steady-state condition is a typical occurrence in coagulation processes and is not specific to our study (Muruganandam et al., 2017; Aldemir et al., 2021). The characteristics of the water being treated, as well as the kind and quantity of coagulant being employed, all affect the ideal dosage for coagulation. Shah et al. (2013) found that a dosage higher than 1.68 g/L failed to improve the removal efficiencies in terms of colour reduction and pollutant concentration due to the establishment of a steady-state condition with increased coagulant and adsorbent dosage.

The last set of preliminary tests was set to determine the range of pH for HS removal. The dosage of 1 g/L and contact time at 120 min were fixed for this purpose. Based on Figure 4.7, the pH range of 5-9 resulted in minimal variations of HS removal ranging from 78.1% to 82.3%.

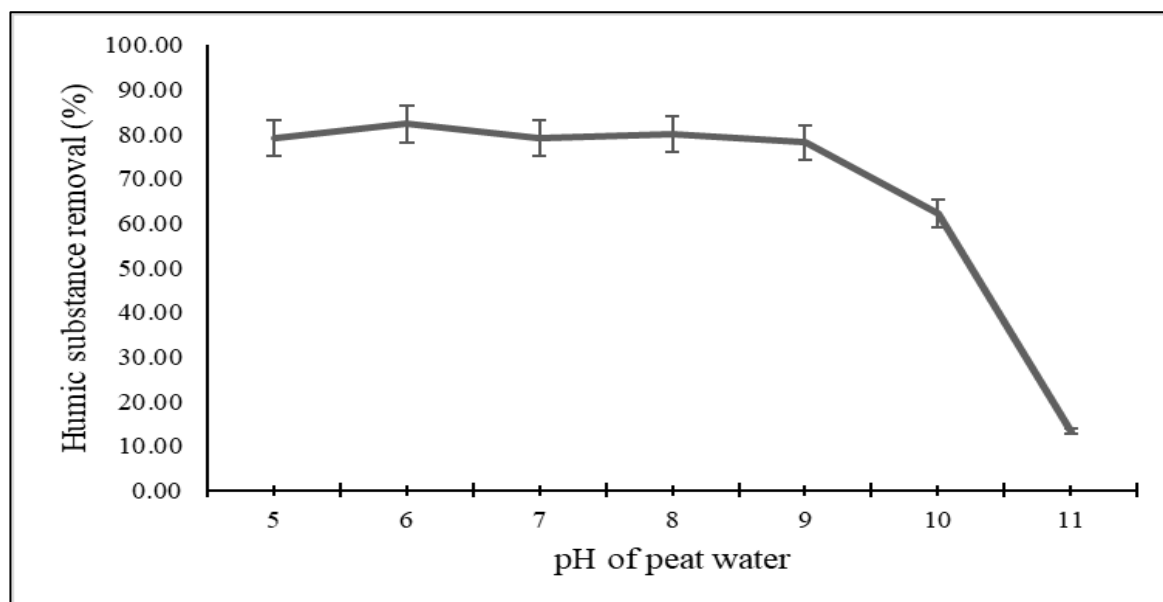


**Figure 4.7:** The removal of humic substance (%) against pH of peat water with constant coagulant dosage (1 g/L) and constant contact time (120 min)

This was consistent with the findings of Desta et al. (2021), who reported that *M. oleifera* seeds powder works best between pH 7 and pH 9 for the elimination of colour, turbidity, and COD in wastewater treatment. The pH of the peat water affects the overall charge of the particles in the water and can therefore impact the coagulation process. At very low pH values, *M. oleifera* may not be able to coagulate the particles effectively, while at very high pH values, the coagulated particles may redisperse back into the water. Due to this, pH values in the medium range are considered ideal for the coagulation process because it allows for optimal charge neutralization and particle destabilization, leading to effective coagulation and removal of impurities (Ernest et al., 2017).

Based on the preliminary data, the following ranges were fixed for the RSM study: coagulant dosage (0.5 g/L to 2 g/L), pH value (pH 6 to pH 10) and contact time (30 min to 120 min). Any increase in the HS content after treatment might be due to the optimal condition that had been exceeded which increases the organic matter in the water sample.

#### 4.4 Colour Degradation of Peat Water Using *M. oleifera* Seeds' Powder

##### 4.4.1 Model Fitting

Table 4.3 shows 15 runs with varying operating conditions that were conducted to investigate the effect of the independent variables (coagulant dosage, pH of peat water, and contact time) against the experimental response (Y), the percentage of HS removal.

**Table 4.3:** Experimental values and predicted values by the experimental model designed by BBD.

| Standard Order | Dosage | pH | Contact time | Actual Value | Predicted Value |
|----------------|--------|----|--------------|--------------|-----------------|
| 1              | 0.5    | 6  | 75           | 47.3         | 47.46           |
| 2              | 2      | 6  | 75           | 80.1         | 79.99           |
| 3              | 0.5    | 10 | 75           | 31.8         | 31.91           |
| 4              | 2      | 10 | 75           | 53.5         | 53.34           |
| 5              | 0.5    | 8  | 30           | 53.2         | 52.93           |
| 6              | 2      | 8  | 30           | 72.3         | 72.30           |
| 7              | 0.5    | 8  | 120          | 50.9         | 50.90           |
| 8              | 2      | 8  | 120          | 85.2         | 85.48           |
| 9              | 1.25   | 6  | 30           | 71.6         | 71.71           |
| 10             | 1.25   | 10 | 30           | 14.6         | 14.76           |
| 11             | 1.25   | 6  | 120          | 41.6         | 41.44           |
| 12             | 1.25   | 10 | 120          | 56.3         | 56.19           |
| 13             | 1.25   | 8  | 75           | 80.9         | 80.23           |
| 14             | 1.25   | 8  | 75           | 80.3         | 80.23           |
| 15             | 1.25   | 8  | 75           | 79.5         | 80.23           |

In this work, three distinct tests, including a sequential model sum of squares, lack of fit tests, and model summary statistics, were conducted to determine the appropriateness of models among several models to reflect the maximal HS removal from peat water; the findings are shown in Table 4.4.

Table 4.4: Fit of summary of various models

| Source                                   | Sum of Squares | DF        | Mean Square        | F Value             | p-value Prob > F | Remarks   |
|------------------------------------------|----------------|-----------|--------------------|---------------------|------------------|-----------|
| Sequential Model Sum of Squares [Type I] |                |           |                    |                     |                  |           |
| Mean                                     | 53892.05       | 1.00      | 53892.05           |                     |                  |           |
| Linear                                   | 2407.88        | 3.00      | 802.63             | 2.39                | 0.1239           |           |
| 2FI                                      | 1373.79        | 3.00      | 457.93             | 1.58                | 0.2678           |           |
| Quadratic                                | 2312.27        | 3.00      | 770.76             | 2977.82             | < 0.0001         | Suggested |
| Cubic                                    | 0.31           | 3.00      | 0.10               | 0.21                | 0.8842           | Aliased   |
| Residual                                 | 0.99           | 2.00      | 0.49               |                     |                  |           |
| Total                                    | 59987.29       | 15.00     | 3999.15            |                     |                  |           |
| Lack of Fit Tests                        |                |           |                    |                     |                  |           |
| Linear                                   | 3686.37        | 9.00      | 409.60             | 830.26              | 0.0012           |           |
| 2FI                                      | 2312.58        | 6.00      | 385.43             | 781.28              | 0.0013           |           |
| Quadratic                                | 0.31           | 3.00      | 0.10               | 0.21                | 0.8842           | Suggested |
| Cubic                                    | 0.00           | 0.00      |                    |                     |                  | Aliased   |
| Pure Error                               | 0.99           | 2.00      | 0.49               |                     |                  |           |
| Model Summary Statistics                 |                |           |                    |                     |                  |           |
| Source                                   | S.D            | R-Squared | Adjusted R-Squared | Predicted R-Squared | PRESS            |           |
| Linear                                   | 18.31          | 0.3950    | 0.2301             | -0.0940             | 6668.21          |           |
| 2FI                                      | 17.01          | 0.6204    | 0.3358             | -0.1740             | 7155.55          |           |
| Quadratic                                | 0.51           | 0.9998    | 0.9994             | 0.9988              | 7.14             | Suggested |
| Cubic                                    | 0.70           | 0.9998    | 0.9989             |                     | +                | Aliased   |

The data shows that a Quadratic model is the best fit for the given experiment with a very small p-value (<0.0001) and the smallest square root of the residual mean square (S.D)

at 0.51 among other models (Entekhabi et al., 2010). This indicates that the simulated and observed data are close to each other, demonstrating more accuracy, and that the model best fits the data. The Lack of Fit tests also supports the Quadratic model (Bo et al., 2015). The model summary statistics show that the predicted error sum of squares (PRESS) was provided, which confirms the fitted model through ANOVA analysis (Behera et al., 2018; Darvishmotevalli et al., 2019). The Linear and 2FI models have relatively low R-squared values, while the Quadratic model has a high R-squared value of 0.9998, indicating a very good fit between the observed and predicted data. The cubic model is aliased and therefore not reliable.

#### 4.4.2 Statistical Analysis

ANOVA is defined as a statistical technique for detecting variations in the means of experimental groups (Sawyer, 2009). Through ANOVA, the test performed can be summarised, including the significance of the regression model, the significance of individual model coefficients, and the test for lack-of-fit (Noordin et al., 2004). Table 4.5 shows the ANOVA analysis for the HS removal quadratic model.

**Table 4.5:** ANOVA of the regression model for the removal of HS

| Source | Sum of square | d | Mean square |
|--------|---------------|---|-------------|
| Model  | 6093.94       | 9 | 677.10      |
| A      | 1455.30       | 1 | 1455.30     |
| B      | 890.42        | 1 | 890.42      |
| C      | 62.16         | 1 | 62.16       |
| AB     | 30.80         | 1 | 30.80       |
| AC     | 57.76         | 1 | 57.76       |

**Table 4.5** continued

|                |         |   |                      |
|----------------|---------|---|----------------------|
| BC             | 1285.22 | 1 | 1285.22              |
| A <sup>2</sup> | 54.49   | 1 | 54.49                |
| B <sup>2</sup> | 1990.20 | 1 | 1990.20              |
| C <sup>2</sup> | 446.09  | 1 | 446.09               |
| Residual       | 1.29    | 5 | 0.26                 |
| Lack of Fit    | 0.31    | 3 | 0.10                 |
| Pure Error     | 0.99    | 2 | 0.49                 |
|                |         | 1 |                      |
| Cor Total      | 6095.24 |   |                      |
| Std. Dev.      | 0.51    |   | R <sup>2</sup>       |
| Mean           | 59.94   |   | Adj. R <sup>2</sup>  |
| C.V. %         | 0.85    |   | Pred. R <sup>2</sup> |
| PRESS          | 7.14    |   | Adeq. Precision      |

The data provided shows the results of a study that aimed to investigate the effects of three independent variables (A, B, C) and their squared terms (A<sub>2</sub>, B<sub>2</sub>, C<sub>2</sub>) on a dependent variable. The results indicate that the model is significant, as shown by the high F-value (677.10) and the low p-value (<0.05) associated with it (Behera et al., 2018). This suggests that the independent variables have a significant effect on the dependent variable.

Further analysis of the model using the sum of squares reveals that the most significant factors are B<sub>2</sub>, A, and BC, as they have the largest mean squares. This means that they have the most significant impact on the dependent variable. On the other hand, the factor with the least significant impact is C, as it has the smallest mean square.

The lack of fit test results shows that the lack of fit is not significant, indicating that the model fits the data well. Additionally, the small value of the residual mean square (0.26) suggests that the difference between the observed and predicted values is small, indicating that the model is accurate (Darvishmotevalli et al., 2019).

The model summary statistics show that the model has a high R-squared value (0.9994), indicating that the model explains most of the variation in the data. The predicted R-squared value (0.9988) also suggests that the model is reliable for predicting future observations (Deng and Chen, 2019).

In conclusion, the study's results suggest that the independent variables have a significant effect on the dependent variable, with B2, A, and BC having the most significant impact. The model is reliable, accurate, and can be used to predict future observations.

"Adequate Precision" measures the signal-to-noise ratio. The range of projected values at design points is compared to the average prediction error to determine adequate precision. Ratios larger than 4 show that the model discrimination is satisfactory in this study, the value is considerably higher than 4 (Noordin et al., 2004).

The degree of precision with which the experiments are compared is indicated by the coefficient of variation (CV). In general, the bigger the CV value, the less reliable the experiment. As the experiment has a lower CV value (0.85%), it implies that the experiments were reliable (Mohan et al., 2014). The reliability of the chosen model is also further reviewed by observing the plot of normal probability residuals and the plot of residuals against the predicted response. The observation of a normal probability plot of residuals is a way to test whether the error terms are normally distributed. The residuals versus predicted response values plot will indicate a problem if a pattern exists.

Based on Figure 4.8, the residuals generally fall on a straight line with no definite patterns, indicating that the residuals are distributed normally (Ferré, 2009). Aside from that, the normal plot of residual reveals that there are only a few deviations from normal, indicating that the model is well-fitting. Therefore, the derived model could be utilised to theorise about the dependent variable. The obtained percentage of HS removal efficiency ranged from 14.46% to 85.2% and the model projected values corresponded satisfactorily with these experimental results.

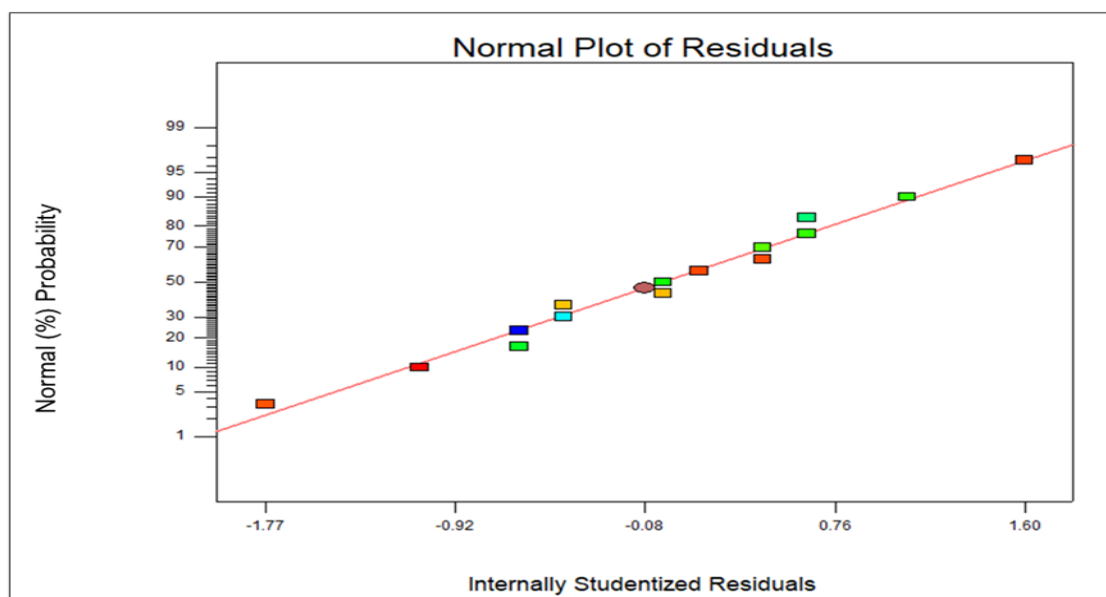


**Figure 4.8:** Normal plot of residuals

Figure 4.9 displays a regression coefficient ( $R^2$ ) value of 0.9998, indicating a reasonably strong correlation between experimental and predicted values. This implies that the model is adequate and proven to be suitable for the study with no reason to suspect any violation of the independence or constant variance assumption (Mohan et al., 2014).

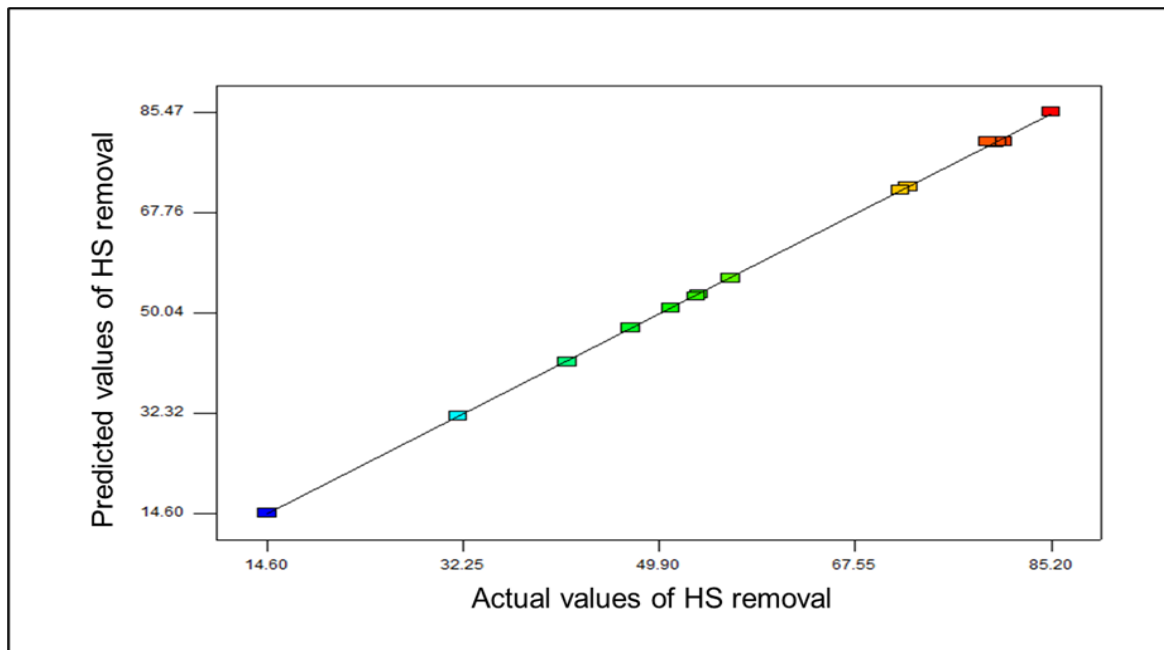


**Figure 4.9:** Predicted versus experimental value of HS removal

The resulting second-order fitting polynomial equation was generated and the equation, in terms of coded factors, is shown in Equation 4.1:

$$Y (\%) = +80.23 + 13.49A - 10.55B + 2.79C - 2.77AB + 3.80AC + 17.93BC - 3.84A^2 - 23.22B^2 - 10.99C^2 \quad \text{Equation 4.1}$$

where, Y is the percentage of colour removal (%); A is the coagulant dosage (g/L), B is the pH of peat water and C is the contact time (min).

#### 4.4.3 Response Surface Plot

The three-dimensional (3D) response surface for the coagulation process of peat water treated with *M. oleifera* seeds powder is illustrated in Figure 4.10a-c. From the plots,

two variables varying within the experimental ranges were plotted to assess the relationships between the variables and the response to HS degradation of peat water. The flexure of the diagram illustrates the interplay between the variables.

Figure 4.10a illustrates the 3D surface of the polynomial model for HS removal with respect to the dosage of *M. oleifera* seed powder and the pH of peat water at a contact time of 75 min. At all pH levels, the HS removal increased with the increasing dosage. For instance, this can be observed clearly at pH 10, where a lower dosage (0.5 g/L) resulted in lower HS removal (31.8%) while a higher dosage (2 g/L) resulted in a higher removal (53.5%). A study by Lee et al. (2015) showed a similar trend with 5 g of biosorbent being the most effective amount in the removal of HS (89.03%). Their findings matched those of Rucka et al. (2019), who discovered that raising the dosage was one of the several approaches to improve the effectiveness of HS removal. The trend is also similar with lower pH (pH 6) as the HS removal in the presence of low dosage (0.5 g/L) was lesser than the larger dosage (2 g/L) which were 47.3% and 80.1%, respectively. Maximum HS removal was achieved at pH 8 and coagulant administration of 1.25 g/L with 80.9% colour removal. This is consistent with a study by Desta and Bote (2021) stating that *M. oleifera* performs well between pH 7 and 9. The slight decrease at a higher coagulant dosage (2 g/L) could be due to a surplus of coagulant at the optimum amount, which caused the colour to slightly increase due to the presence of free ions.

Figure 4.10b shows the interaction between coagulant dosage and contact time at a pH of 8 against colour removal. It was evident that increasing the coagulant dosage along with an increase in contact time improved the removal of HS. The highest removal of 85.2% was obtained with the application of 2 g/L coagulant at 120 min of contact time, followed

by 80.3% removal at dosages of 1.25g/L and 75 contact time. A low dosage (0.5 g/L) produced the lowest removal of 50.9% at the same contact time. This was in agreement with a study by Syafalni et al. (2013) where the highest colour removal in peat water was obtained at a higher coagulant dosage (2.5 g/L). They also noted that the removal efficiency increases with the increment in time, although after a 1 h interval, the removal change was not significant (Syafalni et al., 2013). The study was also concordant with Leszczyński (2019) as their data showed an increasing trend of removal with the increment of coagulant dosage.

In Figure 4.10c, the interaction between pH and contact time at a dosage of 1.25 g/L is depicted. The highest removal of HS was observed at pH 8 with a contact time of 75 min, resulting in an 80.9% removal rate. This finding suggests that at these conditions, there were more available adsorption sites on the surface of coagulants, allowing for greater removal of HS through coagulation. Peat water is known to contain HS, which is a complex mixture of organic compounds, including humic and fulvic acids. These compounds contain functional groups such as phenolic and carbonyl. These functional groups can acquire a negative charge when they become ionised in water. As a result, humic acid ions in the peat water are attracted to the surface of the coagulant particles due to charge neutralisation between the HS and the coagulant's surface, resulting in their removal through coagulation. On the other hand, the presence of hydroxyl,  $\text{HCO}^{-3}$ , and  $\text{CO}_3^{2-}$  ions in the solution phase at higher pH values can cause them to be adsorbed onto the surface of *M. oleifera* seeds, creating a negative surface charge. This negative surface charge can attract positively charged HS and other organic compounds present in the peat water, leading to their removal through adsorption onto the *M. oleifera* seeds.

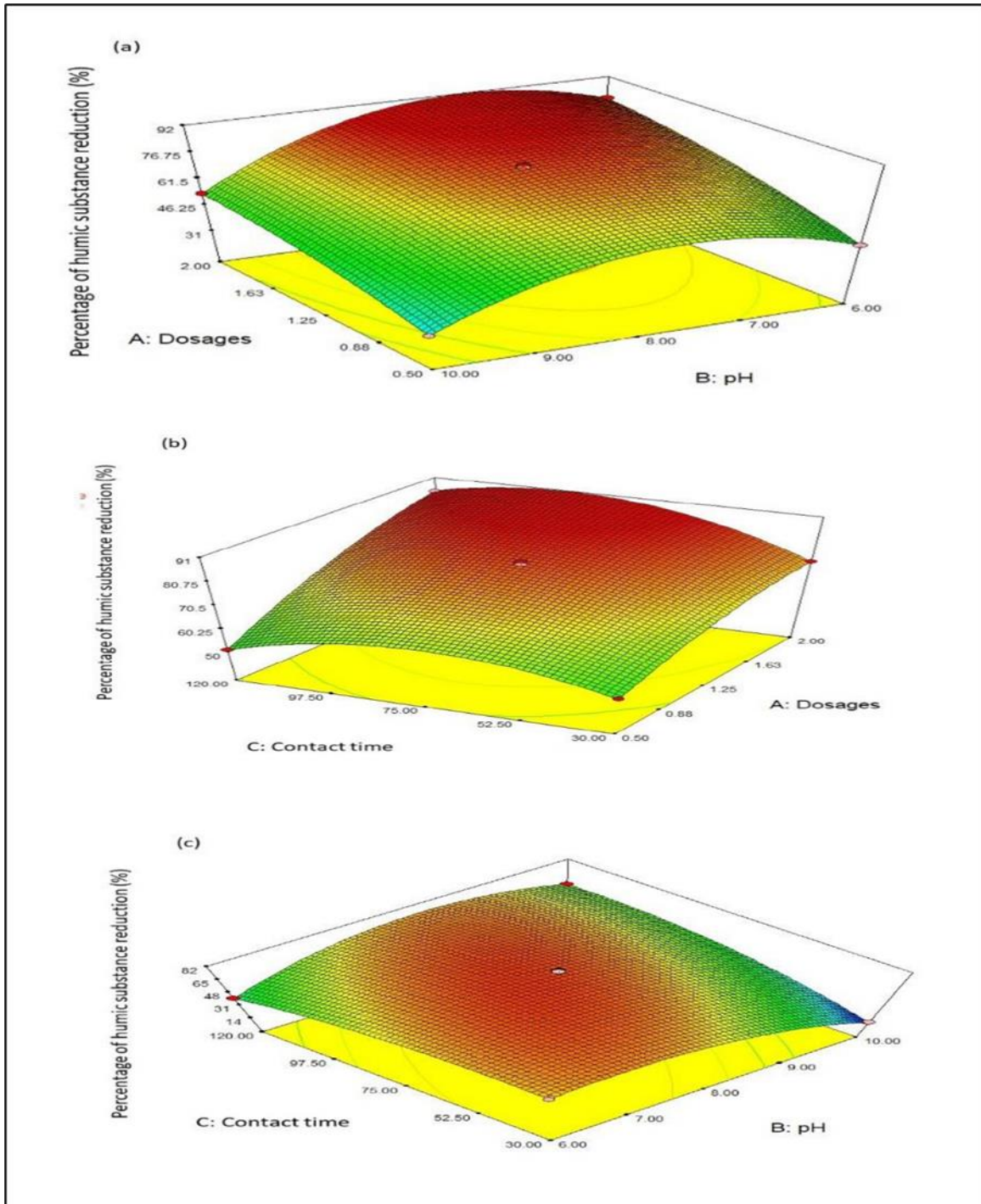


**Figure 4.10:** RSM surface plot: (a) dosages and pH with contact time (75 min), (b) dosages and contact time with pH 8, and (c) pH and contact time with dosages 1.25 g/L; against humic substance removal percentage

#### 4.4.4 Numerical Optimisation

The optimised process condition for HS removal from the peat water was as follows: dosage at 1.94 g/L, pH of peat water at 7.6 and contact time of 86 min. Under these conditions, the predicted HS removal was 91.1%. For their validation of the optimum conditions, triplicate confirmatory experiments were carried out under the optimised conditions and the average HS removal was 88.4%. Two other optimised conditions were tested. The application of 2 g/L, pH of peat water 8.4 and contact time of 120 min resulted in 85.5% removal of HS while the application of 1.86 g/L, pH of peat water 7.5 and contact time of 72 min resulted in 85.5% removal.

The results are closely related to the estimated value, reflecting the predictive performance of the RSM model. Data obtained from optimisation analysis, indicating BBD incorporated with the desirable function could be used to optimise the parameters for HS removal.

Subsequently, treated water under optimal conditions was characterised. Figure 4.11 shows the comparison of peat water before and after treatment. The pH of peat water turning from acidic to neutral condition does benefit the treatment for consumption and daily usage as the permissible pH of water is within pH 6.5 – pH 8.5. Thus, no changes in the pH are needed after the treatment. A marked change can be observed in the turbidity of peat water, from  $32.27 \pm 1.8$  NTU to  $4.96 \pm 0.05$  NTU, which is considered within the permissible range for drinking water. Another major improvement can be seen in the TSS of treated water. The final TSS value of 8.33 mg/L falls within Class I in NWQS. Though the application of *M. oleifera* seeds as coagulants that binds to the particulate of the contaminant in peat water might be one of the reasons for these major changes, another reason might be due to the

measurement process itself. In the water treatment, the supernatant of the treated water was taken for the characterisation study. The flocs at the bottom of the beaker were left undisturbed to sediment. An increase in both COD and BOD was observed after the treatment. The optimum condition that was focused on is specifically HS removal. Thus, the optimum condition for COD might not be the same condition for HS removal. Due to the rise in coagulant doses, the COD elevated beyond the acceptable level. The increase in coagulant doses caused an increase in COD levels due to the excess positive ions from the coagulant neutralizing the negative ions from the water contaminants (Desta & Bote, 2021). The increase in the BOD level is also expected as it is one of the drawbacks of the usage of plant-based coagulants, which is the increase in organic load if no extraction is performed on the coagulant (Sánchez-Martín et al., 2012; El Bouaidi et al., 2022). HS (E4/E6) value was observed to decrease from 6.36 to 3.33.

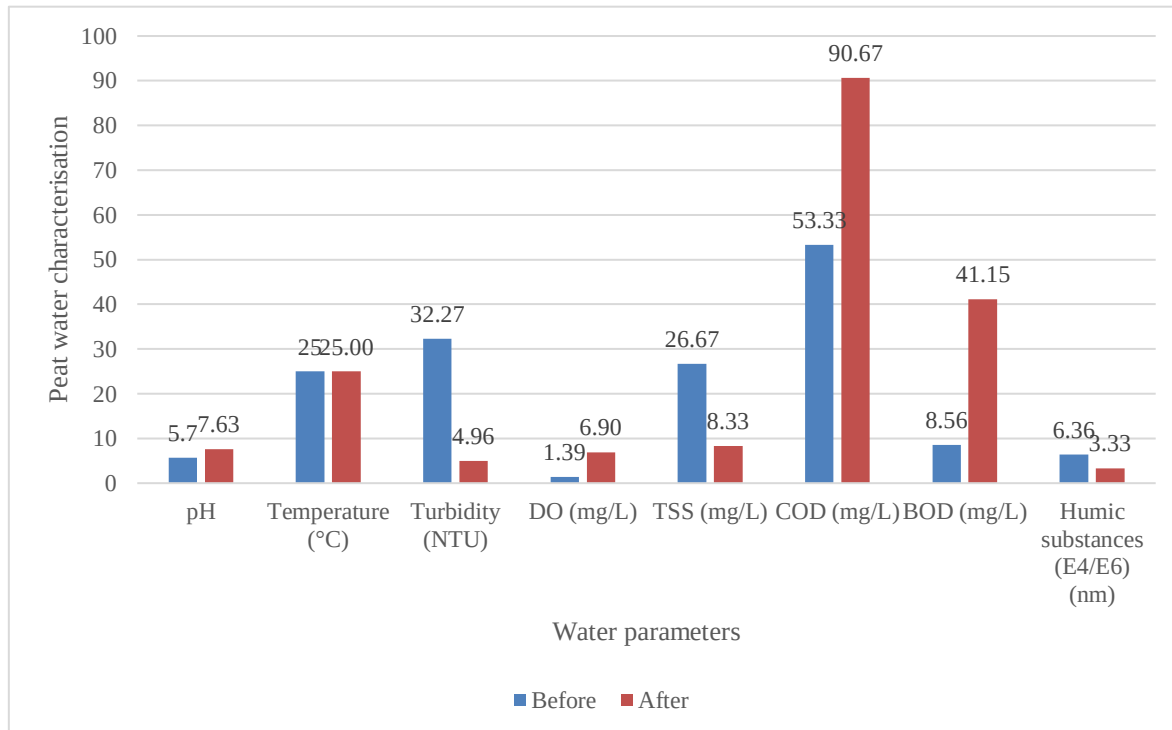


**Figure 4.11:** Peat Water Characterisation Before and After the Treatment with *M. oleifera* seeds powder

#### 4.5 Antibacterial Study

Antibacterial activity of *M. oleifera* was observed against both chosen bacteria with the extraction of organic solvent. Gram-negative and gram-positive effects reported in a single plant extract may be explained by the presence of a broad spectrum of bactericidal chemicals or by the action of plant-derived toxins (Kostova & Dinchev, 2005). Jahn et al. (1986) found that *M. oleifera* contains pterygospermin, moringine, glycosides 4-(L-rhamnosyloxy)-benzylisothiocyanate (RBI) and 4-(L-rhamnosyloxy)-phenylacet, which impart the plant its antibacterial properties. Various bacteria, including *Bacillus subtilis*, *Mycobacterium phei*, *Serratia marcescens*, *E. coli*, *Pseudomonas aeruginosa*, *Shigella*, and *Streptococcus*, were regarded to be susceptible to these compounds (Viera et al. 2010). Jeon et al. (2014) also discovered comparable chemicals, notably 4-( $\alpha$ -L-rhamnosyloxy)-benzyl isothiocyanate (RBI) and another substance known as niazimicin. Several microorganisms, including *S. aureus* and *E. coli*, were subjected to antimicrobial screening utilizing the paper disc diffusion method. RBI and niazimicin were impregnated onto the paper disc, and the diameter of the inhibitory zone around the disc was measured as the antimicrobial activity (Jeon et al., 2014). Both microorganisms exhibited an inhibitory zone, demonstrating that the chemicals suppressed the growth of microorganisms.

Our findings are in accordance with prior studies since all concentrations of *M. oleifera* extract were effective against both *S. aureus* and *E. coli*. The inhibition zone (IZ) is a circular area around the spot of the disc in which the bacteria colonies do not grow as shown in Figure 4.13 to Figure 4.17, where 1, 2, 3 and C are the concentrations of the extracted *M. oleifera* at 2 g/L, 1.25 g/L, 0.5 g/L and control (distilled water), respectively. The largest IZ was registered by the ethanolic extracts against *S. aureus* ( $18.33 \pm 0.58$  mm), while the weakest one was found to be hexane extract against *E. coli* at 5.00 mm. *E. coli*, a

gram-negative bacterium, demonstrates the lowest zone of inhibition, indicating resistance to the plant extract; this may be connected to the cell wall structure. Its cell wall acts as a barrier to a variety of chemicals, including antibiotics (Impey et al., 2020). This would also explain why medicinal plants are typically more effective against gram-positive cultures than gram-negative ones as the former microbes have no cell wall structures.

Few studies observed the antibacterial activity of *M. oleifera* and determined that the activity was attributable to the plants' naturally occurring antioxidants, including ascorbic acid, flavonoids, phenolics, and carotenoids (Yamunadevi et al., 2011; Ndhlala et al., 2014; Amabye & Tadesse, 2016). These natural oxidants are reported to contain novel active compounds with antibacterial properties that may help overcome multidrug resistance. Alkaloids, for example, are naturally occurring chemical compounds composed of basic nitrogen atoms. These compounds are frequently used as medications and recreational drugs due to their pharmacological properties. Flavonoids work in conjunction with Vitamin C to enhance its effects and act as antioxidants. Additionally, they are known to be biologically active against toxins produced by the liver, tumours, viruses, and other microbes (Amabye & Tadesse, 2016).

The results were expressed as the IZ of growth surrounding each disc in millimetres and were categorised as low activity (IZ from 1 mm to 6 mm), moderate activity (7 mm to 10 mm), high activity (11 mm to 15 mm), and extremely high activity (16 mm or beyond) (Figure 4.18) (Khalfaoui et al., 2021). The extraction of *M. oleifera* at a concentration of 2 g/L and ethanol extraction (1.25 g/L) showed high antibacterial activity against *S. aureus*, as their zones of inhibition ranged between 12.67 mm and 18.33 mm, whereas, at all other concentrations, the zones of inhibition were under 12 mm, showing moderate activity to low

activity. This indicates that the concentrations of *M. oleifera* may influence its efficiency against microorganisms, with the higher the concentration, the larger the IZ that may be generated.

Although the seed extracts exhibit antibacterial activity against *S. aureus* and *E. coli*, any quantitative results were observed when amoxicillin (10 mg/mL) (positive control) was applied, whilst no reaction was observed for the negative control (distilled water). As a result, *M. oleifera* is regarded as a novel infection-fighting strategy for the control of multidrug-resistant pathogenic bacteria. Additionally, it possesses a substantial safety margin for human and animal consumption (Stohs & Hartman, 2015; Gopalakrishnan et al., 2016; Brilhante et al., 2017).

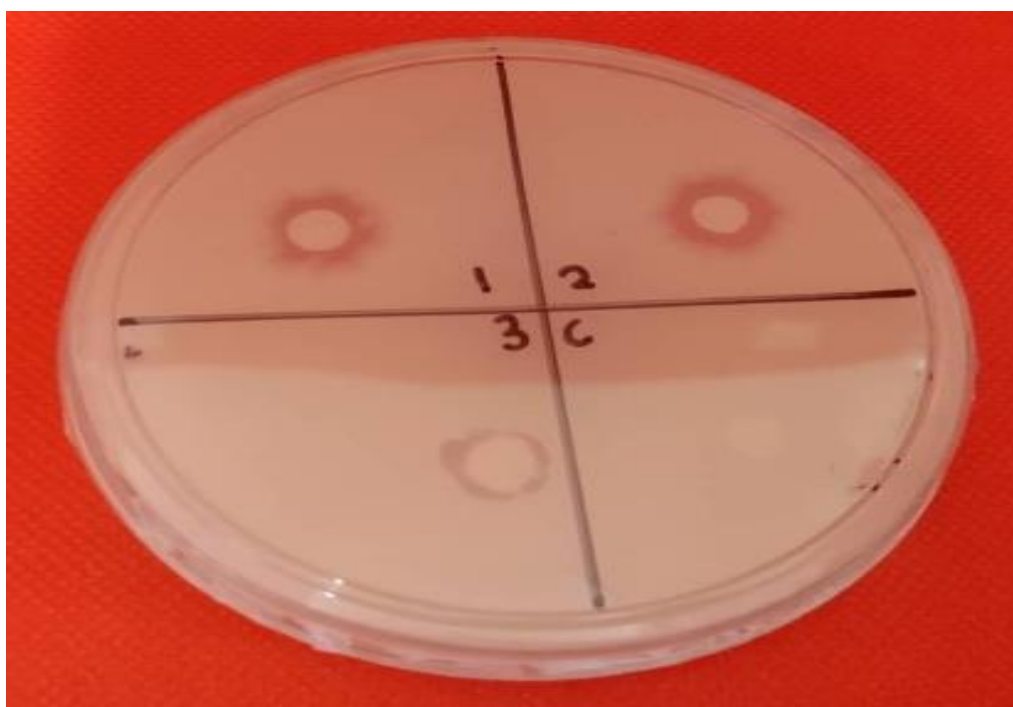


**Figure 4.12:** *E. coli* against *M. oleifera* extracted with ethanol at different concentrations

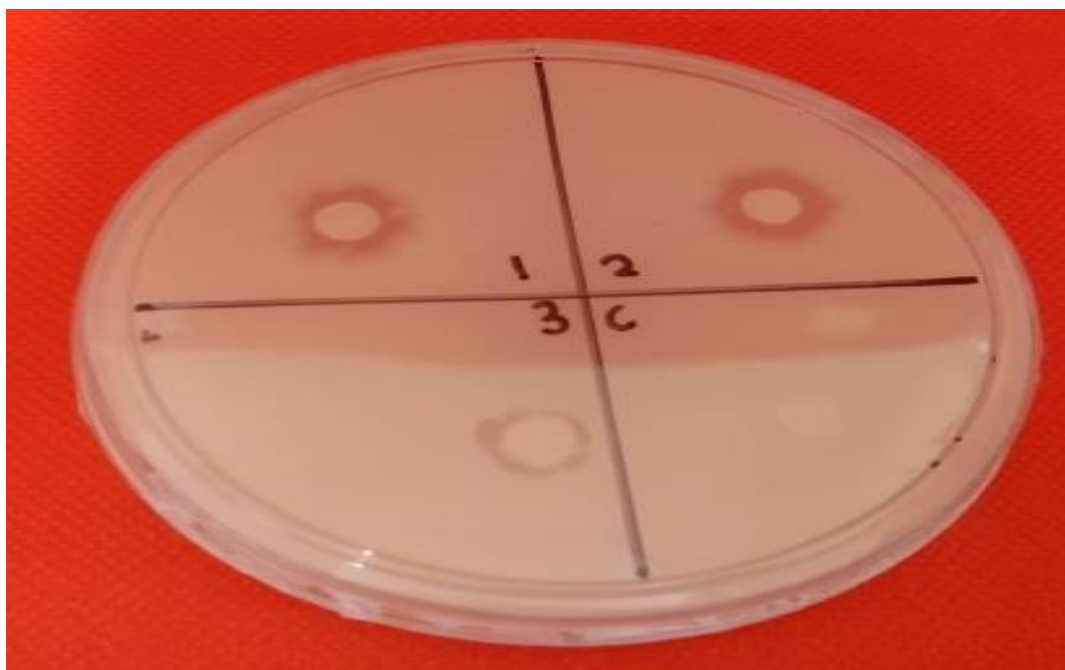


**Figure 4.13:** *E. coli* against *M. oleifera* extracted with methanol at different concentrations

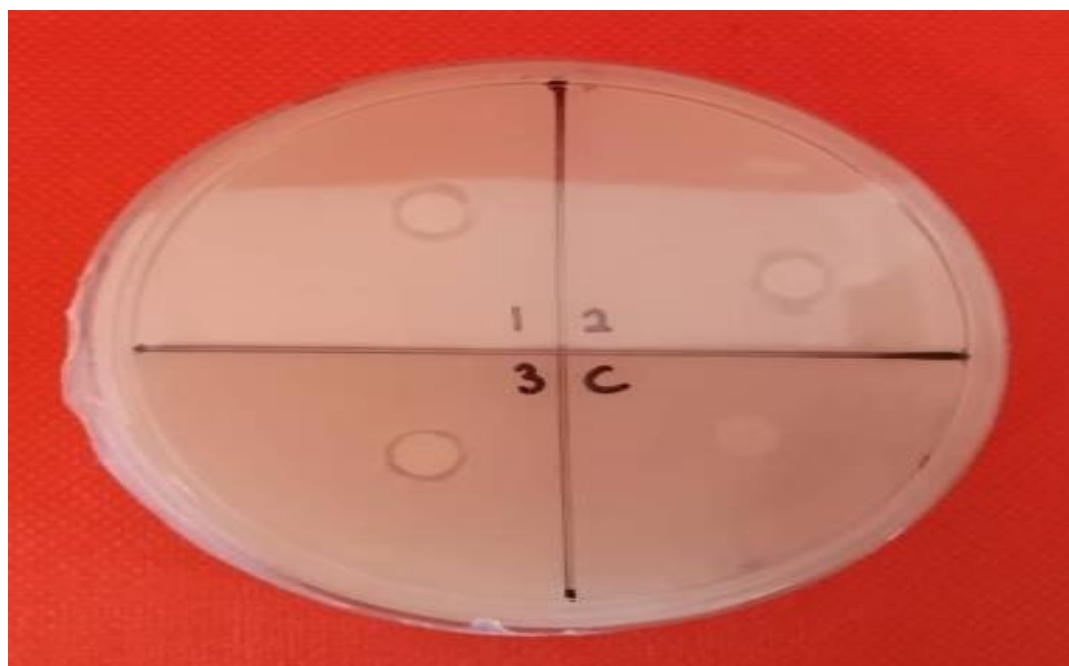


**Figure 4.14:** *E. coli* against *M. oleifera* extracted with hexane at different concentrations

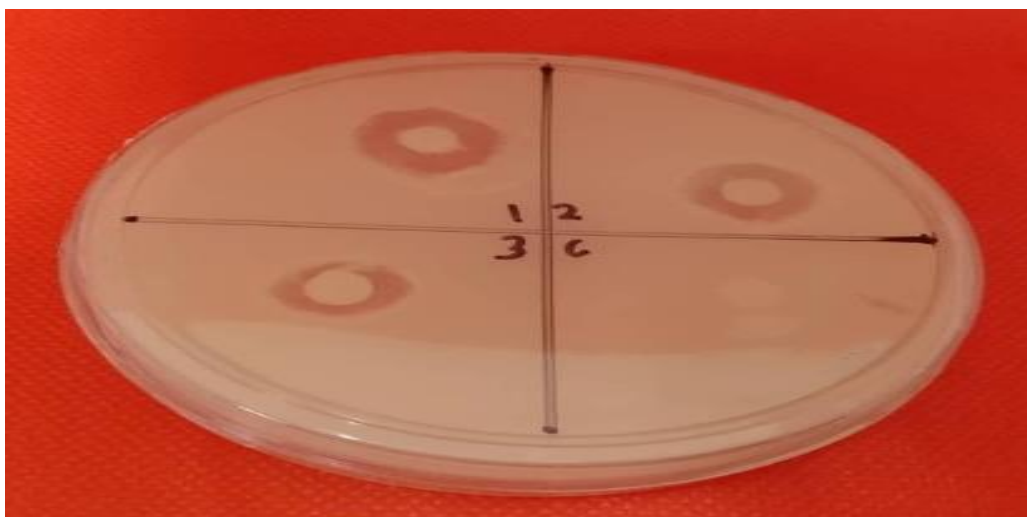


**Figure 4.15:** *S. aureus* against *M. oleifera* extracted with ethanol at different concentrations



**Figure 4.16:** *S. aureus* against *M. oleifera* extracted with methanol at different concentrations

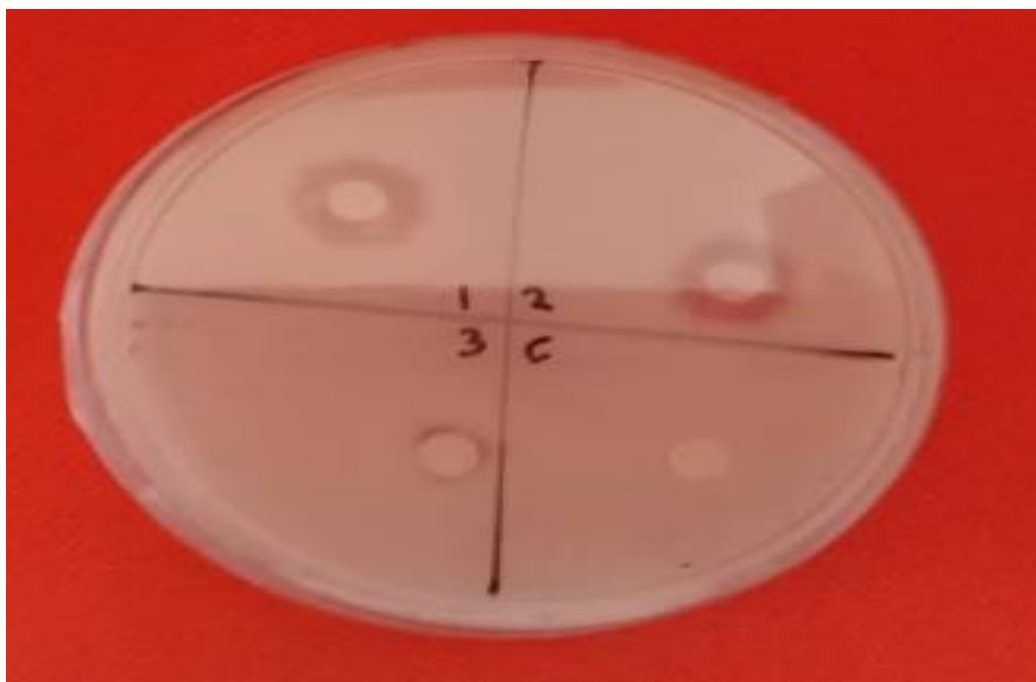


**Figure 4.17:** *S. aureus* against *M. oleifera* extracted with hexane at different concentrations

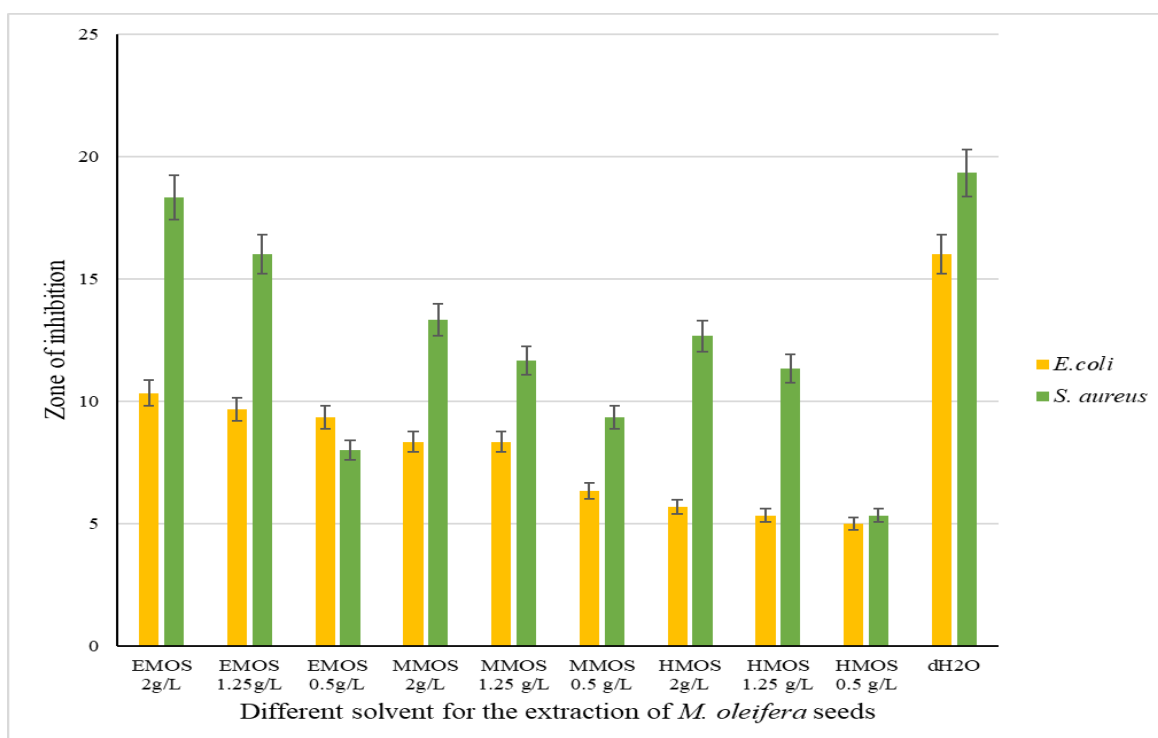


**Figure 4.18:** Zone of inhibition (mm) against *M. oleifera* seeds extraction with different solvents; EMOS (ethanol *M. oleifera* seeds), MMOS (methanol *M. oleifera* seeds), HMOS (hexane *M. oleifera* seeds) at different concentrations

## CHAPTER 5

### CONCLUSION AND RECOMMENDATIONS

#### 5.1 Conclusion

The initial water quality characterisations of peat water revealed that appropriate treatment is necessary prior to any usage. Preparation of coagulant was accomplished through the air-drying method of the seeds and ground into fine powder to obtain coagulant for water treatment. Before the optimisation of peat water treatment was performed, a few preliminary studies were performed to screen the performance of *M. oleifera* seeds powder in the removal of HS from peat water. Its ability was proven through the colour reduction on the treated peat. Therefore, to optimise the treatment of peat water, the appropriate range for coagulant dose, pH of peat water, and contact time were determined as parameters. The range for coagulant dose was from 0.5 g/L to 2 g/L, for pH of peat water it was from 6 to 10, and for contact time it was from 30 min to 120 min. BBD was used to optimise the treatment process based on the range obtained from preliminary experiments. A Quadratic model was chosen as the appropriate model for the data based on its Fit Summary and Lack of Fit Tests, and ANOVA analysis showed a high  $R^2$  value of 0.9998, confirming the model's applicability. Under the optimised RSM conditions, a coagulant dose of 1.94 g/L, pH of 7.6, and a contact time of 86 min resulted in 88.4% colour removal, which is comparable to the predicted optimum result of 91.1%. The antibacterial properties of *M. oleifera* were performed against *E. coli* and *S. aureus* by using a disc diffusion test. *M. oleifera* was extracted by using different solvents, namely ethanol, methanol and hexane. The ethanol extract demonstrated the highest antibacterial activity against *S. aureus*, measuring  $18.33 \pm$

0.58 mm and exhibiting exceedingly strong antibacterial activity. Meanwhile, the lowest antibacterial study was only 5 mm which is the hexanic extract against *E. coli*.

One important point to note is that the study found that the use of *M. oleifera* seeds powder as a coagulant was effective in removing HS from peat water, as demonstrated by the reduction in colour of the treated water. The study also determined the optimum conditions for the treatment of peat water using the coagulant, including coagulant dose, pH of peat water, and contact time. The treated water met the water quality standards for most parameters and showed strong antibacterial activity against *S. aureus*. The results suggest that the use of *M. oleifera* seeds powder as a coagulant could be a simple and effective solution for treating peat water as a feasible water source.

## **5.2 Recommendations**

This study provides opportunities to discover the potential of peat water as a water source. Presently, limited studies are available on peat water treatment, particularly in the usage of plant-based coagulants. The availability of plant-based coagulants to replace hazardous inorganic coagulants should be taken more seriously to reduce the side effect of the coagulant in the future. However, through the application of powdered *M. oleifera* seeds in this experiment, an increase in COD and BOD levels was observed. Thus, it is suggested that *M. oleifera* should be extracted through methods such as dialysis, lyophilization, ion-exchange, and precipitation prior to its usage in coagulation-flocculation treatment. Though the preparation in extracting *M. oleifera* into a crude extract is time-consuming and needs more advanced equipment, it is worth considering so that the treated water can be utilised for consumption. Also, other parameters such as conductivity, salinity, iron, and ammonia

should be considered in the peat water treatment to gain a better understanding of the peat water treatment.

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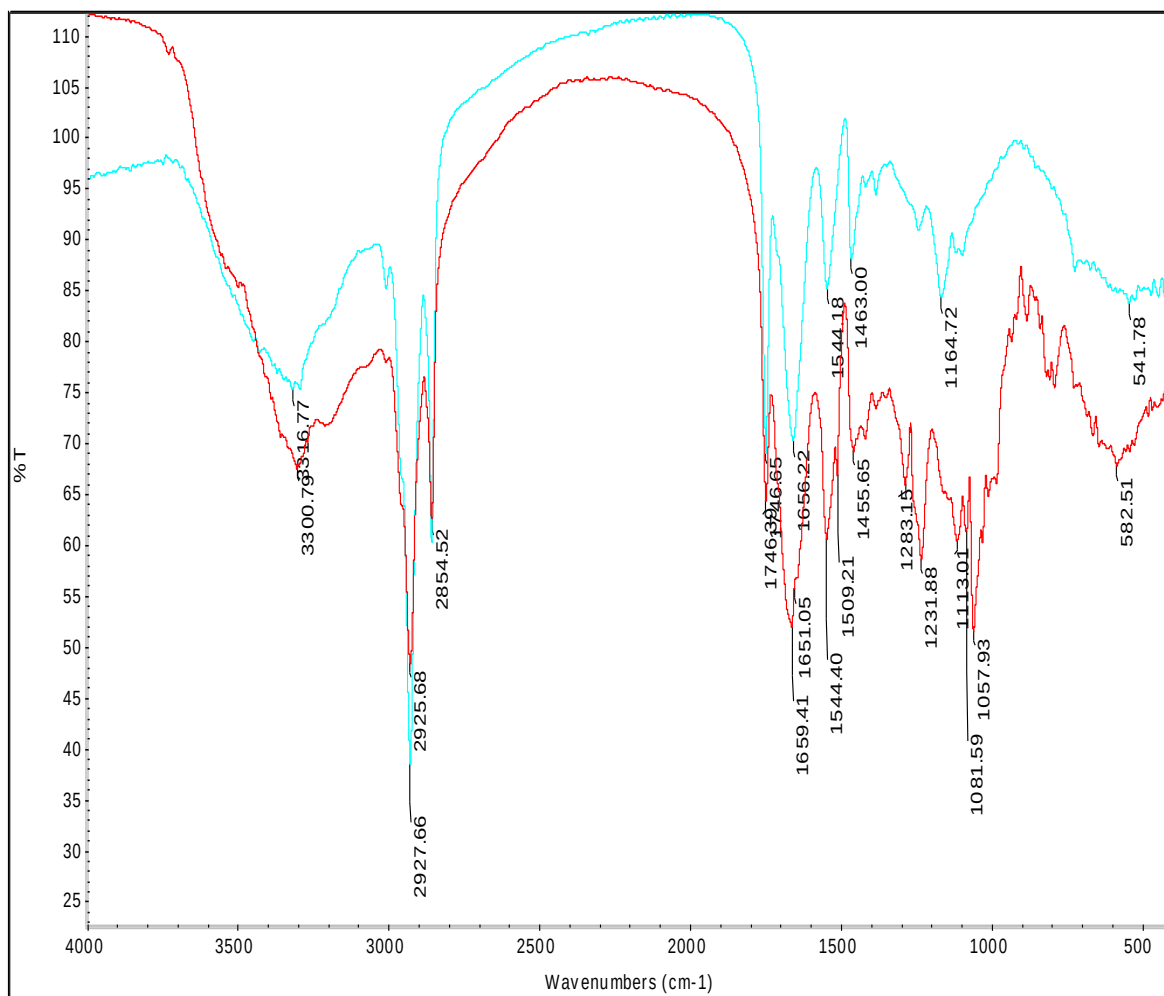
## APPENDICES

**Appendix A:** Raw data for characterisation of peat water sample from Asajaya peat swamp.

| First sampling  |     |                    |     |     |     |       |      |
|-----------------|-----|--------------------|-----|-----|-----|-------|------|
| Reading         | pH  | Turbidity<br>(NTU) | DO  | TSS | COD | BOD   | HS   |
| 1               | 6.8 | 30.19              | 1.6 | 25  | 80  | 8.00  | 6.28 |
| 2               | 6.5 | 29.71              | 1.5 | 25  | 78  | 7     | 6.05 |
| 3               | 6.5 | 29.9               | 1.5 | 30  | 77  | 9     | 6.29 |
| Second sampling |     |                    |     |     |     |       |      |
| Reading         | pH  | Turbidity<br>(NTU) | DO  | TSS | COD | BOD   | HS   |
| 1               | 5.3 | 34.5               | 1.3 | 25  | 48  | 5.00  | 6.83 |
| 2               | 5.2 | 33.1               | 1.3 | 25  | 47  | 3     | 6.38 |
| 3               | 5.3 | 33.4               | 1.2 | 20  | 46  | 14    | 6.55 |
| Third sampling  |     |                    |     |     |     |       |      |
| Reading         | pH  | Turbidity<br>(NTU) | DO  | TSS | COD | BOD   | HS   |
| 1               | 5.3 | 33.17              | 1.4 | 30  | 32  | 10.00 | 6.24 |
| 2               | 5.2 | 33.1               | 1.3 | 25  | 35  | 9     | 6.23 |
| 3               | 5.2 | 33.33              | 1.4 | 30  | 37  | 12    | 6.24 |

\*All values using mg/L except pH and turbidity

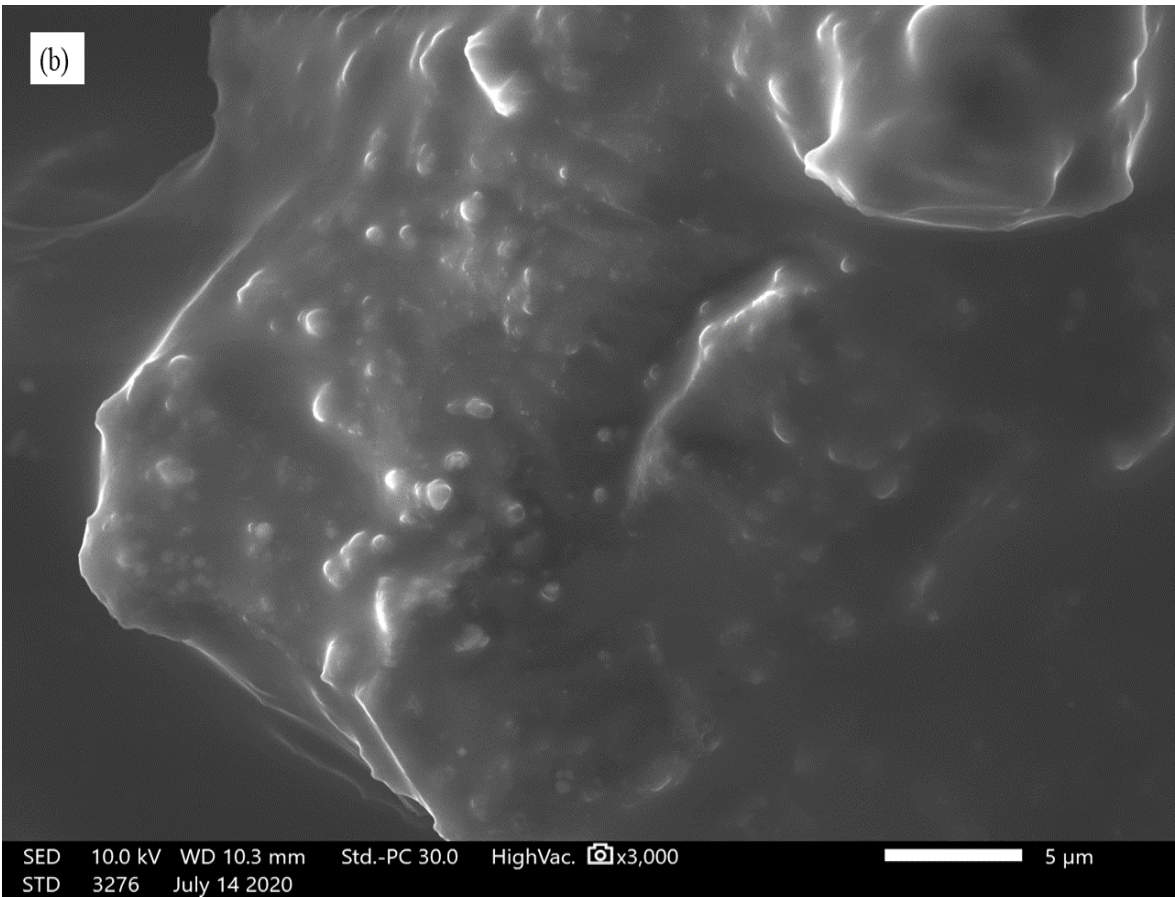
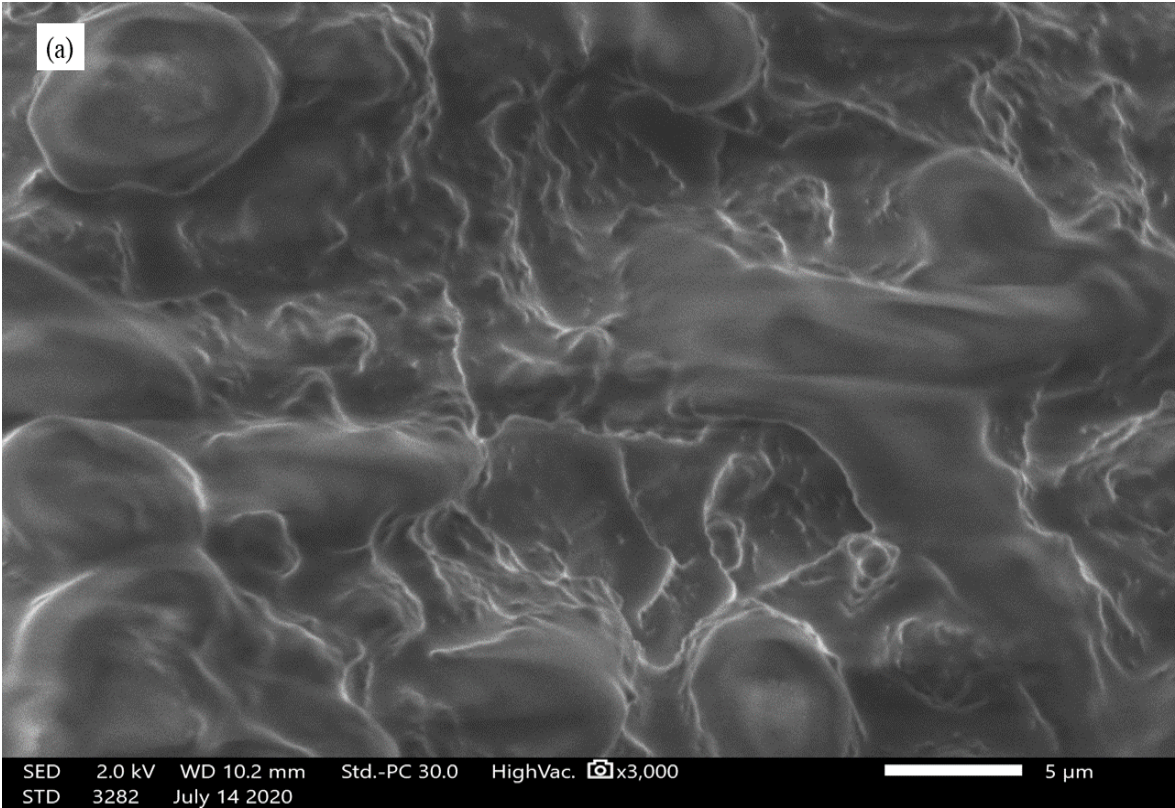
## Appendix B: Stack spectrum of FTIR analysis of *M. oleifera* seeds powder



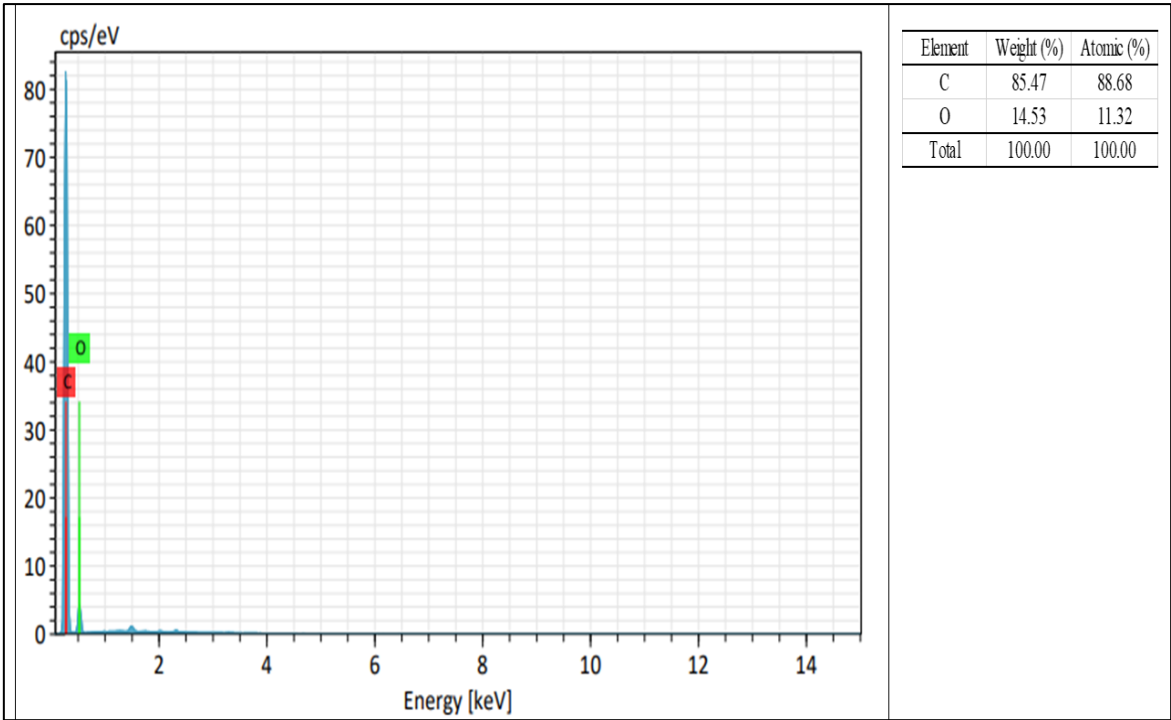
\* Red line = *M. oleifera* before being used for treatment

\* Blue line = *M. oleifera* after being used for treatment

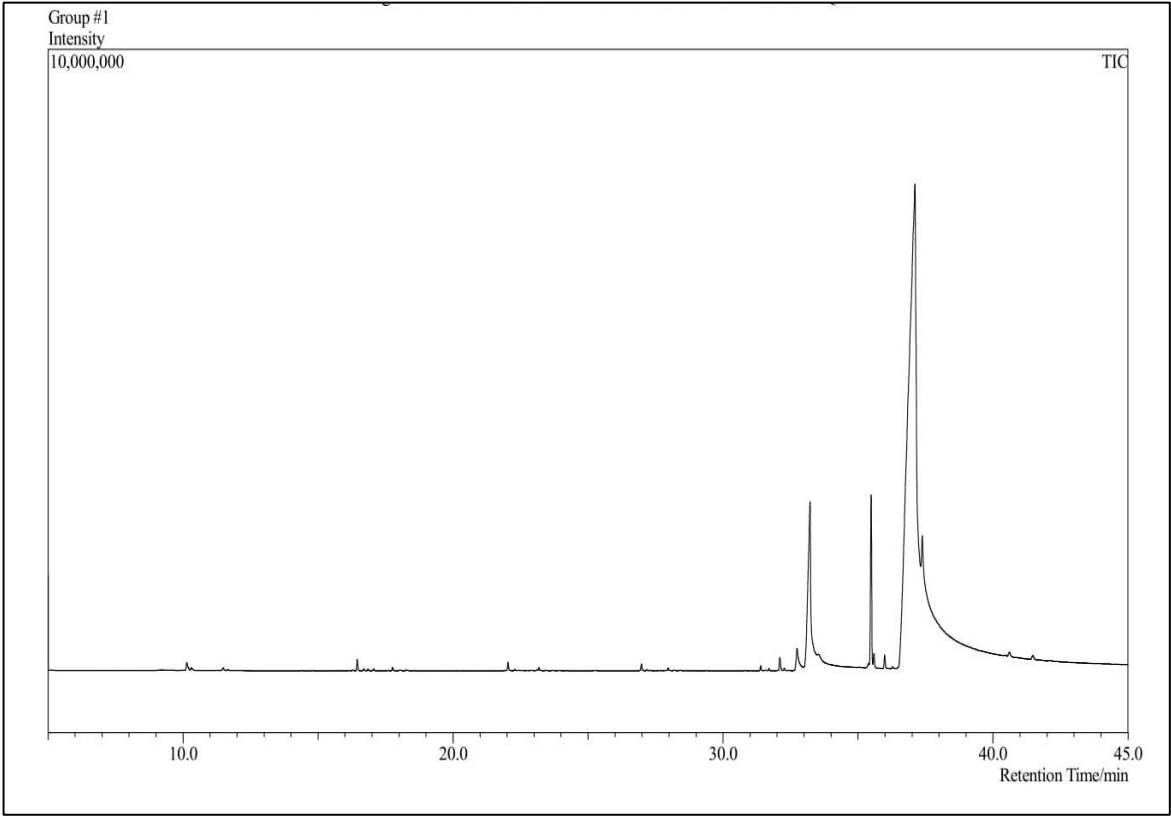
**Appendix C:** SEM analysis of *M. oleifera* seeds powder (a) before, and (b) after peat water treatment (3000× magnification)



**Appendix D:** EDX analysis with only major elements of *M. oleifera*, carbon and oxygen



**Appendix E:** GC-MS Chromatogram of *M. oleifera* seeds extracted with methanol

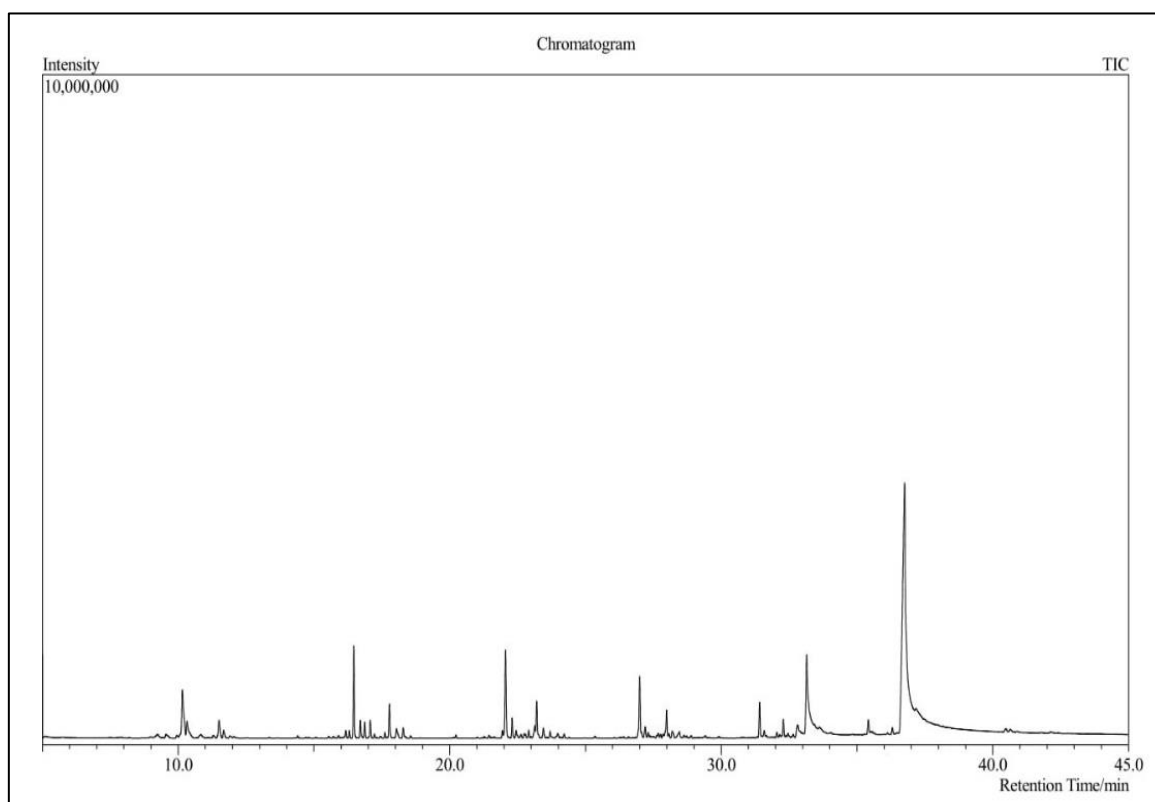


**Appendix F:** GC-MS analysis of *M. oleifera* seeds extracted with hexane

| Peak number | Retention time (min) | Area % | Name                        | Molecular formula               | Molecular weight (g/mol) |
|-------------|----------------------|--------|-----------------------------|---------------------------------|--------------------------|
| 1           | 10.155               | 4.86   | Undecane, 5,7-dimethyl-     | C <sub>13</sub> H <sub>28</sub> | 184.36                   |
| 2           | 10.321               | 1.04   | Dodecane, 2,6,10-trimethyl- | C <sub>15</sub> H <sub>32</sub> | 212.41                   |
| 3           | 11.500               | 1.40   | Nonane, 4,5-dimethyl-       | C <sub>11</sub> H <sub>24</sub> | 156.31                   |
| 4           | 16.171               | 0.35   | Undecane, 2,4-dimethyl-     | C <sub>13</sub> H <sub>28</sub> | 184.36                   |
| 5           | 16.305               | 0.42   | Hexane, 3,3-dimethyl-       | C <sub>8</sub> H <sub>18</sub>  | 114.23                   |
| 6           | 16.469               | 5.51   | Dodecane, 4,6-dimethyl-     | C <sub>14</sub> H <sub>30</sub> | 198.39                   |
| 7           | 16.711               | 1.04   | Nonane, 5-(2-methylpropyl)- | C <sub>13</sub> H <sub>28</sub> | 184.36                   |
| 8           | 16.872               | 0.99   | Dodecane, 4,6-dimethyl-     | C <sub>14</sub> H <sub>30</sub> | 198.39                   |
| 9           | 17.076               | 0.96   | Dodecane, 2,6,10-trimethyl- | C <sub>15</sub> H <sub>32</sub> | 212.41                   |
| 10          | 17.613               | 2.46   | Dodecane, 2,6,11-trimethyl- | C <sub>15</sub> H <sub>32</sub> | 212.41                   |
| 12          | 18.286               | 0.60   | Undecane, 4,8-dimethyl-     | C <sub>13</sub> H <sub>28</sub> | 184.36                   |
| 13          | 21.940               | 0.39   | Nonane, 5-methyl-5-propyl-  | C <sub>13</sub> H <sub>28</sub> | 184.36                   |
| 14          | 22.054               | 5.83   | Eicosane                    | C <sub>20</sub> H <sub>42</sub> | 282.55                   |
| 15          | 22.300               | 1.07   | Heptadecane                 | C <sub>17</sub> H <sub>36</sub> | 240.5                    |
| 16          | 22.445               | 0.65   | Dodecane, 4,6-dimethyl-     | C <sub>14</sub> H <sub>30</sub> | 198.39                   |

|        |        |       |                             |                                   |        |
|--------|--------|-------|-----------------------------|-----------------------------------|--------|
| 18     | 23.134 | 2.37  | Eicosane                    | C <sub>20</sub> H <sub>42</sub>   | 282.55 |
| 20     | 23.450 | 0.55  | Dodecane, 2,6,11-trimethyl- | C <sub>15</sub> H <sub>32</sub>   | 212.41 |
| 21     | 23.700 | 0.34  | 2,6,10-Trimethyltridecane   | C <sub>16</sub> H <sub>34</sub>   | 226.44 |
| 22     | 26.997 | 8.67  | Eicosane                    | C <sub>20</sub> H <sub>42</sub>   | 282.55 |
| 27     | 33.148 | 9.95  | n-Hexadecanoic acid         | C <sub>16</sub> H <sub>32</sub> O | 256.42 |
| 28     | 35.418 | 0.85  | Tetrapentacontane           | C <sub>54</sub> H <sub>110</sub>  | 759.4  |
| 29     | 36.303 | 0.38  | Eicosane                    | C <sub>20</sub> H <sub>42</sub>   | 282.55 |
| 30     | 36.753 | 49.35 | 9-Octadecenoic acid, (E)-   | C <sub>18</sub> H <sub>34</sub> O | 282/46 |
| 100.00 |        |       |                             |                                   |        |

**Appendix G:** GC-MS Chromatogram of *M. oleifera* seeds extracted with hexane



**Appendix H:** Contour plot for humic substance removal against (a) dosages vs pH, (b) dosages vs contact time and (c) pH vs contact time

