

MORINGA OLEIFERA SEED DERIVATIVES
AS NATURAL BIO-COAGULANTS IN WATER
TREATMENT PROCESS



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MORINGA OLEIFERA SEED DERIVATIVES AS NATURAL
BIO-COAGULANTS IN WATER TREATMENT PROCESS



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Thesis submitted in fulfillment of the requirements
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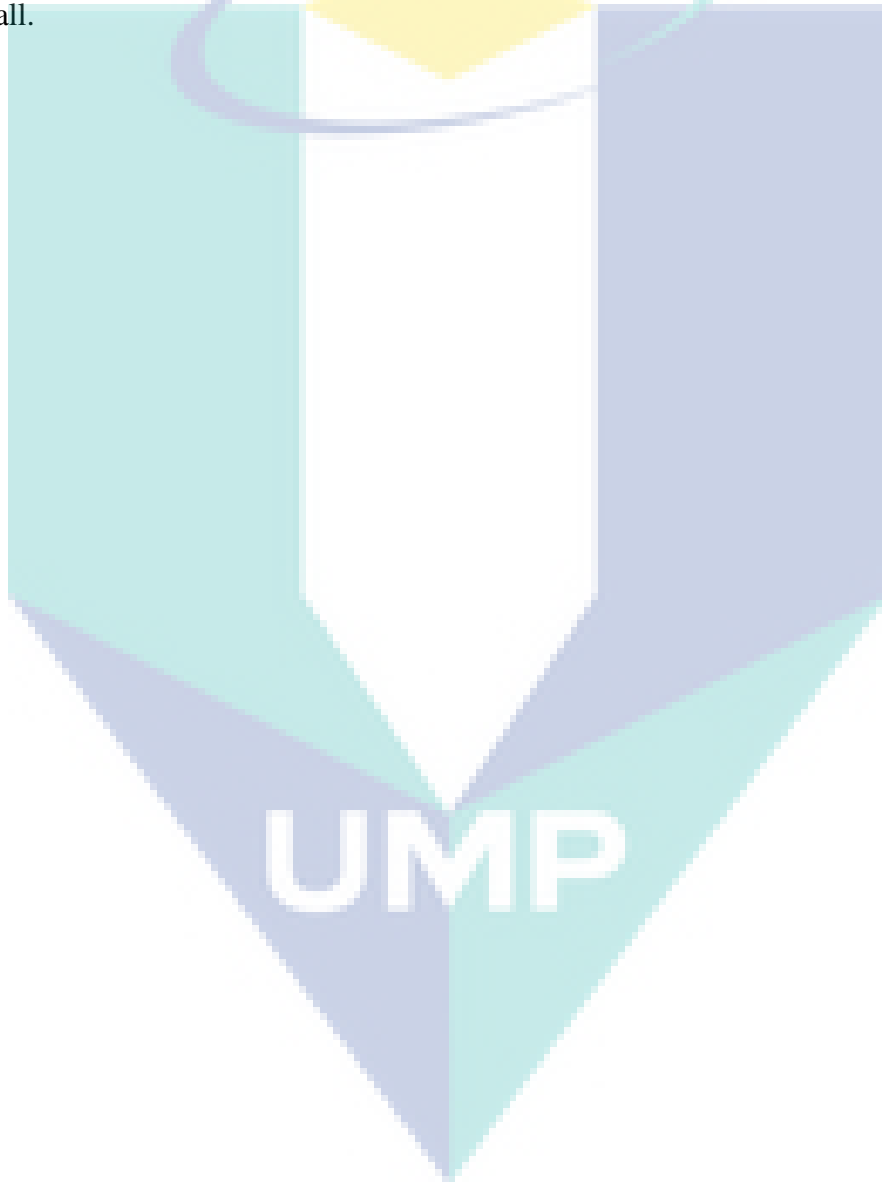
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ABSTRAK

Air sungai di banyak tempat dalam dunia ini digunakan untuk tujuan minuman selepas menjalani beberapa rawatan. Akibatnya, kekeruhan dalam air yang tidak dirawat secara konvensional dikeluarkan dengan merawat air melalui bahan kimia mahal, yang kebanyakannya tidak mesra alam. Malah, dengan menggunakan bahan-bahan kimia dalam rawatan air mempunyai kesan negatif kepada kesihatan manusia. Oleh itu, terdapat keperluan untuk menggunakan bahan semula jadi sebagai alternatif untuk menggantikan bahan kimia. Moringa oleifera adalah tumbuhan komoditi penting yang telah digunakan untuk rawatan air di bahagian tropika di dunia. Dalam kajian ini, potensi benih oleifera Moringa sebagai bio-koagulan untuk rawatan air telah disiasat pada keadaan optimum. Eksperimen ini dijalankan dalam dua peringkat, kaedah yang pertama telah digunakan untuk menentukan saiz zarah terbaik dan jumlah Moringa oleifera benih serbuk (*MOSP*) untuk rawatan air. Ia adalah diperhatikan bahawa saiz zarah 2 mm prestasi yang lebih baik dari segi kecekapan kekeruhan penyingkiran 4.98 NTU, dengan 0.1 g. Tambahan pula, kaedah *MOSP* kedua diletakkan dalam pengekstrakan bidal untuk mengeluarkan minyak dari benih dan dihasilkan *MOCR* digunakan sebagai koagulan biologi dalam rawatan air. Kaedah gerak balas permukaan telah digunakan untuk menentukan kesan parametrik mencampurkan kelajuan, masa dan dos pada kekeruhan sisa. Ketiga-tiga faktor, seperti kelajuan yang rendah, masa kelajuan rendah dan dos disiasat menggunakan Metodologi Response Surface (*RSM*) untuk optimum penyingkiran kekeruhan air. Keputusan yang diperolehi daripada reka bentuk eksperimen menunjukkan keadaan optimum pada kelajuan yang rendah, masa kelajuan rendah dan dos 40 rpm, 60 min, dan 0.75 mg / L, masing-masing. Dalam siasatan ini meramalkan (teori) sisa kekeruhan adalah 4.73 NTU. Hasil daripada ANOVA untuk pengoptimuman kekeruhan sisa menunjukkan bahawa model kuadratik adalah signifikan pada tahap keyakinan 95% ($p < 0.05$). Perbezaan antara kekeruhan teori dan eksperimen menunjukkan bahawa tiada perbezaan signifikan dengan ralat peratusan 1.6913%. Kualiti air yang dirawat telah ditentukan pada keadaan optimum menggunakan ujian kualitatif. Hasil penentuan kualiti air menunjukkan bahawa kekeruhan, kekonduksian, TDS, COD dan BOD dengan masing-masing menunjukkan peratusan sebanyak 99%, 73%, 50%, 75% dan 28%. Akhirnya, keputusan yang diperolehi menunjukkan potensi Moringa oleifera sebagai koagulan semula jadi dalam rawatan berkesan air untuk tujuan minum. Kekeruhan yang lebih rendah (<5 NTU) yang dicapai daripada kajian ini mengesahkan penggunaan produk semulajadi yang mesra alam yang penting ini untuk rawatan air.

ABSTRACT

River water in many parts of the world used for drinking purpose after some treatments. Consequently, the turbidity in untreated water is often conventionally removed by treating the water with expensive chemicals, many of which are not environmentally friendly. In fact, using chemicals materials in water treatments have some negative effects to human health. Therefore, there is a need to use some natural materials as an alternative to replace chemicals. *Moringa oleifera* is an important commodity plants which has been used for the treatment of water in tropical part of the world. In this study, the potential of *Moringa oleifera* seeds as bio-coagulant for water treatment were investigated at optimum condition. The experiment was conducted in two stages, the first method of was employed to determine which of the best particle sizes and amount of *Moringa oleifera* seeds powder (*MOSP*) for water treatment. It was observed that the 2 mm particle size performed better in terms of turbidity removal efficiency 4.98NTU, with 0.1 g. Furthermore, the second method *MOSP* was placed in extraction thimble to remove the oil from the seeds and produced *MOCR* used as bio- coagulant in water treatment. Response surface methodology was applied to determine the parametric effects of mixing speed, time and dosage on the residual turbidity. The three independent factors, such as Low speed, Low speed time and dosage were optimized using the Response Surface Methodology (RSM) for optimal water turbidity removal. The results obtained from experimental design demonstrated the optimum condition at low speed, low speed time and dosage as 40 rpm, 60 min, and 0.75 mg/L, respectively. Under this condition the predicted (theoretical) residual turbidity was 4.73 NTU. The result of the ANOVA for the optimization of the residual turbidity showed that the quadratic model was significant at 95% confidence level ($p < 0.05$). The difference between the theoretical and experimental turbidity showed that no significant variation with a percentage error value of 1.6913 %. The quality of the treated water was determined at optimum condition using qualitative tests. The result of the water quality determination revealed that turbidity, conductivity, TDS, COD and BOD with corresponding percentage changes of 99 %, 73 %, 50 %, 75 % and 28 %, respectively. Finally, the result obtained therefore showed the potential of *Moringa oleifera* as natural coagulants in the effective treatment of water for drinking purpose. The lower turbidity (<5 NTU) achieved from this study confirmed the potential of this important eco-friendly natural product for the treatment of water.

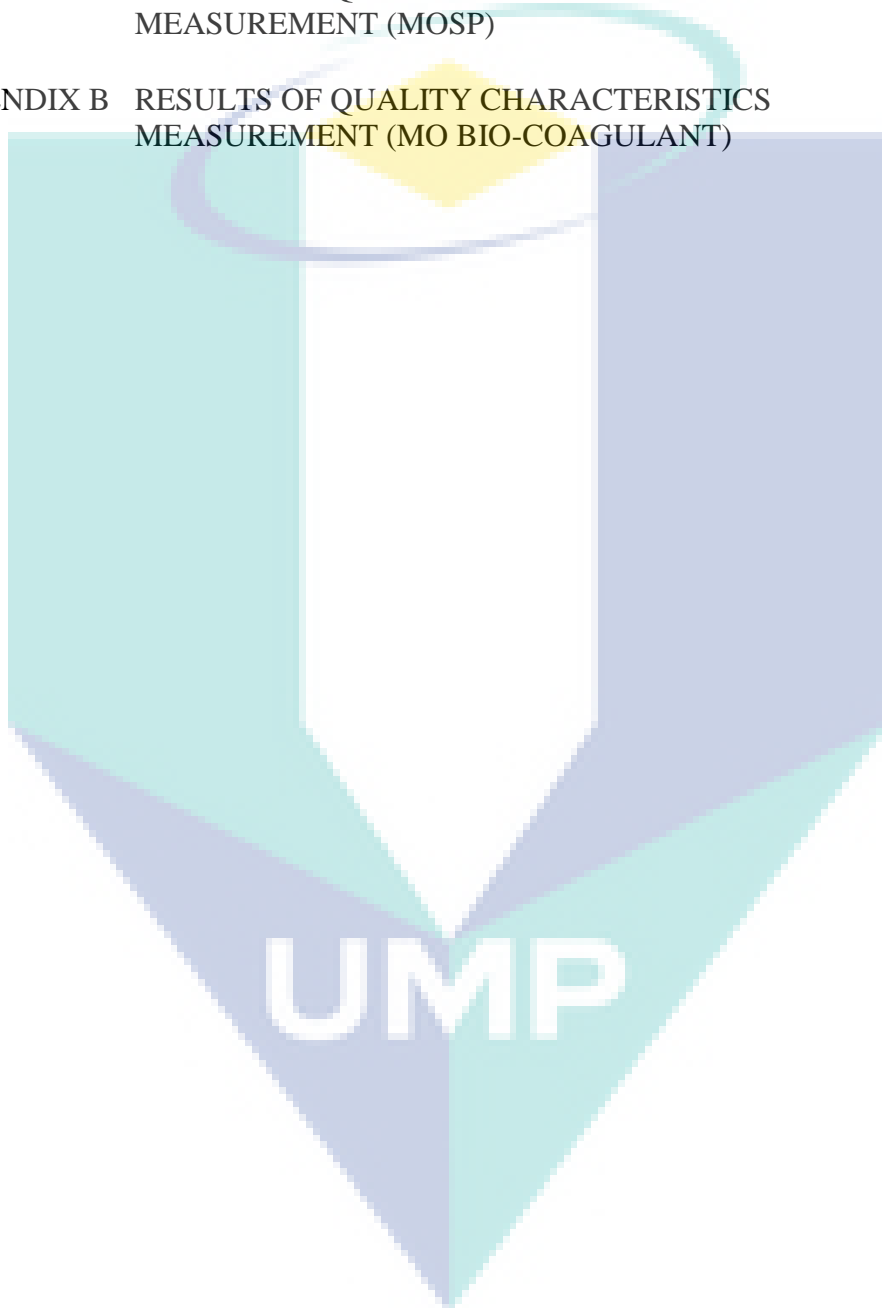
TABLE OF CONTENT

DECLARATION	
TITLE PAGE	
ACKNOWLEDGEMENTS	ii
ABSTRAK	iii
ABSTRACT	iv
TABLE OF CONTENT	v
LIST OF TABLES	ix
LIST OF FIGURES	x
CHAPTER 1 INTRODUCTION	1
1.1 Introduction	1
1.2 Problem Statement	2
1.3 Research Objectives	3
1.4 Research Scopes	4
1.5 Significance of Research	4
CHAPTER 2 LITERATURE REVIEW	6
2.1 Introduction	6
2.2 Water Treatment	7
2.3 Coagulation – flocculation in water and wastewater treatment	9
2.3.1 Mechanism of Coagulation Process	9
2.3.2 Types of Coagulants	10
2.4 <i>Moringa oleifera</i>	16
2.4.1 Application of <i>Moringa Oleifera</i> Seeds in Water Treatment	18
2.4.2 <i>Moringa Oleifera</i> Seeds Oil Extraction	22

2.4.3	Active Component Extraction from (<i>MOCR</i>).	23
2.5	Optimization of coagulation using RSM	23
2.6	Water Quality	24
2.6.1	Turbidity	24
2.6.2	Biological Oxygen Demand (BOD)	25
2.6.3	Chemical Oxygen Demand (COD)	26
2.6.4	pH	26
2.6.5	Conductivity and Total Dissolved Solids (TDS)	27
2.6.6	Overall Water Quality Performance	28
CHAPTER 3 MATERIALS AND METHODOLOGY		30
3.1	Introduction	30
3.2	Sample Preparation	32
3.2.1	<i>Moringa oleifera</i> Seeds Powder (MOSP)	32
3.2.2	Preparation of <i>Moringa oleifera</i> Cake Residue (MOCR) through extraction process	34
3.2.3	Salt extraction	35
3.3	Water sample Collection	36
3.4	Design of Experiment to optimize the study	36
3.5	Jar Test	37
3.6	Water Quality Analysis	37
3.6.1	Turbidity Measurement	37
3.6.2	Chemical Oxygen Demand (COD) Measurement	38
3.6.3	Biochemical Oxygen Demand (BOD) Measurement	40
3.6.4	pH, Conductivity and Total Dissolved Solid Measurement	40

CHAPTER 4 RESULTS AND DISCUSSION	42
4.1 Introduction	42
4.2 Characterizations of Belat River water	42
4.3 Determination <i>MOSP</i> for Water Treatment	43
4.3.1 Turbidity	43
4.3.2 pH	44
4.3.3 Conductivity	45
4.3.4 TDS Measurement	46
4.3.5 COD Measurement	47
4.3.6 BOD Measurement	48
4.4 <i>Moringa oleifera</i> Cake Recovery (MOCR)	49
4.5 Optimization Study of Operating Parameters in Water Treatment Using (RSM).	50
4.6 Model Fitting and Analysis of Variance	51
4.6.1 Validation of Predictive Model	53
4.6.2 The Effects of Variables on the Optimal Settings	55
4.7 Quality Analysis of Treated Water	56
4.7.1 pH Measurement	56
4.7.2 Total Dissolved Solid (TDS)	57
4.7.3 Conductivity	58
4.7.4 COD Measurement	59
4.7.5 BOD Measurement	60
4.7.6 Summary of Water Quality Analysis	62
CHAPTER 5 CONCLUSION AND RECOMMENDATION	63
5.1 Overall Conclusions	63
5.2 Recommendation	64

REFERENCES	65
APPENDICES	75
APPENDIX A RESULTS OF QUALITY CHARACTERISTICS MEASUREMENT (MOSP)	75
APPENDIX B RESULTS OF QUALITY CHARACTERISTICS MEASUREMENT (MO BIO-COAGULANT)	78



LIST OF TABLES

Table 2.1	Advantages and disadvantages of some methods for water treatment	8
Table 2.2	Uses of various part from <i>Moringa oleifera</i>	17
Table 2.3	Summary of previous research on <i>Moringa oleifera</i> and their significant information and results.	19
Table 2.4	Percentages of Oil Extracted with Three Different Solvents	22
Table 2.5	Ministry of Health Malaysia (2014)	28
Table 2.6	National Water Quality Standards for Malaysia (WEPA, 2017)	28
Table 2.7	National Water Quality Classes for Malaysia (WEPA, 2017)	29
Table 3.1	Coded and actual design range	37
Table 4.1	Summary of result for water quality characteristics	42
Table 4.2	Design range, experimental design and results obtained from optimization	51
Table 4.3	Analysis of variance for response surface quadratic model	52
Table 4.4	Theoretical optimal conditions	54
Table 4.5	Validation of experimental best condition	54
Table 4.6	Summary of result for water quality characteristics Using <i>Moringa oleifera</i> bio-coagulant	62

The logo of UMPA (Universiti Malaysia Perlis) is a large, downward-pointing arrow shape. It is composed of several overlapping geometric shapes in shades of teal, light blue, and white. The letters 'UMP' are prominently displayed in white, bold, sans-serif font across the center of the arrow's shaft.

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LIST OF FIGURES

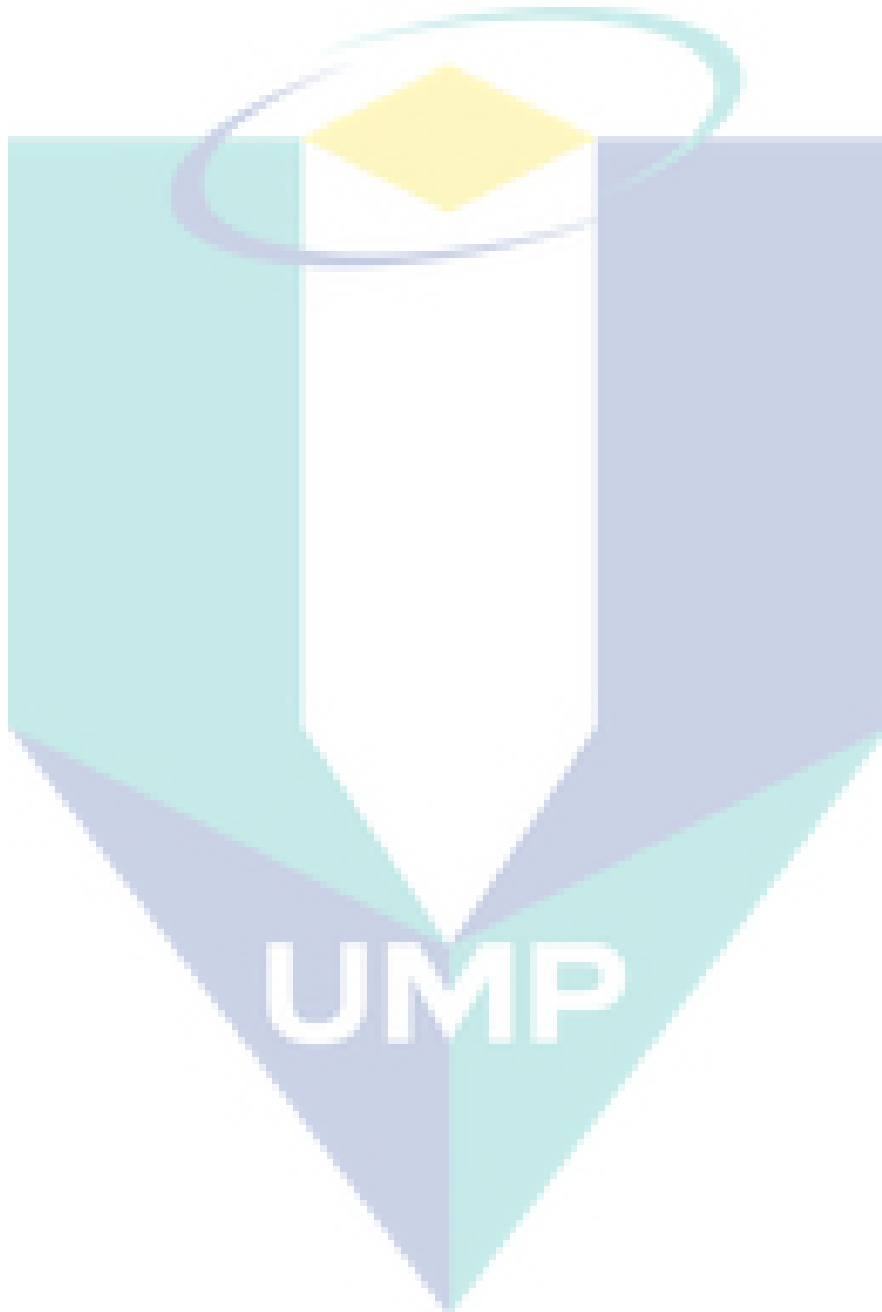
Figure 2-1	Drinking water treatment plant	7
Figure 2-2	<i>Parkinsonia aculeate</i>	12
Figure 2-3	<i>Cicer arietinum</i> and <i>Dolichos lablab</i>	13
Figure 2-4	<i>Cassia alata</i> Leaves	14
Figure 2-5	Peanut seeds	15
Figure 2-6	<i>Jatropha curcas</i> seeds	16
Figure 2-7	<i>Moringa oleifera</i> Pods and Seeds	17
Figure 2-8	pH Value Range	27
Figure 3-1	Process Flow Chart of Experiment	31
Figure 3-2	Undehusk MO seeds and Dehusk MO seeds	32
Figure 3-3	Domestic grinder to grinding MO	33
Figure 3-4	Electronic sieve shaker	33
Figure 3-5	MOSP after sieved and packed into clean, polythene bags and sealed	33
Figure 3-6	Soxhlet Extractor	34
Figure 3-7	Salt extraction	35
Figure 3-8	River water sample collection	36
Figure 3-9	Paddle Jar Test Apparatus	37
Figure 3-10	Digital turbidity meter (TB-500G) and (DTC-4DG)	38
Figure 3-11	COD rack and COD reactor HACH DRB200	39
Figure 3-12	HACH spectrophotometer DR2800	39
Figure 3-13	Manual Calibration for pH before measurement	41
Figure 3-14	pH measurement for the mixture	41
Figure 4-1	Turbidity removal of water treated with <i>MOSP</i>	43
Figure 4-2	pH values for water treated with <i>MOSP</i>	45
Figure 4-3	Water conductivity treated with <i>MOSP</i>	46
Figure 4-4	TDS of water treated with <i>MOSP</i>	47
Figure 4-5	COD of water treated with <i>MOSP</i>	48
Figure 4-6	BOD of water treated with <i>MOSP</i>	49
Figure 4-7	The interaction plot (a) low speed and low speed mixing time (b) Dosage and low speed (c) Dosage and low speed time	53
Figure 4-8	pH Values after Treatment	57
Figure 4-9	TDS Values after Treatment	58
Figure 4-10	Conductivity Values after Treatment	59

Figure 4-11 COD Values after Treatment

60

Figure 4-12 BOD Values after Treatment

61



CHAPTER 1

INTRODUCTION

1.1 Introduction

Water resources such as rivers, lakes and groundwater might contain a lot of pollution and unwanted molecules which make it unfit for daily consumption. Water has to undergo multiple phases of treatment before it is safe to be consumed or to be used for daily activities. The purpose of water treatment is to purify water by eliminating pollutants such as nitrates pesticides, heavy metals and organic materials, and also to improve the taste of the water quality (Thatai et al., 2019). In many parts of the world river water, which can be highly turbid, is used for drinking purposes. This turbidity is conventionally removed by treating the water with expensive chemicals, many of which are imported at great expense. The diseases due to consumption of polluted water containing protozoa, viruses, bacteria and intestinal parasites is known as “Waterborne Diseases” that kill most of the people around the world (Fletcher et al., 2012). The most commonly known diseases are diarrheal disease, hepatitis A, cholera, botulism, typhoid, dysentery, cryptosporidiosis and polio.

Conventional method in water treatment technology since early 20th century is made up of coagulation, sedimentation and filtration. In coagulation process, the chemical coagulants used in this process are aluminium sulphate, ferric sulphate and ferric chloride. The coagulants’ positive charge will neutralise the negative charge of the suspended and dissolved particles in the water (Chen et al., 2018). During this process, the particles will bind together to form larger particles. The particles which become heavy and able to settle quickly at the bottom of the water supply are known as sedimentation. The next step of conventional water treatment known as filtration, this step consists of filters with different pore size and is often made up of gravel, charcoal and sand which plays an important role

in removing particulate matter from water by forcing the water to pass through the porous media (Cheremisinoff, 2001).

Natural coagulants have been used for centuries in traditional water treatment practices throughout certain areas of the developing world. Natural coagulants can be divided into plant, microorganism or animal-based. However, available sources of plant-based coagulants are much more widespread than animal-based coagulants, thus plant-based coagulants could be potential alternatives to chemical coagulants and have gradually gained in importance over the years (Choy et al., 2015). Examples of natural coagulant are *Parkinsonia*, *Moringa oleifera*, *Cicer arietinum*, *Cereus peruvianus*, *Jatropha curcas* and *Cassia*.

Nowadays, there is a need to find and use a natural plant materials coagulants for water treatment. Therefore, researchers have shown a lot of interest on *Moringa oleifera* (*MO*) (Hoa et al., 2018), (Nouhi et al., 2018). *MO* is a unique nutritional value which has excellent coagulation property (Maizuwo et al., 2017). Moreover, *MO* is a rapidly-growing tree which is usually found in the sub-Himalayan region of India, Pakistan, Bangladesh and Afghanistan. *MO* is a cosmopolitan tropical multipurpose tree which contains water soluble and positively charged proteins that can function effectively as a coagulant for water treatment (Shan et al., 2017). Therefore, in this research *MO* seeds was used as natural bio-coagulant for water treatment. Moreover, the advantages of *MO* are environmentally friendly, low cost, biodegradable sludge, does not produce harmful by-products, not corrosive and no effect on the water pH (Idris et al., 2016).

1.2 Problem Statement

The increasing population of Malaysia has led to a rise in the demand for drinking water. According to Department of Statistics Malaysia report, the Malaysia's population for the first quarter of 2019 is estimated at 32.66 million, a significant increase from 2013's estimate of 29,791,949 million (Peng et al., 2019). Safe drinking water is essential to the health and welfare of a community and water from all sources must have some form of purification before consumption.

Various conventional water treatment methods are used to make water safe for the consumer. In fact, using chemical water treatment methods are unhealthy and may causes some diseases. For instance, excess concentration of aluminium sulphate (alum) which is

a chemical coagulant in water treatment has been reported to cause Alzheimer's or other neuro-degenerative diseases (Paula et al., 2014). Drinking water supplies around the world have been identified to have thousands of chemicals considered potentially hazardous to human health at relatively high concentrations (Brusseau & Artiola, 2019).

In addition, cost of the chemical used in conventional method is too expensive for most of the developing country (Wei et al., 2016). The operational expenditure of chemical cost at RM 40/1000 m³ of treated water. In 2013, the estimation of chemical cost for conventional treatment was RM11, 595,049.2 (Chew et al., 2016). Hence, there is a need for a cheaper plants based material for the treatment of water for drinking purpose. An example of plant based material is *Moringa oleifera* (*MO*). The choice of *MO* is due to its potential grow well in tropical area of which Malaysia rightly has (Nouman et al., 2014). According to Idris et al. (2016), *MO* seeds provide a lot of advantages compared to conventional coagulants towards the water treatment. The advantages of *MO* are cost effective, eco-friendly, no pH alteration required, no necessity for alkalinity addition and reduction of sludge volumes.

However, previous researches worked was show a good performance of *MO* towards turbidity, but there are no studies were conducted to evaluate the optimum condition of using *MO* seeds and study the effective of the process parameters viz mixing speed and mixing time as well as dosage. In this study the using *Moringa oleifera* seeds as bio-coagulant will be investigate the optimum condition for water treatment which can be used in water treatment plant.

1.3 Research Objectives

The objectives of this study are stated below:

- i. To investigate the effects of particle size and amount of *Moringa oleifera* seeds powder (*MOSP*) on water treatment using qualitative tests.
- ii. To determine the effects of water treatment parameters such as speed, time and dosage on the water turbidity and determine the optimum conditions of the bio-coagulants using the Response Surface Methodology (RSM).
- iii. To determine the quality of the water treated at optimum condition using qualitative tests with different dosage of bio-coagulant.

1.4 Research Scopes

The following scopes of the work were divided into three to achieving the above three research objectives. Firstly, determine the best particle size and amount of *MOSP* among (2mm, 1mm, 0.5mm, 0.25mm) and (0.05, 0.10, 0.50, 0.75, 1.0, 2.5, 5.0, 7.5, 10.0 g) that produced the highest turbidity removal efficiency.

Secondly, produce bio-coagulant from *MOSP* using Soxhlet extraction method and investigate the effects of some operating parameters such as speed, mixing time and dosage on the turbidity of treated water using the response surface methodology (RSM).

Thirdly, evaluate the effects of the bio-coagulant produce on chemical oxygen demand (COD), biochemical oxygen demand (BOD), conductivity, total dissolved solids (TDS) and pH values at optimum condition. In addition, Belat River located at Gambang area was used as a case study of this research.

1.5 Significance of Research

Water is very important for all the living organism and essential for human to survive. However, these resources become very limited in its pure state because it has been contaminated with different industrial advancements made throughout the year. Recently, the consumptions of water have been increased due to the increase of population in the world wide. Meanwhile, large amount of wastewater containing contaminant such as suspended solid, pathogens, nutrients and heavy metals which may be harmful and dangerous are generated from the field of agriculture, domestic and industrial. Besides, pollution of water has received special attention as an environmental issue and cause shortage of water supply especially in the developing countries and third world countries which have inadequate financial resources.

However, most of the chemical water treatment has some disadvantages such as incomplete metal removal and toxic sludge disposal or treatment problem. Besides, country like Malaysia which having frequently land development and rainy season may cause increasing in the cost of water treatment due to unstable quality of water and high turbidity. To solve this problem, natural coagulants which are more economical and environmental friendly are being researched. Natural coagulants are biodegradable coagulants and can be locally grown. Therefore, this research proposed to produce natural

bio-coagulant from *Moringa oleifera* seeds for water treatment and investigate the optimum solution. *MO* seeds have been chosen as the best coagulant because of its advantages such as environmentally friendly, low cost and no harmful by-products produced. Water treatment with Alum is having some problems including the high cost and side effects for human health. Thus, in this work, water quality parameters are measured such as turbidity, pH, conductivity, BOD, COD, and TDS. In addition, in this work *MO* seeds powder was investigated to help community to increase water quality and studied the effects of operating condition parameters on water treatment using bio-coagulant to produce higher effected water quality with cost effect and environment friendly.



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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Clean drinking water has become scarce nowadays due to poor management of consumption and treatment. About 75% of the present world population lives in the developing countries of the world. The amount of resources available to living creatures are limited. Globally approximately only 1% of the water is fit for human consumption (Batool et al., 2015). Contaminated water and poor sanitation are linked to transmission of diseases such as cholera, diarrhoea, dysentery, hepatitis A, typhoid, and polio. World Health Organization reported that 829,000 people are estimated to die each year from diarrhoea as a result of unsafe drinking-water. Diarrhoea is the most widely known disease linked to contaminated food and water but there are other hazards. In 2017, over 220 million people required preventative treatment for schistosomiasis an acute and chronic disease caused by parasitic worms contracted through exposure to infested water. About 1.2 billion people still lack of safe drinking water and more than 6 million children die from diarrhoea in developing countries every year (Eman et al., 2014). However, it is untenable and unbelievable under all situations that waterborne diseases still kill on the average 25,000 people every day in developing countries while millions suffer the debilitating effects of these diseases (Al-Kalifawi, 2016).

Safe and pure drinking water is essential to the health and welfare of a community, and water from different sources must have some standard form of purification and treatment before consumption. Different water purification and treatment methods are used to make water safe and healthy to the consumer. The treatment method used depends on the character of the raw water. A major problem with treatment of surface water is due to large seasonal variation in turbidity (McConnachie et al., 1999). Water treatment

method in many developed countries is based on dosage of chemicals which is not a good approach. Commonly used method for water treatment for drinking purpose is coagulation-flocculation followed by sedimentation, filtration and disinfection to make it safe for drinking purpose (Ali et al., 2009).

2.2 Water Treatment

Conventional treatment consists of the following unit processes: coagulation, flocculation, sedimentation, and filtration, and is typically followed by disinfection at full-scale as shows in figure 2.1.

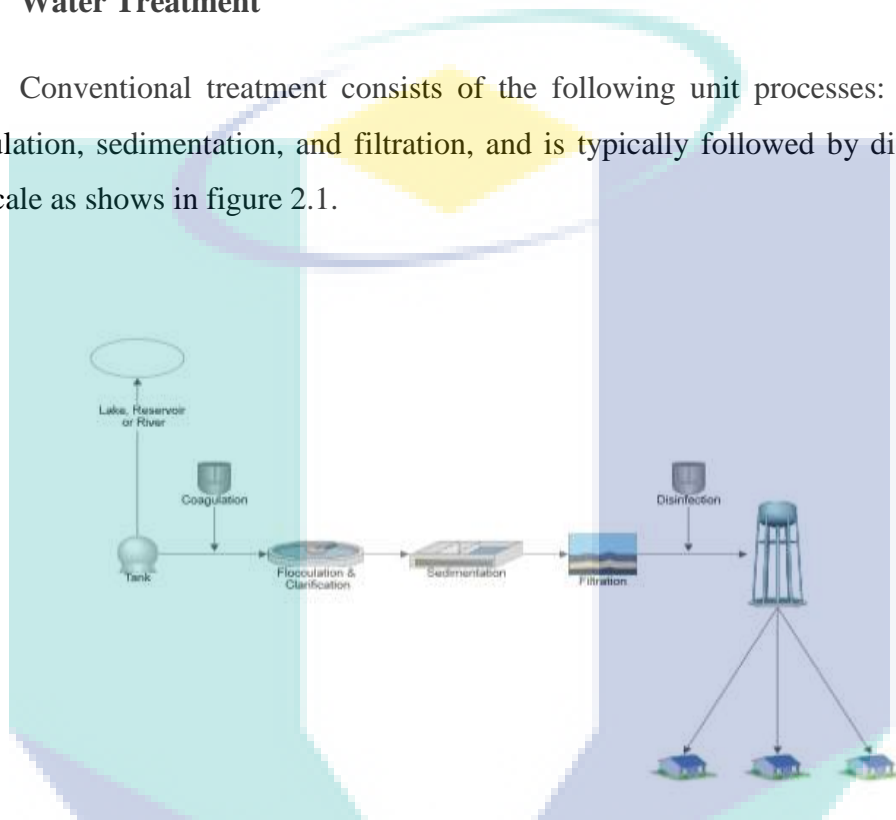


Figure 2-1 Drinking water treatment plant

In coagulation, a positively charged coagulant (usually an aluminum or iron salt) is added to raw water and mixed in the rapid mix chamber. The coagulant alters or destabilizes negatively charged particulate, dissolved, and colloidal contaminants. Coagulant aid polymers and acid may also be added to enhance the coagulation process. Then, sedimentation the heavy particles (floc) settle to the bottom and clear water move to filtration stage. At the filtration process the water passes though filters, some made of layers of sand, gravel and charcoal that help to remove smaller particles. After that, a small amount of chlorine or some other disinfection material add in order to kill any bacteria or microorganisms that may be present in water. In the last stage, the water placed in a closed tank or reservoir for disinfection to take place. The water then flows though pipes to homes and businesses in the community.

Chemical coagulants for water treatment are used in a wide-range. Aluminium sulphate is an example of the chemical coagulant, which is the most commonly, used coagulant in the developing countries (Farooq et al., 2010). However, aluminium sulphate is reported to cause some neurological diseases for instance pre-senile dementia or Alzheimer's disease (Baskan & Pala, 2010). The currently used techniques contain several constraints in the water and wastewater treatment (Monmaturapoj, 2017), such methods showed to not be effective and not economically possible for treatment of low concentrations. Therefore, new alternative methods need to be explored to find the best ecological and economical techniques to treat the water. A number of effective bio-coagulants from plant resources have been investigated which will be mentioned in this chapter. Table 2.1, illustrates the advantages and disadvantages of some methods for water and wastewater treatment.

Table 2.1 Advantages and disadvantages of some methods for water treatment

Method	Advantages	Disadvantages	References
Chemical Precipitation	Inexpensive Simple Most of the metals can be removed	Disposal problems High solid waste produced	(Monmaturapoj, 2017).
Ion-exchange	Metal selective High regeneration of Materials	Fewer numbers of metal ions removed High cost	(Srikanth et al., 2010)
Chemical Coagulation	De watering Sludge settling	Large consumption of chemicals High cost	(Demirbas & Kobya, 2017).
Membrane Process and ultra-filtration	High efficiency Less soil waste produced Less chemical consumption	High running cost Low flow rates	(Fu and Wang, 2011)
Natural zeolite	Relatively less costly materials Most of the metals can be removed	Low efficiency	(Fu and Wang, 2011)
Electrochemical methods	Pure metals can be achieved No consumption of chemicals	High running cost High capital cost	(Srikanth et al., 2010)

2.3 Coagulation – flocculation in water and wastewater treatment

The suspended material in waters and wastewaters mostly arise from land erosion, the dissolution of minerals, decay of vegetation and from several domestic and industrial waste discharges. Water or wastewater, may comprise suspended, dissolved organic and or inorganic matter, as well as several biological organisms, such as bacteria, algae or viruses (Tzoupanos & Zouboulis, 2008). This material has to be removed, as it causes deterioration of water quality. The particles are very small and difficult to see with the naked eye and cannot be easily removed. Due to the very small size, the only way for settling and the subsequent separation is to come closer, to make contacts and to form larger particles, which can be settle easier. This procedure however, is hindered due to the homonymous negative charge this material carries. The electrostatic repulsive forces constrain the particles from approaching each other and the suspension is characterized as stable; therefore, long time period is required for settling. In order to accelerate the settling time, destabilization is required, denoting the importance of coagulation.

2.3.1 Mechanism of Coagulation Process

In water treatment, coagulation and flocculation are treatments that aim to optimize the removal of suspended particles by decantation and filtration processes. Inorganic coagulants used for the treatment of potable water exhibit the following characteristics such as nontoxic at the working dosage, they have a high charge density and they are insoluble in the neutral pH range (Lachin et al., 2019). There are four mechanisms of coagulant employed to destabilize natural water suspensions and these include compression of the electric double layer, adsorption and charge neutralization, adsorption and inter-particle bridging, and enmeshment in a precipitate (Bodlund, 2013). These mechanisms are discussed as follows:

- i. **Compression of the Double Layer.** If the electric double layer is compressed, the repulsive force is reduced and particles will come together as a result of Brownian motion and remain attached due to van der Waals forces of attraction. Both the ionic strength and the charge of counter ions are important in the compression of the double layer.

- ii. **Adsorption and Charge Neutralization:** Hydrolyzed metal salts, pre-hydrolyzed metal salts, and cationic polymers have a positive charge. They destabilize particles through charge neutralization.
- iii. **Adsorption and Inter-Particle Bridging:** Schematically, polymer chains adsorb on particle surfaces at one or more sites along the polymer chain. The adsorption is a result of columbic, charge-charge interactions, dipole interaction, hydrogen bonding, and van der Waals forces of attraction (Green & Schaeffer, 2018). Other sites on the polymer chain extend into solution and adsorb on surfaces of other particles, thus creating a “bridge” between the particles. This bridge results in a larger particle that settles more quickly and forms a more dense sludge.
- iv. **Enmeshment in a Precipitate.** With doses exceeding saturation for the metal hydroxide, aluminium and iron salts form insoluble precipitates and particulate matter is entrapped in the precipitate. This type of destabilization has been described as sweep coagulation.

2.3.2 Types of Coagulants

A wide range of coagulants can be used for water and wastewater treatment. The most common coagulants used are ferric sulphate, aluminium sulphate, and ferric chloride which called synthetic coagulant (Abiola, 2019). On the other hand, there is a natural coagulant which is recommended to be used instead of using chemical coagulant, because the chemical coagulant have side effect to environment due to chemical involve in this coagulant. Recently, there is an increase on the research of plant to be used as natural coagulant for various traditional water purification system (Ju et al., 2016). However, there are also several other plants which can act as natural coagulant in water treatment such as *Parkinsonia aculeate*, *Cicer arietinum* and *Dolichos lablab*, *Cassia alata*, peanut seeds and *Jatropha curcas* (Saritha, 2017).

2.3.2.1 Chemical Coagulants

2.3.2.1.1 Aluminium Sulphate

Aluminium sulphate is a chemical compound with the formula $Al_2(SO_4)_3$. It is solvable in water and is principally utilized as a coagulating. Aluminum sulfate is utilized in water cleansing and as a mordant in dyeing and printing textiles, in water treatment, it

causes impurities to coagulate which are expelled as the particulate settles to the base of the container or more easily filtered (Ghaly, 2014). This process is called coagulation or flocculation. Aluminium sulphate is sometimes used to reduce the pH of garden soil, as it hydrolyses to form the aluminium hydroxide precipitate and a dilute sulfuric acid solution (Hicks et al., 2009). An example of what changing the pH level of soil can do to plants is visible when looking at the *Hydrangea macrophylla*. The gardener can add aluminium sulphate to the soil to reduce the pH level which in turn will result in the flowers of the *Hydrangea* turning a different colour. Aluminium sulphate may decrease the pH of stream, thus representing a potential threat to aquatic fauna and flora (Abdalla et al., 2018).

2.3.2.1.2 Ferric Sulphate

Ferric sulphate, also known as iron (III) sulphate, is an inorganic salt used in water purification for industrial wastes and also as oxidizing agent. Solutions are used in dyeing as a mordant, and as a coagulant for industrial wastes, it is also used in pigments, and in pickling baths for aluminium and steel. Generally, ferric sulphate is used in the industry in the water and wastewater treatment operations due its capacity as flocculants and coagulant and to eliminate odour from sulphur compounds also, ferric sulphate used as solids separation agent and oxidizing agent (Rubio et al., 2002). Moreover, this salt is used in pigment industry. In medicine, it can be used as astringent and styptic. Ferric sulphate may cause eyes, throat and nose irritation by inhalation and mouth and stomach by ingestion. It is not flammable, however, when heated, ferric sulphate emits toxic fumes of iron and sulphur oxides (Menezes et al., 2017).

2.3.2.1.3 Ferric Chloride

Ferric chloride is known as a flocculants. Flocculants are chemicals that help make tiny particles clump together so they can be removed from water. Even after large pieces of debris are removed using filters, natural surface water still may be full of tiny particles that are too small to sink. These simply float through the water, giving water a cloudy appearance. Adding ferric chloride to a tank of brown, cloudy water causes tiny pieces of stuff to come together. Eventually, the clumps grow large enough to sink down to the bottom of the tank, clearing the water above 60 percent of all ferric chloride is used for wastewater treatment (Bratby, 2016). Wastewater is the water that flows down our

drains after we use it for washing, bathing and flushing. Before it is released to rivers, lakes and oceans, wastewater takes a detour of its own--to the wastewater treatment plant. There, ferric chloride is used to clear the water of particles. And chlorine and other disinfectants destroy harmful germs.

2.3.2.2 Natural Coagulants

2.3.2.2.1 Parkinsonia aculeata

Parkinsonia aculeata is a species of perennial flowering tree in the pea family. It is a short shrubby tree which can reach a height of 10m. The tree has yellow flowers that have been pollinated by the bees' will produce fruits containing one or more seeds.



Figure 2-2 *Parkinsonia aculeate*

This tree can grow in many different types of soils namely clay soils, sand dunes, strongly alkaline, chalky and mildly salty soils. Mainly, the tree produced two types of seeds, 25% of the seeds are soft and are able to germinate without pre-treatment while the hard-shell seeds requires a process in order to germinate. According to research, the crude extracted from *P. aculeata* seeds are very effective polyelectrolyte coagulants for treating the natural and synthetic turbid water (Saritha, 2017). The extracted coagulant protein are cationic proteins which can be purified by using a simple technique to scale up the quality of the coagulant in treating the water for drinking purpose especially in poor communities.

2.3.2.2.2 *Cicer arietinum* and *Dolichos lablab*

Cicer arietinum and *Dolichos lablab* are also among the coagulant used in water treatment. The amount of *Cicer arietinum* powder used for water treatment is always maintained approximately less than 600µm in order to have the same quantity of solubilisation of active ingredients in the seed.

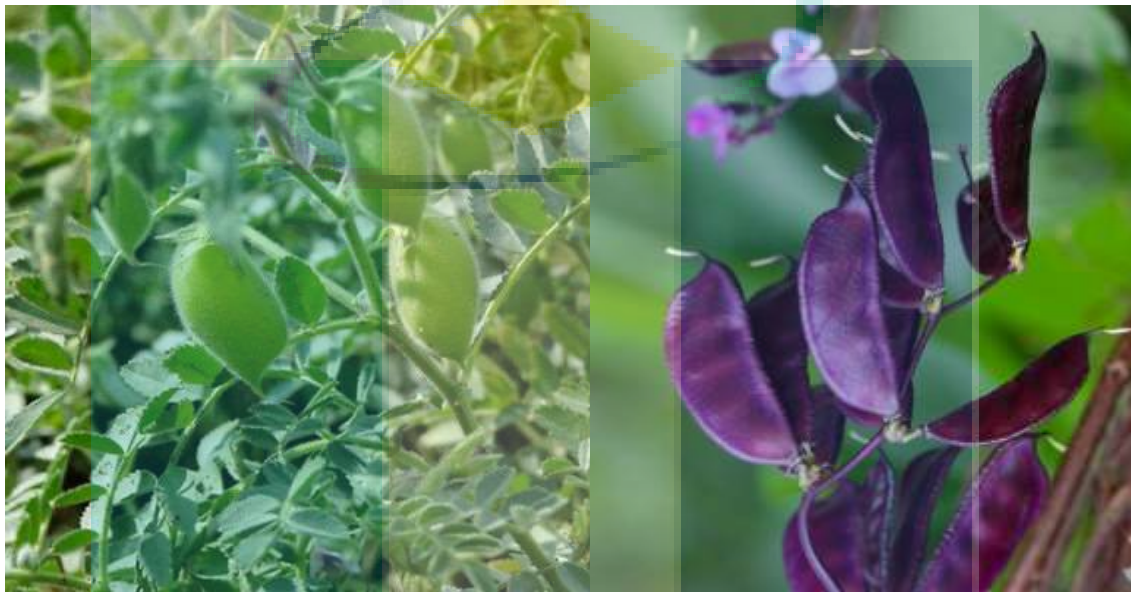


Figure 2-3 *Cicer arietinum* and *Dolichos lablab*

Saritha in 2017 reported that the most effective coagulant dose for *Cicer arietinum* to be used for higher, medium and lower turbidity range is 100 mg/L at 12 min slow mixing time and 30 min settling time according to Bangladesh drinking standard. While, *Dolichos lablab* is able to act as a coagulant in reducing water turbidity and the most effective dose for coagulation is 100 mg/mL with the results for turbidity of 20 (low), 40 (medium) and 80 (high) NTUs considerably decreases after treatment. The main purpose of this coagulants to be used in Bangladesh because it is easily cultivable, cheap and its availability (Saritha, 2017).

2.3.2.2.3 *Cassia alata* Leaves

Cassia alata is a wild legume species which is an important medicinal tree, as well as an ornamental flowering plant in the subfamily Caesalpinioideae.



Figure 2-4 *Cassia alata* Leaves

Cassia leaves can be used as a coagulant in water treatment. Rak et al, (2012) reported that the turbidity and other physico-chemicals of surface water were treated by cassia leaves which carried out with coagulant dosage between (0.5 - 3 mg/L) with the intervals of 0.5 mg/L. The results have shown that *Cassia alata* leaves can remove turbidity up to 93.33% at the optimal dosage of 1.0 mL/L. In addition, the potential of *Cassia alata* leaves to remove other pollutants presence in the river water like suspended solids, ferum, manganese and pH was also identified. On the other hand, the leaves of *Cassia alata* can remove suspended solids by 56.4%.

2.3.2.2.4 *Peanut* Seeds

Peanut, tis a legume crop grown mainly for its edible seeds. It is widely grown in the tropics and subtropics (Birima, 2013).



Figure 2-5 *Peanut seeds*

Peanut seeds has a potential an environmental friendly and natural coagulant for the treatment of high turbid water. The *peanut seeds* have been used to treat synthetic water of 200 NTU turbidity. The results of *peanut* extracted with NaCl was obtained 92% turbidity removing using 20 mg/l. While *peanut seeds* extracted with distilled water could remove only 31.5% of the same turbidity with the same dosage. The coagulant dosage did not affected by the concentration of the salt solution, however, residual turbidity was decreased with increasing the concentration of the salt; and the relationship was found to be a second order polynomial curve with R² of 0.9312. The other salts tested were also found to be good solvents to extract the active coagulation component with no much difference from NaCl solution in terms of efficiency (Birima, 2013).

2.3.2.2.4 *Jatropha curcas*

Jatropha curcas is belonging to the family Euphorbiaceae and is grown in subtropical regions such as Central America, Africa, the Indian subcontinent, and other countries in Asia. It is a drought-resistant perennial plant which grows well in poor soil. It is easy to establish, grows relatively fast, and lives for 50 years. *Jatropha curcas*, the wonder plant, produces seeds with an oil content of 37% (Verma et al., 2019).



Figure 2-6 *Jatropha curcas* seeds

The seed and press cake powder of *Jatropha curcas* were used as coagulant in wastewater treatment. According to research, the coagulant derived from its seeds are able to reduce turbidity of range between 100NTU to 8000NTU with up to 96% at *pH* between 1 to 3 and as well as between 11 to 12. The highest turbidity removal recorded is at *pH* 3 with dosage 120mg/L. Apart from that, the coagulant also able to reduce the time taken for sedimentation process and smaller amount of sludge volume produced. However, the coagulant does not have a significant effect on *pH* (Saritha, 2017).

2.4 *Moringa oleifera*

Moringa oleifera (*MO*) seeds contain low molecular weight water-soluble protein which have high amount of positive charge. Furthermore, the positively charged protein will bind with negatively charged particles in order to reduce the turbidity of raw water. Hence, the seed has been reported to have the capability of reducing low and high values of turbidity (Saritha, 2017). *MO* is tropical tree from Moringaceae family with multipurpose uses and grow mostly at dry and hot climate such as India (Bodlund, 2013). The seed, leave and pod of *MO* are always used in medical field because of its rich nutritional profile and antioxidants galore (Foidl et al., 2001). Some research also proved that the seed, bark and leave of *MO* can used in water treatment to reduce the turbidity and heavy metal (Alfarra et al., 2014). For example, its seeds have been used for few generation in Sudan and India for treatment of drinking water (Ravikumar & Sheeja, 2013). In addition, the leaves and fruits of the *MO* can be consumed or eaten as vegetables (Madzvamuse, 2015). The leaves of the *MO* (Figure 2.7) contain a lot of vitamins and minerals (Anjorin et al., 2010).



Figure 2-7 *Moringa oleifera* Pods and Seeds

Most of the part of *Moringa oleifera* can be used in medical treatment due to its high nutrient value. Besides, some of the part which contains active coagulant (polyelectrolyte) are commonly used as water purification (Yongabi, 2012). Table 2.2 shows some of the uses from each part of the *Moringa oleifera*.

Table 2.2 Uses of various part from *Moringa oleifera*

Part	Applications	References
Seed	Water treatment Fertilizer Treatment on Venomous Bite	Madzvamuse, 2015; Anwar et al., 2007
Leave	Animal Fodder Vegetables Water Treatment Antitumor	Madzvamuse, 2015; Bodlund, 2013; Anwar et al., 2007
Press cake	Water purification Fertilizer	Madzvamuse, 2015; Alfarra et al., 2014
Fruit	Vegetable	Madzvamuse, 2015
Oil (Extraction from seed)	Cooking Soap manufacture Lubricant and Cosmetics production	Madzvamuse, 2015; Yongabi, 2012.
Roots	Pickle with vinegar Antilithic Anti-inflammatory	Madzvamuse, 2015; Anwar et al., 2007
Bark	Water treatment Food seasoning	Madzvamuse, 2015
Wood	Heavy metal removal (act as active carbon)	Madzvamuse, 2015

Therefore, this research, would like to evaluate the performance of water treatment using natural coagulant of *Moringa oleifera* water quality including turbidity, pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Dissolved Oxygen (DO), Total Dissolved Solids (TDS) and conductivity (Saritha, 2017).

2.4.1 Application of *Moringa Oleifera* Seeds in Water Treatment

The use of natural coagulants for the clarification of water and wastewater has been recorded throughout human history since ancient times till today. Natural coagulant plays an important role in water treatment process. Coagulation and flocculation process are the two processes when natural coagulant is added for water treatment. *Moringa oleifera* is one type of natural coagulant can be used in water treatment. It is a tropical plant found in Asia, India, Africa and Latin America (Yongabi, 2012). *Moringa oleifera* is reported that its seeds are used to clean the turbidity of river water in rural communities in African countries. Table 2.3 lists the summary of previous research works related to the *Moringa oleifera* in water purification process and their significant information and results obtained.



UMP

Table 2.3 Summary of previous research on *Moringa oleifera* and their significant information and results.

Type of <i>Moringa Oleifera</i>	Significant Results from previous studies			Other miscellaneous results	Reference
	Dosage (mg/L)	Turbidity (NTU)	pH		
Seeds powder	0.3	From 465 to 100	From 5.5 to 7.2	BOD from 3400 to 692 mg/L COD from 8800 to 1248 mg/L TDS from 605 to 553 ppm TSS from 24663 to 1120 ppm	(Neethu et al., 2017)
Seeds powder	15	From 36 to 3.2	From 6 to 6.1	Temperature from 20.7°C to 24.97°C Conductivity from 92 to 119 µs/cm	(Tunggolou & Payus, 2017)
Seeds powder	80	From 49.92 to 5.44	From 6.96 to 7.38	Temperature from 28.4°C to 26.9°C 54% reduction of BOD Conductivity from 227 to 219 µs/cm removal of metal contents (Cd, Cr, Mn)	(Hendrawati et al., 2016)
Seeds powder	0.1	From 289.5 to 5	From 7.41 to 7.4	Reduction COD from 8000 to 4928 mg/L Enhance in BOD, COD, TSS and TDS	(Patil et al., 2015)

Table 2.3 Continued

Type of <i>Moringa Oleifera</i>	Significant Results from previous studies			Reference	
	Dosage (mg/L)	Turbidity (NTU)	pH		Other miscellaneous results
Seeds powder	90	From 339 to 4.10	From 8.1 to 7.9	Temperature from 26.6°C to 26.8°C Dissolved oxygen from 0.30 mg/L to 0.10 mg/L Hardness from 212 mg/L to 300 mg/L Conductivity from 1.75 to 1.78 µs/cm	(Yusuf et al., 2015)
Seeds powder	90	From 63 to 5	From 4.8 to 6.38	Reduction of TDS from 1000 to 160 ppm Conductivity from 3002 to 60 µs/cm	(Emmanuel et al., 2011)
cake residue	80	From 44 to 5	From 7.64 to 7.7	Enhance in BOD and COD. 82% decreased of chemical oxygen demand (COD). 99% removal of Escherichia coli Reduction of bacteria Improvement of dissolved oxygen (DO)	(Hoa et al., 2018)

Table 2.3 Continued

Type of <i>Moringa Oleifera</i>	Significant Results from previous studies			Reference	
	Dosage (mg/L)	Turbidity (NTU)	pH		Other miscellaneous results
Cake residue	3	From 67.2 to 5.07	From 8 to 8.1	BOD from 100.1 to 76.65 mg/L COD from 132 to 164 mg/L Conductivity from 347 to 390 $\mu\text{s}/\text{cm}$ TDS from 307 to 400 ppm	(Shan et al., 2017)
Cake residue extracted	3	From 44 to 5.1	From 6.69 to 6.61	BOD from 7.7 to 2.8 mg/L COD from 140 to 48 mg/L Conductivity 109.8 to 110.5 TDS from 67.5 to 67.1 ppm	(Thomas, 2016)
Cake residue extracted	3	From 148.3 to 4.19	From 7.15 to 7.1	BOD from 120 to 250 mg/L COD from 520 to 739 mg/L Conductivity 402 to 451 $\mu\text{s}/\text{cm}$ TDS from 412 to 411 ppm	(Eman et al., 2014)

From previous studies, it can easily say that *Moringa oleifera* seeds powder is good natural coagulant which can be choose for water treatment in Malaysia. *Moringa oleifera* tree is resistant to dryness and grows in arid and semiarid areas. Hence, it can be planted in Malaysia. In addition, *Moringa oleifera* trees grow easily from seeds or cuttings. Besides, the production of *Moringa oleifera* fruits is very high compared to other crops. It can reach up to 3 ton/hectare. From all the research works done before, it can be concluded that *Moringa oleifera* extract has no effect on pH and alkalinity after treatment. Furthermore, sludge produced with *Moringa oleifera* is reported to be four to five times less than that produced with alum. Lastly, the extract of *Moringa oleifera* seed removes 60% to 70% of hardness as well as 99% of low turbidity (Sasikala, 2016).

2.4.2 *Moringa Oleifera* Seeds Oil Extraction

Oil is the main component of the seed and represents 36 % of the seed weight. The oil can be extracted almost entirely by solvent extraction, generally n-hexane, whereas less yield is obtained by cold press extraction. In fact, only 69% (on average) of the total oil contained in seeds can be extracted by cold press (Ogunsina et al., 2014). In Table 2.4, the research is conducted with the same amount of MO seeds powder by using three different type of solvents namely methanol, ethanol and n-hexane. The experiments were carried out by using a soxhlet extractor which involves many cycle of oil extraction. After each extraction cycle was completed, the *Moringa oleifera* cake residue (*MOCR*) was dried in the 50°C oven for 1 hour. After the drying process, the *MOCR* was weighted by using an electronic weighing balance (Eman et al., 2014).

Table 2.4 Percentages of Oil Extracted with Three Different Solvents

Solvents	Amount of solvents (mL)	Weight of seeds (g)	Percentages of oil yield (%)			
			Bath 1	Batch 2	Batch 3	Average
Methanol	200	30	16.2	17.5	15.7	16.5
Ethanol	200	30	18.9	19.8	20.8	19.8
n-Hexane	200	30	33.2	34.5	35.1	34.3

Source: Siddhuraju & Becker (2003).

It can therefore be concluded that n-hexane is the best solvent for oil extraction as it has the highest percentages of oil extracted which is around 34.3% while methanol is the least recommended solvent to be used for oil extraction as methanol has the lowest amount of oil extracted which is around 16.5% (Onoji et al., 2016).

2.4.3 Active Component Extraction from (MOCR).

It is well known that solubility of proteins increase with salt concentration at low salt ionic strength (Brunet et al., 2015). Okuda et al., (1999) have used aqueous solutions of sodium chloride, sodium nitrate, potassium chloride and potassium nitrate, which were individually tested for extraction of the active component from *Moringa oleifera* seeds. There was no difference in the coagulation efficiency observed for extracts using any of four salts (NaCl, NaNO₃, KCL and KNO₃). This indicates that NaCl is not a unique salt to enhance solubility of the active component in *Moringa oleifera* seeds. Ionic strength, however, could be the main factor for the improvement of extraction efficiency rather than specific chemical characteristics of the salt ions. Purification and isolation of the active component confirmed that the active component of salt solution method was mainly proteins which come in agreement with results obtained by Okuda et al., (1999). The 1.0 M of NaCl solution acted as a better solvent than pure water in breaking protein-protein or protein-polysaccharide or other associations in the seed powder, which led to increase protein solubility in the salt solution (Eman et al., 2014).

2.5 Optimization of coagulation using RSM

The traditional experimental method, one factor at a time approach, can hardly be used to establish relationship among all the experimental input factors and output responses. Even though, the traditional approach can be useful in finding predominant factors in this situation, it is a time and energy consuming method. Furthermore, since the results are valid only under fixed experimental conditions, the prediction for other conditions is uncertain (Robson & McCartan, 2016). To solve this problem, design of experiment (DOE) offers better alternative to study the effect of variables and their responses with minimum number of experiments. Using design experiment based one response surface methodology (RSM), the aggregate mix proportions can be arrived with minimum number of experiments without the need for studying all possible combinations experiments.

Further the input levels of the different variables for a particular level of response can also be determined. The response surface methodology (RSM) is the collection of mathematical and statistical techniques in designing, formulating, developing, and analysing scientific studies (Bezerra et al., 2008). The main objective is to optimize response(s) that is being influenced by various independent factors. The experiments are done in runs whereby the independent factors are varied to observe the effect on the responses. Response surface methodology is mostly applied in Food, Industrial, Biological, Clinical, Physical, Social, and Engineering Sciences. Hill and Hunter reported that RSM was introduced by G.E.P. Box and K.B. Wilson in 1951 (Bezerra et al., 2008). They suggested the use of a first-degree polynomial model to approximate the response factor. For the second-order models, several researchers had worked on central composite designs (CCDs) and three-level designs by Box and Behnken (Bezerra et al., 2008). Central composite design involves $2N$ factorial points, $2N$ axial points and 1 central point. The importance of RSM is to reduce the cost of expensive analysis methods and associated numerical noise (Bezerra et al., 2008). However, a face-centered central composite design (FCCCD) is mostly used for generating the experimental design matrix since FCCCD is one of the efficient designs in composite central design (Seidi et al., 2011). RSM also quantifies relationships among one or more measured responses and the vital input factors (Design Expert 7.0 software® Version 7.1.6, Stat-Ease Inc., Minneapolis, USA)). Therefore, DOE could be used in water treatment with minimum numbers of experimental runs, and to determine the optimum conditions using RSM (Chattoraj et al., 2018).

2.6 Water Quality

In this research, *Moringa oleifera* seeds is the natural coagulant chosen to be used in treating the water quality. The parameters which will be used in determining the quality of treated water are Turbidity, BOD, COD, pH and TDS as well as conductivity.

2.6.1 Turbidity

Turbidity is caused by suspended solids in water which makes light scattered making the light appeared cloudy or murky. Therefore, turbidity is the degree of measurement to which the water losses its transparency because of the suspended particles presence. The suspended solids are made up of sediment such as clay and silt,

organic and inorganic matter, soluble coloured organic compounds, algae and also other microscopic organisms (Harisha et al., 2010). The suspended solids will absorb heat from sunlight which makes the turbid water warmer and indirectly reduces the oxygen concentration in water. The increase in turbidity will increase the tendency of light to be scattered (Choi et al., 2011). Sediment is a natural component in a stream but the present of excessive sediment is the main factor which contributed towards the formation of suspended solids in water sources. The sources of excessive sediment usually come from the erosion of unstable stream banks, construction sites, agricultural activities and as well as urban runoff. Sediment in water sources are usually present in the form of suspended load and bed load. Suspended load is the particle which suspended in the water column such as clay and colloid particles in suspension while bed load refers to the sediment pushed along to the bottom of the channel such as sand and gravel.

Therefore, turbidity with the value lower than 10 NTU is considered as clear water, 50 NTU is considered as cloudy and values between 100 to 500 NTU or greater are considered as very cloudy to muddy. High level of turbidity in water resources will increase the cost of treating water and have a great harmful impact on freshwater fish. Moreover, turbidity which has high amount of deposited fine sediment filling up the gravel spaces will reduce the spawning habitat for some fish species and invertebrate species which the food source of many fish (Kemp et al., 2011).

2.6.2 Biological Oxygen Demand (BOD)

BOD is a measure of total amount of oxygen consumed by bacterial while decomposing the organic substances under aerobic conditions. BOD measurement may be affected by the present of various contaminants such as organics, chlorine, heavy metal and bacteria. The present of organic matter in water will affect BOD because it will increase the demand for oxygen. Next, chlorine is used in water with the purpose to control microbial contamination; therefore; chlorine will affect the accuracy of BOD test because chlorine will interfere the growth of microorganism in water. The amount of heavy metals will also affect the results for BOD test because heavy metals containing compound which will disturb the growth of microorganisms. The bacteria present in water is the must have component in conducting BOD test but it is recommended to minimise the level of bacteria in water as bacteria will have the tendency to release organics compound (Hooda et al.,2010).

There are two stages of decomposition for BOD measurement which are carbonaceous stage and nitrogenous stage. The first stage of decomposition is carbonaceous stage which indicates the portion of oxygen demand involved in converting organic compound to carbon dioxide. The second stage of decomposition in BOD is basically the combination of carbonaceous and nitrogenous demand which involve the process of converting organic nitrogen, ammonia and nitrite into nitrate. The nitrification process usually takes place between 5 to 6 days if ammonia, nitrite and nitrifying bacteria present (Delzer et al., 2003).

Usually, for BOD measurement, the water sample will be sealed for five days with the purpose to measure the amount of oxygen loss. The samples are often diluted before the incubation process in order to avoid the oxygen from being depleted by bacteria. By the end of the treatment, the BOD content in water must be reduced because the excess bacteria presence will be removed as sludge and the waste will be disposed on land (Kemp et al., 2011). Generally, the results obtained by the end of treatment can be categorised into high BOD represent a high amount of organic material which is easily degradable, low BOD represent a low volume of organic materials which are difficult to break down. BOD value obtained can also be compared with the COD value. A small difference between BOD and COD value shows that a large proportion of organic compound which can be easily degraded while a large difference might indicate that the organic compound cannot be broken down easily (Chamarro & Esplugas, 2001).

2.6.3 Chemical Oxygen Demand (COD)

COD is a measurement of total amount of oxygen required for oxidation of all organic material into carbon dioxide and water. The time needed to obtain COD measurements only takes a few hours while the time needed to obtain BOD measurements take five days. It is necessary to make sure the value of COD is higher than the BOD value because the higher the BOD content in water, the higher the rate of bacterial growth and oxygen consumed in the water (Kim et al., 2003).

2.6.4 pH

pH is the measure of the amount of free hydrogen ions in water. Generally, water with pH lower than 7 is considered as acidic while water with pH greater than 7 is considered as basic (Figure 2.4). The pH of water will affect the solubility of toxic and

nutritive compound in water. The increase in acidity will increase the tendency of metals to be more soluble in water and indirectly increases the toxicity of water.

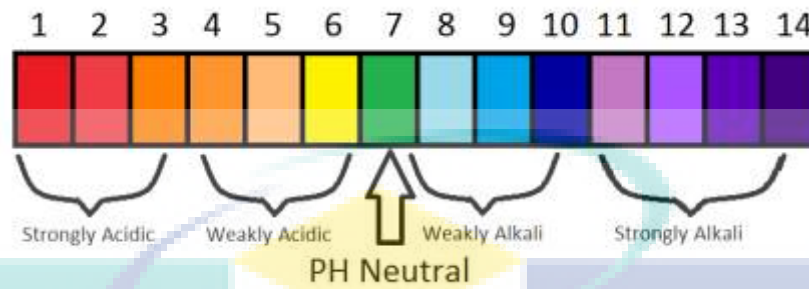


Figure 2-8 pH Value Range

Alkalinity is the tendency of water to neutralise acid such as hydroxide, carbonate and bicarbonate (Rafferty, 1999). Based on research, the normal range of pH for surface water is between 6.5 to 8.5 while the normal range for groundwater is between 6 to 8.5. The pH value for pure water at 25°C is 7 but the pH will decrease to 5.2 when being exposed to carbon dioxide. Basically, pH value which is lower than 6.5 can cause corrosion in plumbing fixtures and piping because of acidity. Furthermore, pH values decrease because of elevated level of toxic metals such as iron, manganese, copper, lead and zinc. The negative impacts of low pH value are staining of laundry, metallic or sour taste and “blue-green” staining of sinks.

However, water with pH value which is greater than 8.5 is categorised as hard water because of high alkalinity. Hard water does not cause health problem but can cause problem aesthetically such as formation of “scale” in piping systems, causes alkaline taste in water, decreases the efficiency of electric water heaters and difficulties in getting detergents to foam (Oram, 2014).

2.6.5 Conductivity and Total Dissolved Solids (TDS)

Conductivity is the measurement of dissolved ions concentration which gives substance the ability to conduct electricity. Conductivity measurements can be used as a quick indication in locating potential water quality problem sources. The conductivity is usually measured in terms of conductivity per unit length which typically uses the units’ microsiemens/cm (Bookter, 2009). Moreover, the TDS measurement will determine the amount of dissolved solids because of dissolution. TDS can be measured by evaporating a known volume of water and weighing the weight of the obtained residue. The higher

the TDS, the higher the harm of the consumed water towards health of living organism (Rhoades, 1996).

2.6.6 Overall Water Quality Performance

In determining the quality of water, the most important aspect that must be considered is term of chemical, physical and biological. Moreover, the quality of water will change with time because of both natural and human factors (Gelda et al., 2009). For example, temperature, photosynthesis activities of living organism in water and flows will vary with season especially during raining season which can daily affect the amount of suspended sediment formed. In conclusion, it is necessary to always obtain water quality data with the purpose to maintain the parameters to be measured within the standard range for safe consumption or usage of water (Rhoades, 1996). Table 2.5, 2.6 and 2.7 show the standard values for some water quality parameters:

Table 2.5 Ministry of Health Malaysia (2014)

PARAMETER	RECOMMENDED RAW WATER QUALITY	DRINKING WATER QUALITY STANDARDS
	Acceptable Value (unless otherwise stated)	Maximum Acceptable Value (unless otherwise stated)
Turbidity	1000 NTU	5 NTU
pH	5.5 - 9.0	5.5 - 9.0
TDS	1500 ppm	1500 ppm
BOD	6 mg/L	-

Table 2.6 National Water Quality Standards for Malaysia (WEPA, 2017)

PARAMETER	UNIT	CLASS					
		I	IIA	IIB	III	IV	V
Turbidity	NTU	5	50	50	-	-	-
pH	-	6.5– 8.5	6– 9	6– 9	5– 9	5– 9	-
Conductivity	μ s/cm	1000	1000	-	-	6000	-
TDS	ppm	500	1000	-	-	4000	-
COD	mg/L	10	25	25	50	100	> 100
BOD	mg/L	1	3	3	6	12	> 12

Table 2.7 National Water Quality Classes for Malaysia (WEPA, 2017)

CLASS	USES
Class I	Conservation of natural environment. Water Supply I – Practically no treatment necessary. Fishery I – Very sensitive aquatic species.
Class IIA	Water Supply II – Conventional treatment. Fishery II – Sensitive aquatic species.
Class IIB	Recreational use body contact.
Class III	Water Supply III – Extensive treatment required. Fishery III – Common of economic value and tolerant species; livestock drinking.
Class IV	Irrigation
Class V	None of the above.



The diagram is a large downward-pointing arrow shape composed of several colored segments. The top part is a light blue trapezoid. Below it are two vertical rectangular segments, one light blue on the left and one light purple on the right. The bottom part is a large downward-pointing triangle, split into two segments: light blue on the left and light purple on the right. The letters 'UMP' are written in white, bold, sans-serif font across the bottom of the triangle.

UMP

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Introduction

This chapter briefs the materials and experimental methods for the production of natural bio-coagulant from *Moringa oleifera* seeds. The experimental procedure was conducted in two methods, before started the experiments *Moringa oleifera* seeds were removed from its shell and the obtained seeds were grind to *Moringa oleifera* seeds powder (MOSP). The first method MOSP was sieved with different particle sizes of 2 mm, 1 mm, 0.5 mm and 0.25 mm. Then, the experiment was conducted using *Moringa oleifera* seeds powder (MOSP) as natural coagulant for water treatment to get the best particle sizes and amount, while in the second method the MOSP were placed in the extraction thimble for the *Moringa oleifera* seeds oil extraction process and *Moringa oleifera* cake residue (MOCR) was formed after the oil is completely extracted. After that, salt extraction was prepared by 1M of NaCl to extract the active coagulation component. The Jar test was conducted in order to obtain clear water sample. Then, the water treatment process was conducted by analysing the water sample turbidity, COD, BOD, conductivity, pH and TDS (Eman et al, 2014). The overall process flow is presented in Figure 3.1.

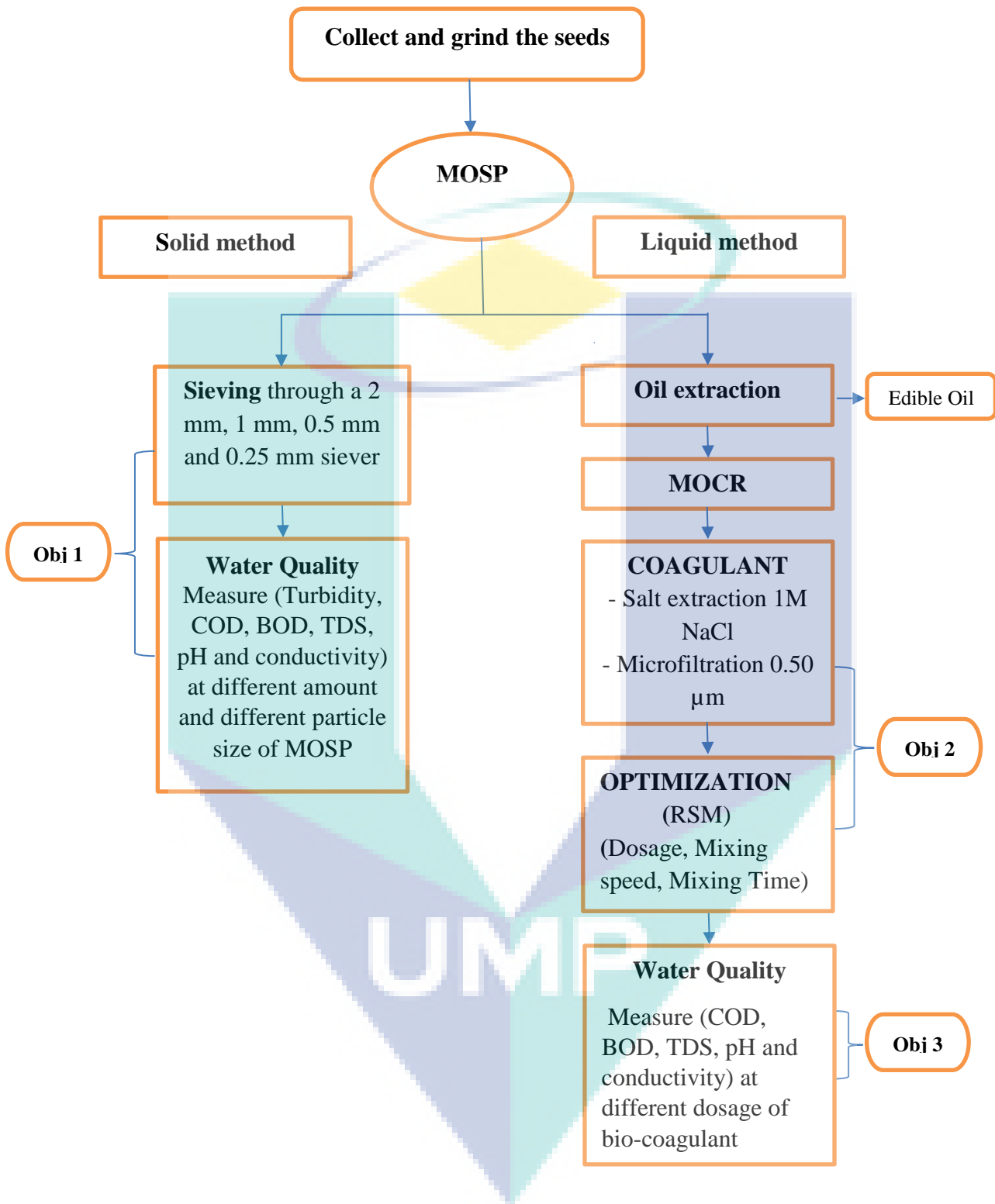


Figure 3-1 Process Flow Chart of Experiment

3.2 Sample Preparation

3.2.1 *Moringa oleifera* Seeds Powder (MOSP)

The *Moringa oleifera* (MO) seeds were purchased from Mitomasa Sdn. Bhd., Kuala Lumpur. The MO were dehusked to get the kernels. The kernel of MO seeds was then grinded to a fine powder using a domestic grinder. Fine powders were then sieved through a 2 mm, 1 mm, 0.5 mm and 0.25 mm sieve and used as coagulant (Kalibbala, 2007). MO seeds powder strongly attracts moisture and the product can reabsorb humidity during or after grinding. Hence, MO seeds powder were dried at 50°C for 30 minutes to reduce moisture content. After drying, MO powder was left to cool and packed into clean polythene bags and sealed. The bags were kept at chiller at 4°C to maintain freshness and dryness for to further use.

Figure 3.2, 3.3, and 3.4 show undehusked and dehusked MO seeds, domestic grinder used to grind MO seeds to powder formed and electronic sieve shaker used to sieve for different powdered sizes. Figure 3.5 shows *the Moringa oleifera* seeds powder (MOSP) after sieving and packed into clean, single-use polythene bags and sealed.



Figure 3-2 Undehusk MO seeds and Dehusk MO seeds



Figure 3-3 Domestic grinder to grinding MO

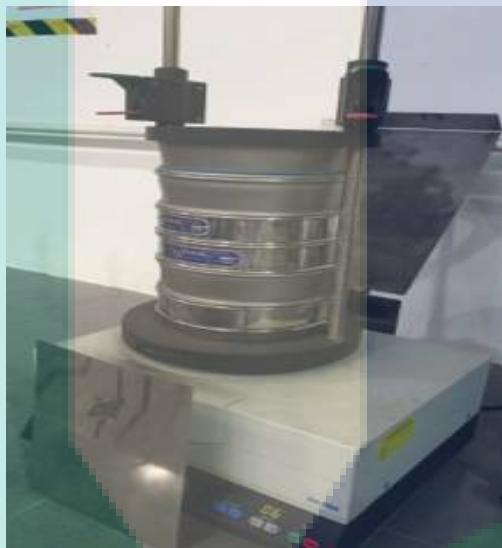


Figure 3-4 Electronic sieve shaker



Figure 3-5 MOSP after sieved and packed into clean, polythene bags and sealed

3.2.2 Preparation of *Moringa oleifera* Cake Residue (MOCR) through extraction process

Moringa oleifera seeds powder (MOSP) with 20 g was placed in the extraction thimble with 250mL of n-hexane solvent poured into the round bottom flask (Figure 3.6). Then, the soxhlet extractor was placed into the heating mantle. Running cold tap water was supplied to the condenser and the water was continuously flowing in and out of the condenser. The heating mantle was set to 100°C until the solvent starts to boil. Temperature 100°C was chosen with the purpose to ensure n-hexane to vaporise and able to pass through the siphon and react with the *Moringa oleifera* seeds powder in the thimble. The temperature of the heating mantle was reduced to around 70°C (Eman et al, 2014). The boiling temperature of n-hexane is 68.75°C.



Figure 3-6 Soxhlet Extractor

After the soxhlet apparatus is set up, the hexane solvent was heated up for 45 minutes in order to extract the oil (Da Porto et al., 2016). The percentage cake recovered was estimated using Equation (3.1):

$$\text{Percentage cake recovery} = \frac{W_0 - W_1}{W_0} = \frac{20 - 6.82}{20} \times 100\% = 65.9\% \quad 3.1$$

Where W_0 is the mass of *Moringa oleifera* seeds powder before extraction, W_1 is the mass of *Moringa oleifera* seeds oil produced after extraction

After the oil extraction process, *Moringa oleifera* cake residue was collected from the thimble and dried it overnight in an oven for 50°C. The seeds cake residue can be used for water treatment once the oil is removed from the *Moringa oleifera* seeds. The purpose of the oil extracted from the seeds because the presence of oil in the *Moringa oleifera* seeds powder will affect the coagulation process. The presence of oil will increase the quantity of organic matter added to water and oil extraction is recommended as a method to purify the extract (Sancho et al, 2016). The performance of the *Moringa oleifera* cake residue in water treatment process will decreases as the oil content in the *Moringa oleifera* cake residue increases (Eman et al, 2014).

3.2.3 Salt extraction

The purpose of salt extraction was to extract the active coagulant component. Birima et al. (2013) reported that NaCl can be used to extract the ingredient coagulant. 1 Molar of NaCl was prepared by added 58.44 g of NaCl to 1 L of distilled water and mixing until dissolve all the NaCl in the distilled water then added 5 grams of *Moringa oleifera* cake residue and mixed for 30 minutes using the magnetic stirrer (ERLA, ERLA Technologies (M) Sdn. Bhd. Malaysia). The sample was filtered using filter paper. Lastly, microfiltration was conducted with 0.50 µm Filter as shown in Figure 3.7 (Ali et al., 2010).



Figure 3-7 Salt extraction

3.3 Water sample Collection

The river water sample was collected using high density polyethylene (HDPE) container from few spots in Belat River, which is located at housing area in Gambang, Kuantan, Pahang State, Malaysia (Figure 3.8). The water quality status of this river was investigated before conducted the experiments to determine the initial turbidity, pH, TDS, conductivity, COB and BOD of the river water. Then, the river water tested with different dosages of *Moringa oleifera* bio-coagulant under jar test to evaluate the performance of *Moringa oleifera* as a bio-coagulant on river water. The experiments were conducted at the Environmental Engineering Laboratory, Universiti Malaysia Pahang.

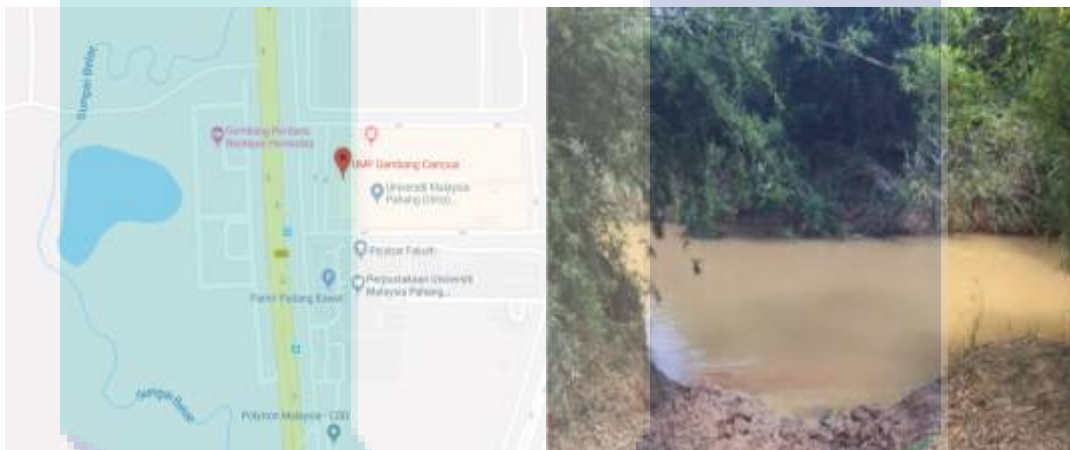


Figure 3-8 River water sample collection

3.4 Design of Experiment to optimize the study

In this study, the effect of variables such as dosage, speed and mixing time on the residual turbidity were evaluated using the Response Surface Methodology (RSM). Design Expert 7.0 software® (Version 7.1.6, Stat- Ease Inc., Minneapolis, USA) was used for the experimental design and analysis of the results. The high speed and the high mixing time were fixed at 100 rpm and 6 min, respectively (Ali et al, 2010). However, according the results obtained from the previous study conducted by Ali et al., (2012) three independent factors, viz: Low speed (40-80 rpm), Low mixing time (20- 60 min) and dosage (0.25- 1.25 mg/L) were optimized using the Response Surface Methodology (RSM) for optimal water turbidity (Table 3.1).

Table 3.1 Coded and actual design range

Independent factor	Designation	Units	Coded level	
			-1	+1
Low speed	A	rpm	40	80
Low mixing time	B	min	20	60
Dosage	C	mg/L	0.25	1.25

3.5 Jar Test

The clear water sample is prepared by placing 500 mL of water sample into each 6 labelled beakers and then placed into the Jar Test apparatus (Figure 3.9). Next, correct concentration of *Moringa oleifera* bio-coagulant was placed into Jar Test apparatus respectively and operated with fixed high speed and high mixing time at 100 rpm and 6 min, and low speed and low mixing time between (40-80 rpm), (20-60 min) respectively. Then, stop the paddles and the water was left to settle down for 1 hour. The clear water was collected into conical flask and stored at 4°C for further analysis (Shan et al, 2017).



Figure 3-9 Paddle Jar Test Apparatus

3.6 Water Quality Analysis

3.6.1 Turbidity Measurement

Digital turbidity meter (TB-500G) in Figure 3.10 was used in this experiment to measure high turbidity. The measurement range of this turbidity meter is from 20 NTU to 500 NTU. 10 mL of sample was used to inspect each time for the turbidity. Transmitted light measurement method is used for the device to measure turbidity and it is important

to make sure that the temperature of the sample is in 20°C - 25°C before measurement. Digital turbidity meter (DTC-4DG) in Figure 3.11 was used in this experiment to measure low turbidity after treatment with MO bio-coagulant. The measurement range of this turbidity meter is from (0.0 NTU to 20.0 NTU). 10 mL of sample was used to inspect each time for the turbidity. Transmitted light measurement method is used for the device to measure turbidity and it is important to make sure that the temperature of the sample is in ambient temperature, which is between 20°C - 25°C.



Figure 3-10 Digital turbidity meter (TB-500G) and (DTC-4DG)

3.6.2 Chemical Oxygen Demand (COD) Measurement

In this study, test for Chemical Oxygen Demand (COD) was performed by Reactor Digestion Method. The results in mg/L COD are defined as the milligrams of O₂ consumed per liter of sample under the conditions of this procedure. The sample is heated for 2 hours with sulfuric acid and a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion (Cr²O₇²⁻) to green chromic ion (Cr³⁺) COD reactor in Figure 3.12 was preheated to 150°C. 2 mL each of de-ionized water (control) and samples after jar test was added into COD Digestion Reagent Vials. Digestion reagent vials used for measurement test were low range reagent vials ranging from 3 mg/L to 150 mg/L while for high range reagent vials ranging from 20

mg/L to 1500 mg/L. The samples were then inserted into COD reactor HACH DRB200 and heated for 2 hours at 150°C with strong oxidizing agent (potassium dichromate solution). Then the solutions were cool down to room temperature. The vials were cleaned to remove any fingerprint before measuring the COD with HACH spectrophotometer DR2800 (Figure 3.13) (Kasmawati, 2007).



Figure 3-11 COD rack and COD reactor HACH DRB200



Figure 3-12 HACH spectrophotometer DR2800

3.6.3 Biochemical Oxygen Demand (BOD) Measurement

Five-days Biochemical Oxygen Demand (BOD) test were performed using in house method based on APHA 5210B (Tariq et al., 2012). BOD measurement requires taking two measurements. One is measured immediately for dissolved oxygen (initial), and the second is incubated in the lab for 5 days and then tested for the amount of dissolved oxygen remaining (final). This represents the amount of oxygen consumed by microorganisms to break down the organic matter present in the sample during the incubation period. A dissolved oxygen (DO) meter was used for BOD measurement. 1 liter of diluted water sample was prepared by adding 1 mL each of phosphate buffer, magnesium sulfate, calcium chloride and ferric chloride solution into 1 L of volumetric flask then distilled water was added to volumetric flask till 1 L. About 10 mL of samples after jar test was transferred into each BOD bottle. Then, 300 mL of diluted water was added into the BOD bottle. Besides that, the control was prepared from 300 mL diluted water in BOD bottle. The DO was measured for all samples using DO meter. After that, the diluted water was added to the flared mouth of the bottle and covered with aluminum foil to prevent evaporation of the solution. All bottles was put into the BOD incubator for 5 days at 20 °C. The DO value was measured after 5 days (Suhartini, 2013).

3.6.4 pH, Conductivity and Total Dissolved Solid Measurement

Milwaukee MW801 Standard Combination Meter in Figure 3.14 and Figure 3.15 were used in the experiment to measure pH, Conductivity and Total Dissolved Solid (TDS). The measurement range for pH for this meter is from 0.0 to 14.0, for conductivity is from 0 to 1990 $\mu\text{m}/\text{cm}$ and for TDS is from 0 to 1990ppm, which is an ideal tool for drinking water measurements. SE600 combination interchangeable probe was used to measure pH, conductivity and TDS. The probe was washed with distilled water using washing bottle and manual calibration was performed each time before the second reading was taken. In addition, reading was taken 3 times to ensure accuracy of result.



Figure 3-13 Manual Calibration for pH before measurement



Figure 3-14 pH measurement for the mixture

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the results of the experiments in three sections and a discussion of the results. The first section presents the effects of particle and amount of *Moringa oleifera* seeds powder (*MOSP*) toward the river water quality. The second section presents the optimum condition of the parameters such as speed, time and bio-coagulant dosage using The Response Surface Methodology (RSM). The third section discusses the effects of *Moringa oleifera* seeds bio-coagulant dosages to the quality of the treated water at the optimum condition.

4.2 Characterizations of Belat River water

The water treatment parameters of the water before analysis were conducted to determine the initial turbidity, pH, TDS, conductivity, COB and BOD properties. The results obtained are presented in Table 4.1.

Table 4.1 Summary of result for water quality characteristics

Parameters	Units	Value measured Before Treatment
Turbidity	NTU	415
pH	-	6.4
TDS	ppm	100
Conductivity	$\mu\text{s}/\text{cm}$	220
COD (mg/L)	mg/L	158
BOD	mg/L	25

4.3 Determination *MOSP* for Water Treatment

This investigation was conducted to determine the optimum particle sizes of *MOSP* for the water treatment process using different measured quantity. Different sizes and dosages of *MOSP* (2 mm, 1 mm, 0.5 mm and 0.25 mm);(0.05, 0.10, 0.50, 0.75, 1.0, 2.5, 5.0, 7.5 and 10.0 g) were added to 500 mL of river water in a jar test experiment. It was important to ensure that the turbidity was almost the same in each beaker before each *MOSP* was added into different beakers filled with river water.

4.3.1 Turbidity

The results obtained clearly revealed that at 2 mm particle size, the coagulant performed better in terms of turbidity removal efficiency (98.80%) when treated with 0.1g of *MOSP* as presented in Figure 4.1. The experiment was repeated with different particle size (i.e. 1 mm, 0.5 mm and 0.25 mm) of *MOSP*. Figure 4.1 shows the effects of changes in the amount of *MOSP* on the turbidity removal at different particle sizes.

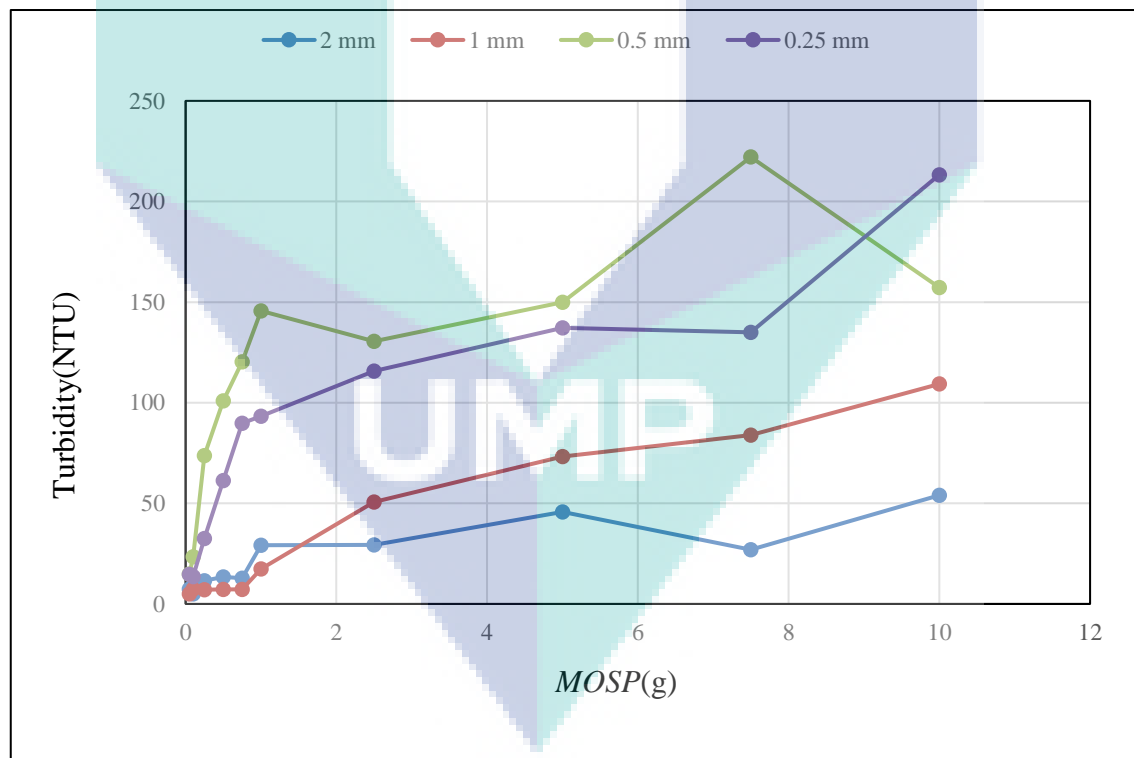


Figure 4-1 Turbidity removal of water treated with *MOSP*

Goudeli et al., (2015) reported that particle sizes in different sizes contributed differently to the water turbidity after the process of coagulation. The result obtained revealed that the turbidity removal was very low when 0.5 mm and 0.25 mm particle sizes

were. Latifah, (2015) reported that the choice of optimum particle sizes allows for a higher chances of efficient reaction between the constituents of *Moringa oleifera* seeds and the treated water. This is consistent with the chemical reaction theory which states direction proportionality between the surface area and the reaction rate. This indicated that in water, the basic amino acids present in *Moringa oleifera* protein have the capacity to accept a proton from the water using the best particle size at a controlled dosage (Levallois & Villanueva, 2019). Moreover, Sharma et al. (2006) conducted test to determine the effect of different particle size on the efficiency of *Moringa Oleifera* seeds powder as coagulant. The test indicates that larger sized powder 2000 micron (2 mm) is more effective than 1000 and 420 micron sized powder.

Meanwhile, in this study, the lowest turbidity (i.e highest turbidity removal) was therefore obtained at an average particle size of 2 mm at 0.1 g which is consistent with previous studies (Sharma et al., 2006). However, for particle sizes below 2 mm, agglomeration of the MOSP tends to occur due to the sticking together of the particles. This led to a reduction in the turbidity removal efficiency when the particle sizes of 1 mm, 0.5 mm and 0.25 mm when compared with 2 mm. Singh et al., (2014) corroborated the occurrence of agglomeration where sintering (coalescence) takes place instead of settling as in the case of coagulation. Moreover, the optimal particle size for the *Moringa oleifera* powder presented a better coagulation site for the effective interactions between the negative and positive charged ions. However, care must be taken to select the best particle size without resulting to agglomeration of the particles (Chupin et al., 2015). The result obtained therefore indicated that the coagulant concentration exceeded the optimum dosage with an increased turbidity due to the neutralization and precipitation of all the colloidal suspension at optimum dosage (Adeniran et al., 2017). On the other hand, using an excess mass of *MOSP* coagulant beyond 0.1 g reduces the interaction between the treated water and the oppositely charged colloidal particles.

4.3.2 pH

Figure 4.2 shows the effect of changes in the amount of *MOSP* at different particle sizes on the pH value. The amount of *MOSP* was varied from 0.25 to 10 g using different particle sizes (2 mm, 1 mm, 0.5 mm and 0.25 mm). The result obtained shows the pH at 7.0 using a particle size of 0.5 mm. This result is an improvement over the investigation

conducted by Dehghani et al., (2016), who obtained the best pH as 6.0 with COD, turbidity, and TSS decreased by 39.78%, 62.16%, and 61.05% respectively. This is consistent with the standard pH level of drinking water which is expected to be between 6-8.5 as the human body could maintains pH equilibrium on a constant basis (Swistock et al., 2013).

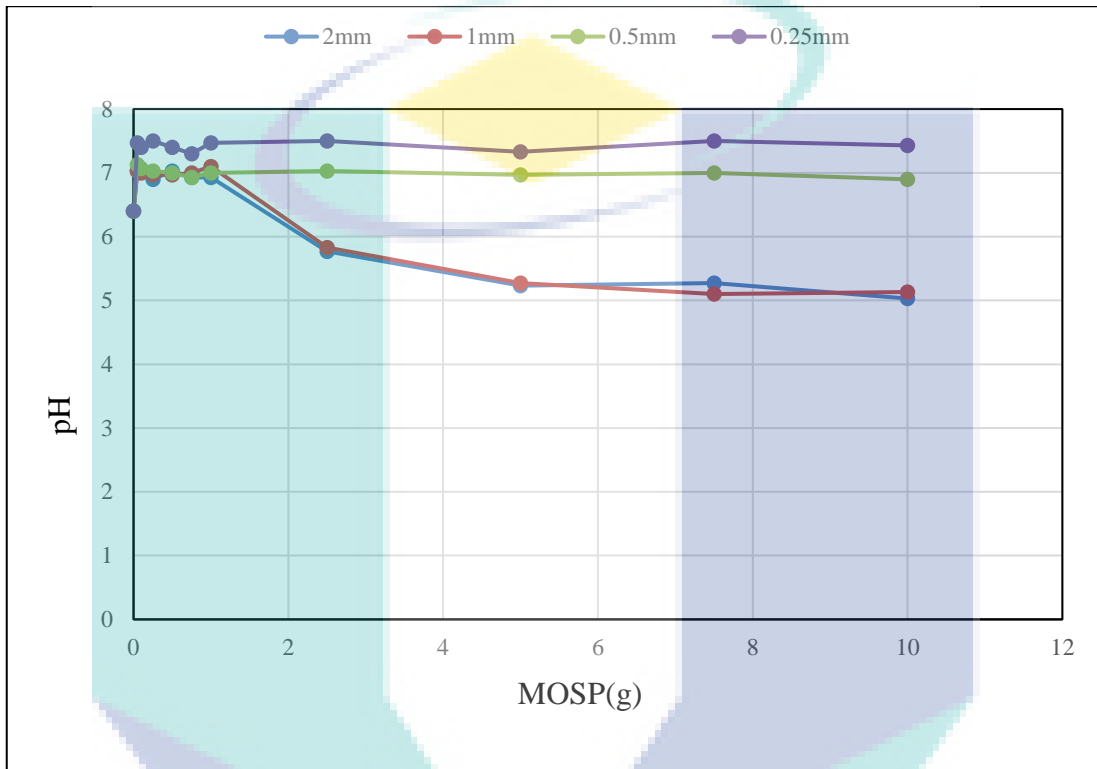


Figure 4-2 pH values for water treated with MOSP

Therefore, by increasing *Moringa oleifera* seeds powder will lead to increase the acidic concentration of the water by increasing the dosing solutions. The basic concentration of the water can be increased by the same process, but the acid concentration increases more when compared to basic concentration. More amount of the dosing solution has to be used in treating the water which has more acidic content in it (Amagloh et al., 2009).

4.3.3 Conductivity

Figure 4.3 shows the variation of MOSP amounts in grams on the water conductivity using different particle sizes. The result obtained revealed that at MOSP above 1.0 g the conductivity increased due to the presence of unbound ions (Lawrence et al., 2019). Dhanorkar and Kamra (2001) buttressed that the reason for the increase the

polar conductivity could be attributed to the increasing of ions. The result shows that the conductivity obtained was within the standard allowable range of $800 \mu\text{s}/\text{cm}$ as set by Queensland Health Forensic and Scientific Services (QHFSS), (Ikonomopoulou et al., 2011).

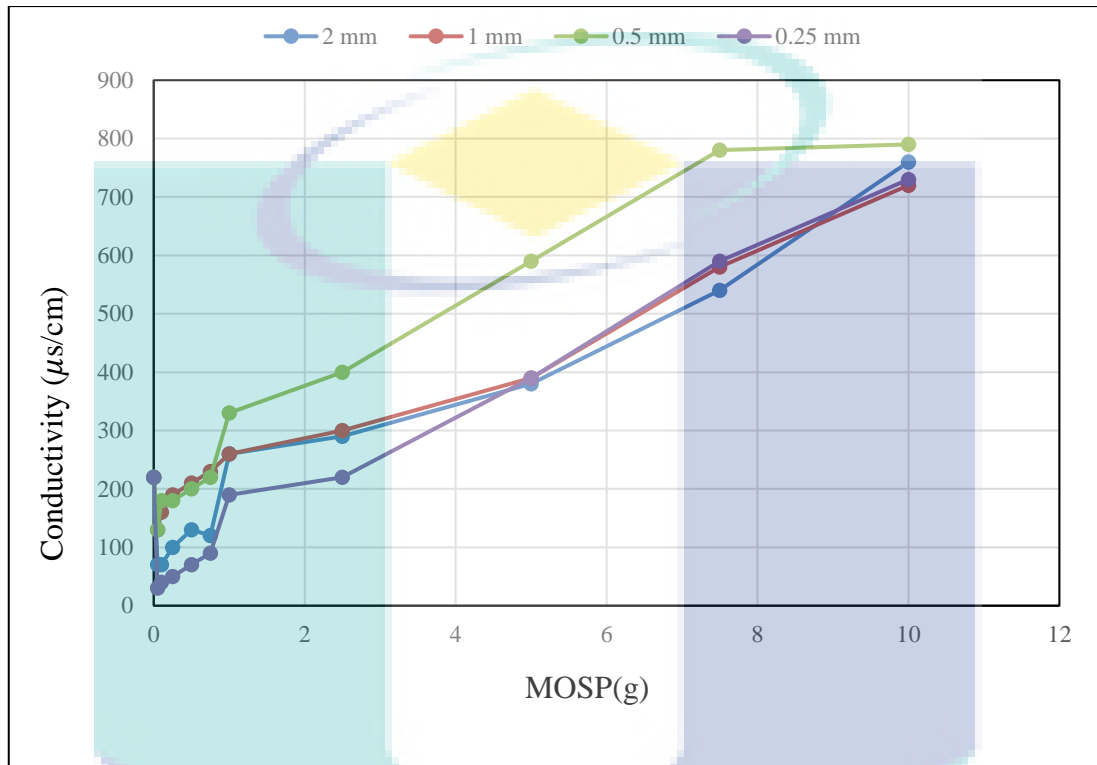


Figure 4-3 Water conductivity treated with MOSP

4.3.4 TDS Measurement

From the result obtained, the total dissolved solid in river water after treatment with MOSP is increasing for different particle size of MOSP used (Figure 4.4). However, the maximum TDS measured was $450\text{mg}/\text{L}$ when 10 g of MOSP used for 0.5 mm particle size of MOSP, which is still in the TDS standard level set by EPA. High TDS results in undesirable taste, which could be salty, bitter, or metallic. For drinking water, the maximum TDS level set by Environment Protection Agency (EPA) is 500 ppm. The effect of MOSP usage on the TDS for the treated water is presented in Figure 4.4.

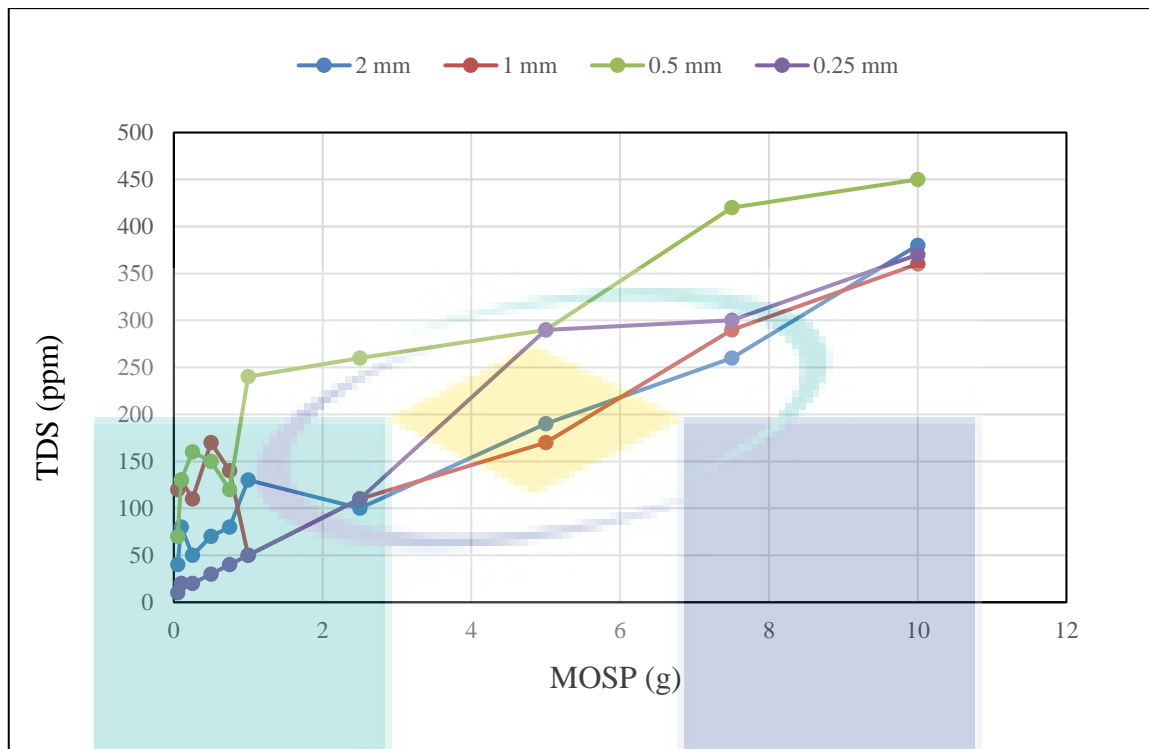


Figure 4-4 TDS of water treated with MOSP

4.3.5 COD Measurement

Figure 4.5 shows the result of COD from Belat river samples at different doses of MOSP. The initial COD before treatment (i.e 0.00 g of MOSP) was obtained to be 158 mg/L and these decreases correspondingly to 100.33 mg/L when 0.10 g of MOSP with 2 mm particle size of MOSP was applied. This indicated that 36.7% of COD removal was attained with 0.10 g of MOSP using 2 mm particle size. This is an improvement over the investigation made by Dehghani et al., (2016) who obtained the best COD at 31.78 % which could be attributed to the proper choice of the coagulant particle sizes. Therefore, the increasing in COD is partly due to the presence of little oil in the *Moringa oleifera* seeds powder which lead to a corresponding increase the organics in the water which more oxygen will required, thus the COD value will increased.

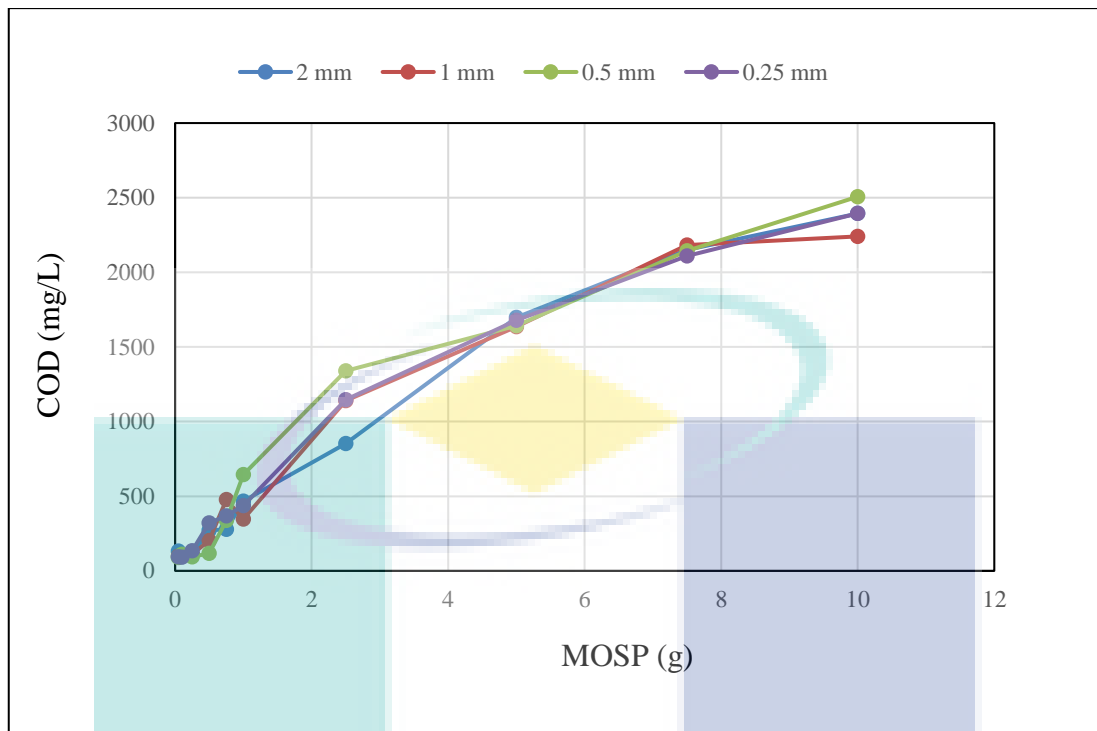


Figure 4-5 COD of water treated with MOSP

4.3.6 BOD Measurement

The BOD for river water collected from Belat was 25 mg/L. When particle size of 2 mm MOSP used for treatment, 0.05, 0.10 and 0.50 g of MOSP used showed a decrease in BOD value and others dosage show an increase in BOD value (Figure 4.5). When particle size of 1mm MOSP used for treatment, 0.05 g, 0.10 g and 0.25 g of MOSP used showed a decrease in BOD value and others dosage show an increase in BOD value. The higher the MOSP dosage used the higher the BOD value in river water. The BOD removal was 40% when 0.05 g of 2 mm MOSP was used. When compare with COD, it shows that BOD result has the same trend with COD result. The possible reason for the increase of the BOD value after treatment may due to the presence of and organic matter in MO seeds (Nordmark et al., 2018).

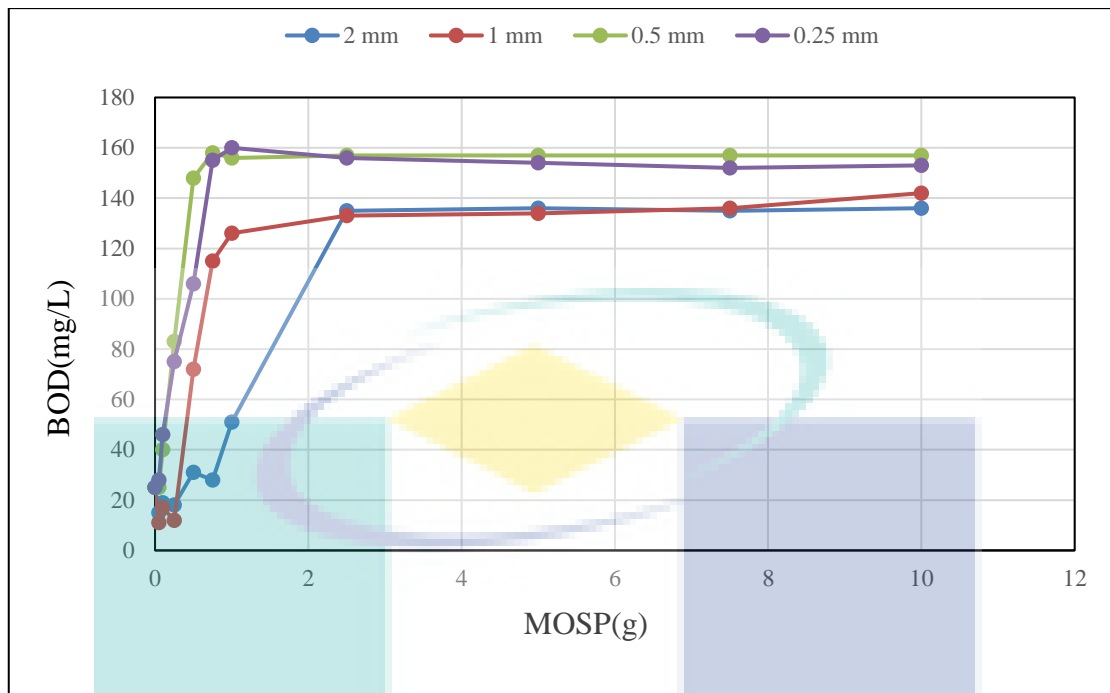


Figure 4-6 BOD of water treated with MOSP

The effect of MOSP usage on the BOD for the treated water is presented in Figure 4.6. Generally, when BOD levels are high, there is a decline in DO levels. This is because the demand for oxygen by the bacteria is high and they are taking that oxygen from the oxygen dissolved in the water. If there is no organic waste present in the water, there will not be as many bacteria present to decompose it and thus the BOD will tend to be lower and the DO level will tend to be higher. By using more than 1g of MOSP for river water treatment, BOD parameters does not meet drinking water quality requirement set by Ministry of Health Malaysia (Al-Mamun & Zainuddin, 2013).

4.4 *Moringa oleifera* Cake Recovery (MOCR)

Oil was extracted from *Moringa oleifera* seeds powder using n-hexane as extracting solvent. The result obtained showed higher recovery for the *Moringa oleifera* cake (65.9%). The higher cake recovery confirmed the efficiency of n-hexane in the extraction of oil from Moringa seed powder (Ahmed, 2016). The oil extraction

n was conducted due to its effect in increasing the pH, COD, and TDS. Hence the presence of oil affects these quality characteristics which explains the reason for their removal to form cake residue (Sancho et al, 2016).

4.5 Optimization Study of Operating Parameters in Water Treatment Using (RSM).

Experimental design plays an important role in several areas of science and industry. Experimentation is an application of treatments applied to experimental units and is then part of a scientific method based on the measurement of one or more responses. One of the most commonly used experimental designs for optimization is the response surface methodology (RSM). A study on the bio-coagulant of *Moringa oleifera* on to turbidity removal was conducted and the process parameters were optimized by response surface methodology (RSM).

The Response Surface Methodology (RSM) based on Central Composite Design (CCD) was used to evaluate and optimize the effect of low speed, low speed time and dosage of *Moringa oleifera* seeds bio-coagulant as independent variables on the turbidity removal as the response function. According to the previous studies conducted the ranges of the three independent factors were low speed (40-80 rpm), low speed time (20-60 min) and dosage (0.25-1.25 mg/L). The interaction effects and optimal parameters were obtained by using MODDE software. The significance of the independent variables and their interactions was tested by means of analysis of variance (ANOVA) with a 95% confidence level. The three independent factors were optimized using the Response Surface Methodology (RSM) for optimal water turbidity (Table 4.2). However, the high speed and the high speed time were fixed at 100 rpm and 6 min, respectively (Ali et al., 2012). Table 4.3 presents the experimental design and results for the optimization of water turbidity under the effects of three parameters, namely, Low speed (x1), Low speed time (x2) and dosage (x3).

Table 4.2 Design range, experimental design and results obtained from optimization

Independent factor	Designation	Units	Coded level	
			-1	+1
Low speed	A	rpm	40	80
Low speed time	B	Min	20	60
Dosage	C	mg/L	0.25	1.25
Test	Low speed (rpm)	Low speed time (min)	Dosage (mg/L)	Residual turbidity (NTU)
1	60.00	40.00	0.75	4±0.12
2	80.00	20.00	0.25	13±0.14
3	40.00	60.00	1.25	14±0.06
4	60.00	20.00	0.75	7.1±0.03
5	80.00	60.00	1.25	11.62±0.07
6	80.00	40.00	0.75	4.4±0.11
7	80.00	20.00	1.25	7.64±0.19
8	40.00	20.00	0.25	9±0.01
9	40.00	20.00	1.25	10.21±0.04
10	60.00	40.00	0.75	7.44±0.02
11	40.00	60.00	0.25	13.55±0.01
12	60.00	40.00	0.25	14.43±0.21
13	60.00	40.00	0.75	4.2±0.18
14	60.00	40.00	0.75	5.1±0.22
15	60.00	40.00	0.75	5.77±0.05
16	60.00	40.00	1.25	8.46±0.34
17	80.00	60.00	0.25	13.92±0.01
18	40.00	40.00	0.75	5±0.02
19	60.00	40.00	0.75	10±0.01
20	60.00	60.00	0.75	8.8±0.21

4.6 Model Fitting and Analysis of Variance

In this study the quadratic and interaction regression coefficient of the models were evaluated using Design Expert software®. The result of the ANOVA for the optimization of the residual turbidity shows that the quadratic model is significant at 95% confidence level ($p < 0.05$). Moreover, the low speed time (B) and dosage (C) were both significant, whereas the low speeds (A) were not significant factors in the optimization of residual turbidity. Additionally, AB, AC, BC, A^2 , and B^2 were insignificant interactions, while only C^2 has a significant contribution to the response setting. The statistical analysis gives many comparative measures for the model selection and this indicated that the quadratic model has a significant contribution with lower standard deviation (1.93) and higher

coefficient of regression ($R^2 = 0.9847$). The lack of fit is not significant in relation to the pure error. It is pertinent to note that an insignificant lack of fit is desirable for the adequacy of all the model terms. It indicated that the model is adequate to describe the observed data (Table 4.3).

Table 4.3 Analysis of variance for response surface quadratic model

	Sum of squares	df	Mean square	F value	p-value
Model	206.40	9	22.93	26.18	0.0044 ^a
A	0.14	1	0.14	0.038	0.8503 ^b
B	22.32	1	22.32	16.01	0.0341 ^a
C	14.33	1	14.33	33.86	0.0078 ^a
AB	1.48	1	1.48	0.40	0.5420 ^b
AC	10.86	1	10.86	2.93	0.1180 ^b
BC	0.66	1	0.66	0.18	0.6819 ^b
A ²	6.16	1	6.16	1.66	0.2266 ^b
B ²	8.45	1	8.45	2.28	0.1622 ^b
C ²	75.74	1	75.74	20.41	0.0011 ^a
Residual	37.11	10	3.71		
Lack of Fit	10.98	5	2.20	0.42	0.8185 ^b
Pure Error	26.13	5	5.23		
Cor Total	243.51	19			
Std. Dev.		1.93		R-Squared	0.9847
Mean		8.88		Adj R-Squared	0.9810
C.V. %		21.69		Pred R-Squared	0.9795
PRESS		4.16		Adeq Precision	47.444

^a significant, ^b insignificant, A-Low speed, B- Low speed time, C-Dosage

Figure 4.7 (a) shows the result of interaction effects between the low speed and low speed time. The result obtained revealed that the residual turbidity decreased with a low speed time between 50 -70 min when a low speed varied between 30-50 rpm. However, Figure 4.7 (b) indicated that the *Moringa oleifera* bio-coagulant dosage was used between (0.5-1.00 mg/L) against the low speed of 50-70 rpm. The low speed mixing time decreased between 30-50 min when the dosage was between 0.5-1.00 mg/L as presented in Figure 4.7c).

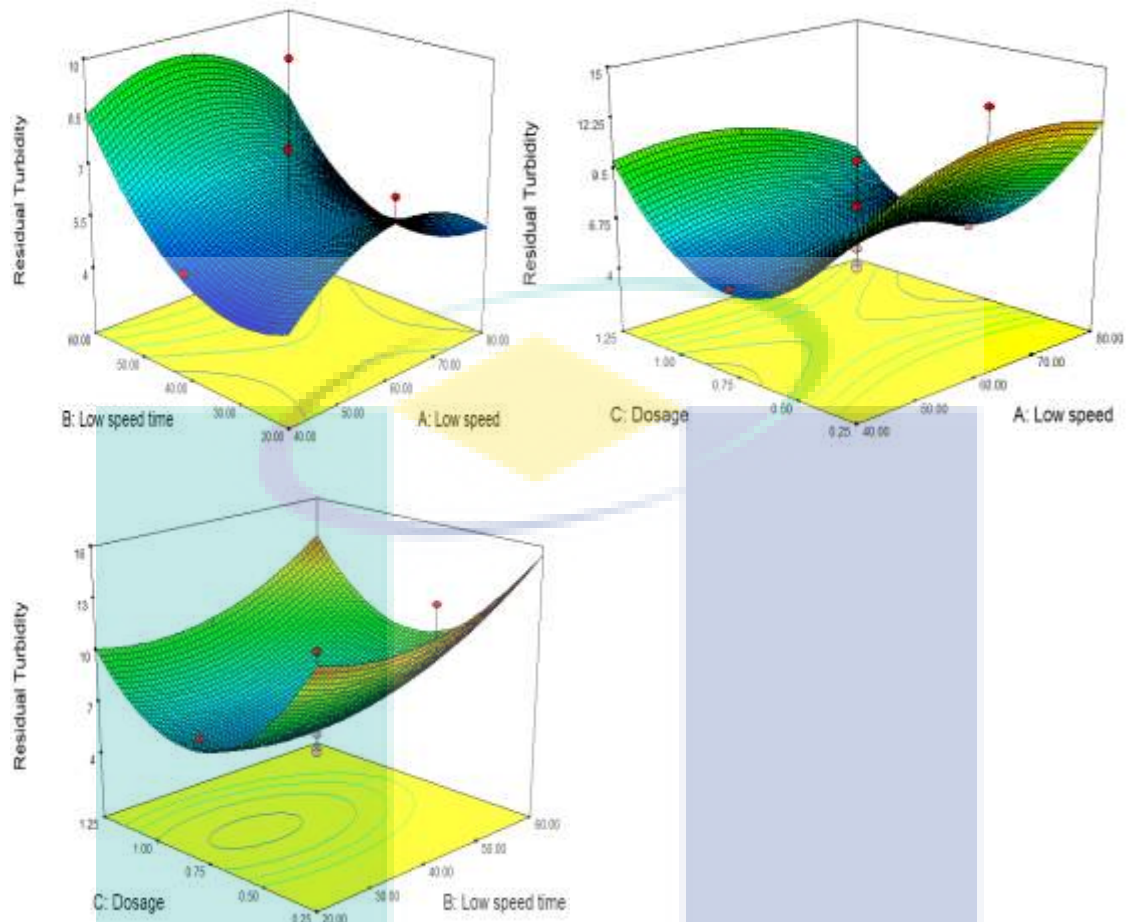


Figure 4-7 The interaction plot (a) low speed and low speed mixing time (b) Dosage and low speed (c) Dosage and low speed time

4.6.1 Validation of Predictive Model

In this study, the response was correlated using three parameter settings with quadratic model and the coefficient of regression. The significant of the three variables (i.e. low speed, low speed time and dosage) on the residual turbidity were evaluated using the Design Expert® software. It should be noted that the single parameters indicated the effect of that particular factor, whereas with two variable represent the interaction between those two factors, while the second order terms indicated their quadratic effects. The positive and negative sign is an indication of synergistic and antagonistic effects of the terms, respectively. From the result generated, the regression model for the residual turbidity is presented in Eq. (4.1) below.

$$\begin{aligned}
 \text{Residual Turbidity} = & 30.342 + 0.0673A - 0.954B - 23.654C + 0.0132B^2 + \\
 & 18.765C^2 - 0.0002AB - 0.0787AC - 0.0463BC
 \end{aligned}
 \tag{4.1}$$

The above model was validated by conducting a replicate experiments for the residual turbidity. The optimal condition was generated from the Design expert software with a single solution. Under this condition a number of replicate experiments were performed and a comparison between the observed and predicted results from the model equation were made, with the minimum residual turbidity selected as the optimum condition. The results obtained from experimental design placed the optimum condition at low speed, low speed time and dosage as 40 rpm, 60 min, and 0.75 mg/L, respectively. Under this condition the predicted (theoretical) residual turbidity was 4.73 NTU as presented in Table 4.4.

Table 4.4 Theoretical optimal conditions

Variables	Units	Values	Predicted residual turbidity (NTU).
Low speed (A)	rpm	40	4.73
Low speed time (B)	min	60	
Dosage (C)	mg/L	0.75	

A close agreement between the theoretical and experimental best residual turbidity was estimated using Equ. (4.2) below.

$$\text{Percentage Error} = \sum_{n=1}^3 \left| \frac{\text{Theoretical}_{\text{turbidity}} - \text{Experimental}_{\text{turbidity}}}{\text{Theoretical}_{\text{turbidity}}} \right| \times 100 \quad 4.2$$

The percentage error was used to examine the validity of the best response setting in the waste water treatment using a residual *Moringa oleifera*. Byrne et al., (2013) reported that for the best condition to be valid, the percentage error must not exceed 10 % at 95% confidence level. The test shows that there is no significant difference between the theoretical and experimental turbidity settings with a percentage error value of 1.6913 as presented in Table 4.5. The percentage error obtained is therefore negligible when compared with the 10 % cut-off value at 95% confidence.

Table 4.5 Validation of experimental best condition

Run	Theoretical value (NTU)	Experimental value (NTU)	Error (%)
1	4.73	4.70	0.6342
2	4.73	4.71	0.4228
3	4.73	4.70	0.6342

4.6.2 The Effects of Variables on the Optimal Settings

In a highly turbid river the degree of colloidal suspension is measured using an incident angular light beam at 90° measured in Nephelometric Turbidity Unit (NTU). The colloidal particles are usually characterized with the Tyndall effects in which light becomes reflected back by the particles (Ali et al., 2012). These particles have their sizes ranging between the dissolved and suspended particles which become too minute to become sediment or removed by normal filtration process. To therefore assist in the removal of colloidal particles from suspension, the velocity gradient (high mixing speed and low mixing speed is of paramount importance when treating turbid water for safe drinking. The mixing speed (low speed and high speed) is one of the important factors in achieving higher turbidity efficiency during the coagulation process (Zhu et al., 2012). In this study, the optimum lower mixing speed achieved during the Jar test experiment was 40 rpm at a dosage of 0.75 mg/L of *Moringa oleifera* bio-coagulant. The Low and high speed time are also an important factor because an excessive speed and time could results in coagulants rupture, while excessive speed time allows the coagulants erosion to occur as reported by Yu et al., (2010). In this study the optimum low speed time of 60 min was used at a constant high speed time of 6 min to achieve an effective turbidity removal. These two durations are sufficient for the binding together of colloidal positive charged ions and the negative charged impurities from the river water. This are the optimum time combination condition required for an efficient bio-coagulation process.

In this study, the dosage is one of the most important variables considered for the determination of the optimum conditions of the coagulation. The mechanism of the coagulation process is such that when the *Moringa oleifera* bio-coagulant dose is too small, there are poor effects in the positive colloidal attractive forces from the bio coagulants and the negatively charged impurities from the water. However, when the coagulant dosage is too high, the colloidal particles in the water becomes oversaturated and thereby resulting in the instability and weakens the Brownian motion and the Zeta-potential value. It is therefore necessary to determine the optimum dosage in order to minimize the dosing cost and obtain the optimum performance in treatment. The results of the optimization obtained gave an optimum dosage at 0.75 mg/L and this implies that as the natural bio-coagulant dosage increases, the quality characteristics such as turbidity and water colour decreased significantly. The result therefore shows that the *Moringa oleifera* bio-coagulant exhibited a highly efficient turbidity reduction when a dose of 0.75

mg/L is applied on the river water. The turbidity obtained (4.73 NTU) using these combination of parameters were in accordance with the recommended standard level (<5.0NTU) of the WHO and this substantiated the efficacy of *Moringa oleifera* as a natural bio-coagulants for safe drinking water.

4.7 Quality Analysis of Treated Water

In this research, the quality characteristics such as pH, TDS, conductivity, COB and BOD test were tested at optimal condition on the effect of bio-coagulant concentration.

4.7.1 pH Measurement

The degree of acidity and alkalinity is one of the most important factors that affecting the use of *Moringa oleifera* as coagulant. The inability of *MO* bio-coagulant to coagulate at optimal pH could affect the quality of the water produced as acidic properties could lead to a poor quality of water produced. In this research the initial pH of the river water collected was recorded as 6.4 which are relatively acidic. Figure 4.8 shows the comparison between the dosage used for water treatment and the pH values obtained after water treatment. From Figure 4.8, the lowest pH value was recorded as 5.2 at a dosage of 2.5 mg/L. Based on the analysis; the optimum pH obtained using *MO* bio-coagulant is 6.5 at 0.75 mg/L. At this value, amino acids ionize to produce carboxylate ion and proton, proton charge attracts electrons (colloids) to form neutral group. These results shows that *Moringa oleifera* bio-coagulant does not significantly impact the pH value which showed agreement with previously conducted research (Eman et al., 2014). Ali et al., (2015) investigated the effect of pH on the bio-sorption of Cd (II). He reported that there was no effect of pH on the bio-sorption of Cd (II). From the statistical analysis obtained from their studies, it was reported that pH has no effect with the p-value greater than the threshold 0.05. Hence, the pH was considered as insignificant factor in water treatment process using *Moringa oleifera* leaves as bio-coagulant. This was supported by other researchers such as Arnoldsson et al., (2008); Hoa et al., (2018) and Shan, et al., (2017).

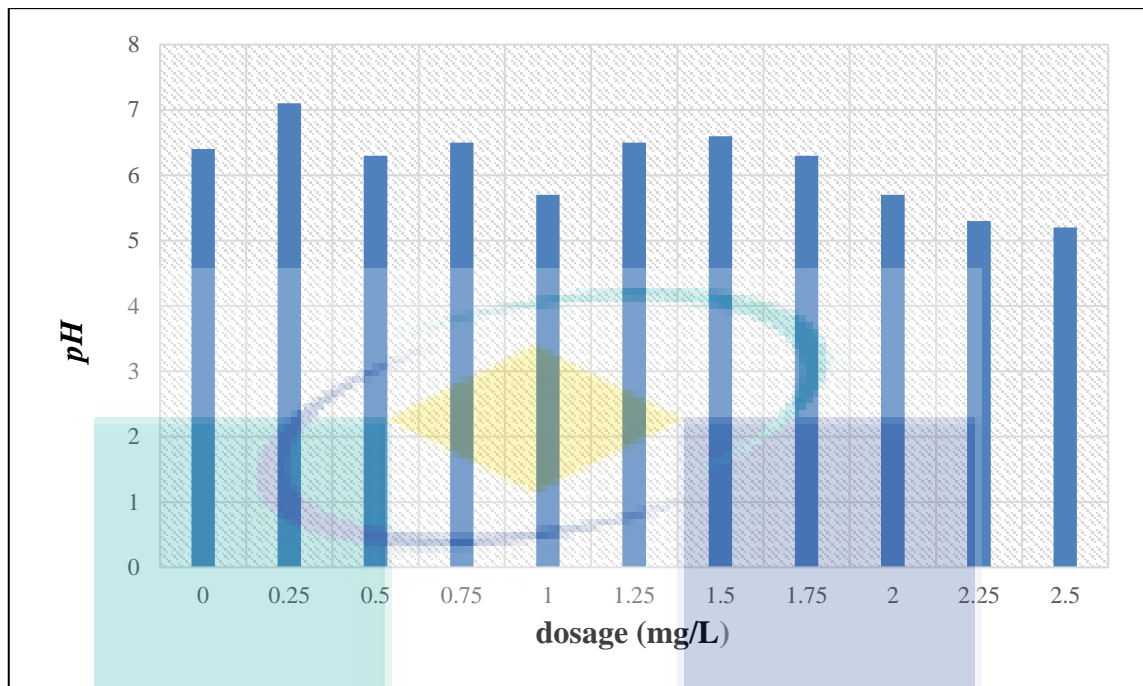


Figure 4-8 pH Values after Treatment

4.7.2 Total Dissolved Solid (TDS)

The total dissolved solids of the river water were recorded at 100 ppm. Figure 4.9 shows the changes in the average dissolved solids with increasing the dosage concentration. The result obtained gave the lowest value as 40 ppm at a dosage of 1.0 mg/L and the highest as 600 ppm at 2.5 mg/L dosage concentration. Based on this trend, it indicates that a reduced total dissolved solids (TDS) is achievable with a dosage concentration of 0.75 mg/L. This suggests that bio-coagulant concentration exceeded the optimum dosage from 1.5 to 2.5 mg/L which led to an increased turbidity. The sudden increase in TDS could be attributed to the precipitation of excess coagulants which had no or little effects beyond the 1.5 mg/L threshold (Adeniran et al., 2017). This is in agreement with Hoa et al., (2018) who reported a reduction in the TDS value after treatment. Nevertheless, this is in agreement with the standard water quality as the required TDS values should be less than 1000 ppm (Farah & Torell, 2018). However, this result opposed the reported reduction in TDS by Shan et al. (2017) from 307 ppm to 400 ppm after treatment with *Moringa oliefera* bio-coagulant.

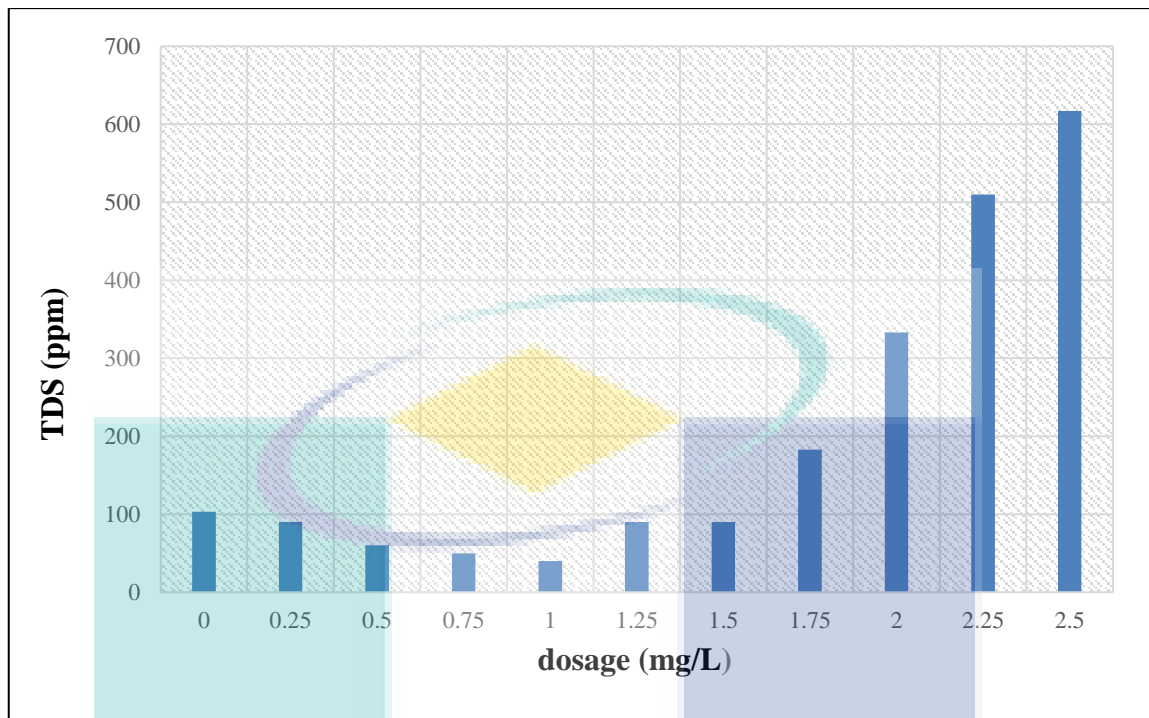


Figure 4-9 TDS Values after Treatment

4.7.3 Conductivity

In this study, the initial conductivity of the river water before treatment was recorded as 220 $\mu\text{s}/\text{cm}$. Based on the iterative investigation of trend in the conductivity of the water after treatment with the *MO* bio-coagulant, the conductivity was reduced from 220 $\mu\text{s}/\text{cm}$ to 50 $\mu\text{s}/\text{cm}$ which is still an acceptable value for drinking water, as the maximum value should be less than 1055 $\mu\text{s}/\text{cm}$ according to WHO. Hence with a corresponding dosage of 0.5 mg/L, a conductivity reduction accounting for 77.3 % reduction was estimated (Figure 4.10). The sudden rise in the conductivity beyond the 1.5 mg/L concentration is as a result of precipitation in excess coagulants which had no or little effects beyond the 1.5 mg/L threshold

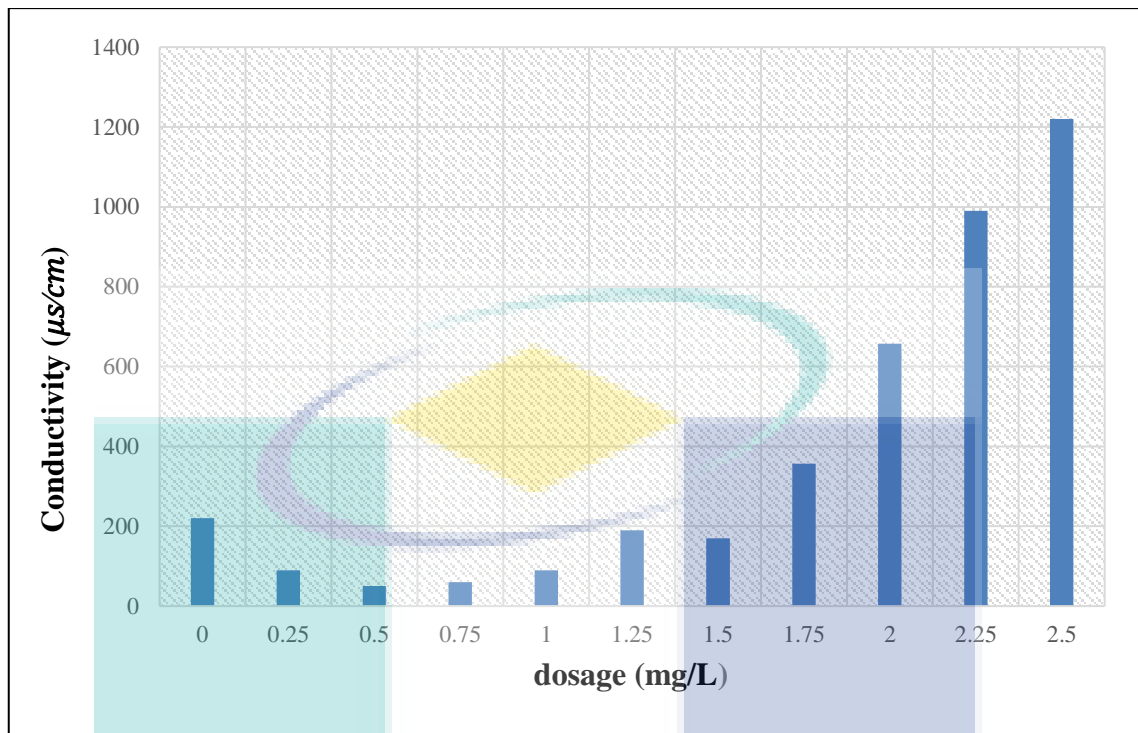


Figure 4-10 Conductivity Values after Treatment

This is consistent with Hendrawati et al., (2016) which reported a reduction in the conductivity to 227 µs/cm from the addition of a *Moringa oleifera* as bio-coagulant to ground water. Miraji, (2014) also buttressed that the increase in conductivity indicated an increase in the amount of free ions obtained after dissolution of salt, ionic proteins and ionization of water. However, the excess use of *MO* bio-coagulant beyond optimum dosage made conductivity value rise again due to the presence of unbound ions (Hendrawati et al., 2016). The addition of bio-coagulant may result in the dispersion of some mineral ions and inorganic compounds into a floc which will then be precipitated and separated from the solution. This caused the reduction of electrical conductivity.

4.7.4 COD Measurement

In this analysis, the initial COD value before treatment of the river water was recorded as 158 mg/L. However, there was an improvement in the COD value after treatment especially with the use of *MO* bio-coagulant dosage, with the value increasing progressively with an increase in bio-coagulant concentration as shown in Figure 4.11. The rapid increase in the COD from 1000 mg/L to 2000 mg/L could attributed to the accumulation of organic matter due to the concentration increase from 2 to 2.5 mg/L.

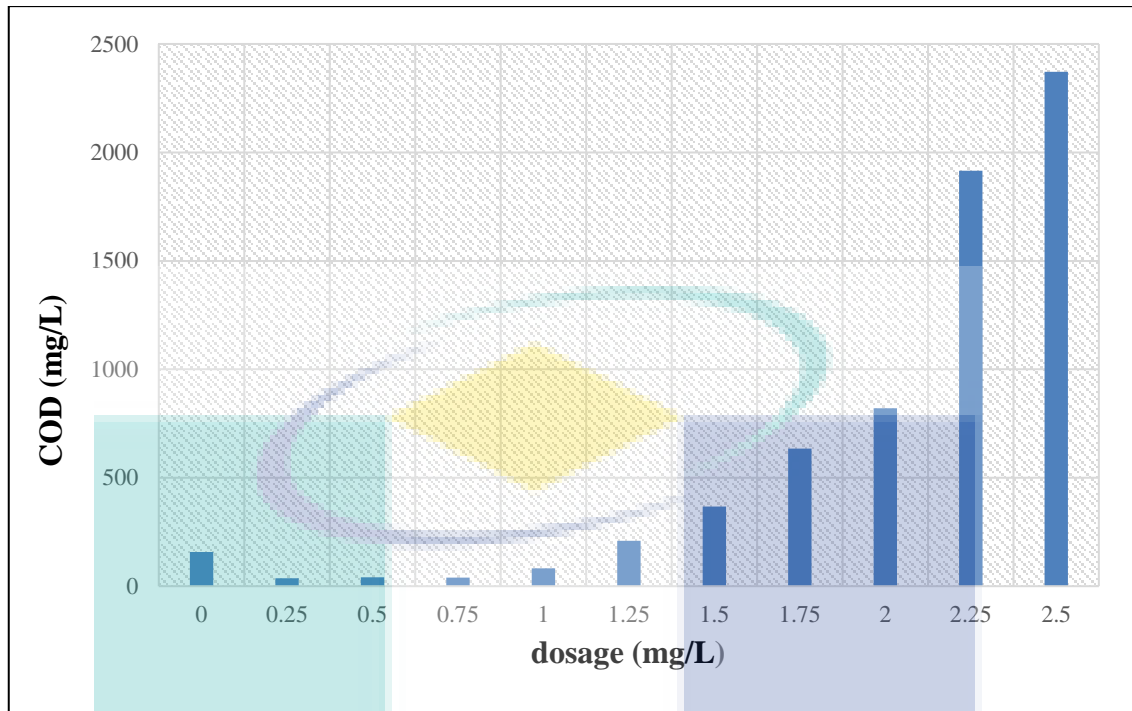


Figure 4-11 COD Values after Treatment

Hence at a dosage value of 0.75 mg/L, the corresponding COD value was recorded as 40 mg/L. This result is not in conformity with the standard COD for water quality standard (WHO) This result is related to Shan et al., (2016) with COD before and after treatment recorded 132 ± 2.84 mg/L and 164 ± 2.83 mg/L, respectively. The higher COD value is regarded as a threat to human health as reported by Arnodsson et al., (2008). In organic matter, the sugar, proteins and fats are most reduction which depends on the nature of the organic compound. Therefore, more oxygen is required for the complete oxidation of fats and fat type compounds. The *Moringa oleifera* was reported as non-toxic agent utilized for water purification (Amagloh & Benang, 2009). However, the increase in COD is partly due to the excess of bio-coagulant which lead to increase the proteins in the treated water resulted to a corresponding increase in the COD value (Karri et al., 2016).

4.7.5 BOD Measurement

In this section, the effect of *Moringa oleifera* bio-coagulant dosage on the biochemical oxygen demand was investigated and the result is shown in Figure 4.12. The initial BOD before treatment of the dirty water was recorded as 25 mg/L while the value after treatment was measured at 18 mg/L using a dosage of 0.75 mg/L. The increase in concentration from 1.25 to 2.5 mg/L resulted in the decline in BOD values until constant

trend due the presence of natural organic compound in the MO bio-coagulant as buttressed by Shan et al., (2017). However, the result obtained showed a 28 % reduction in the BOD value. There was a significant reduction in the BOD value and this is consistent, if not better than the result obtained by Shan et al. (2017) which recorded a 20 % reduction in BOD value from 100 mg/L to 80 mg/L. This indicated that the *Moringa oleifera* bio-coagulant dosage greatly influence the BOD value of the river water. This is a great improvement over results from previous studies which reported a lower reduction or no BOD reduction at all (Karri et al., 2016), (Prasad, 2016).

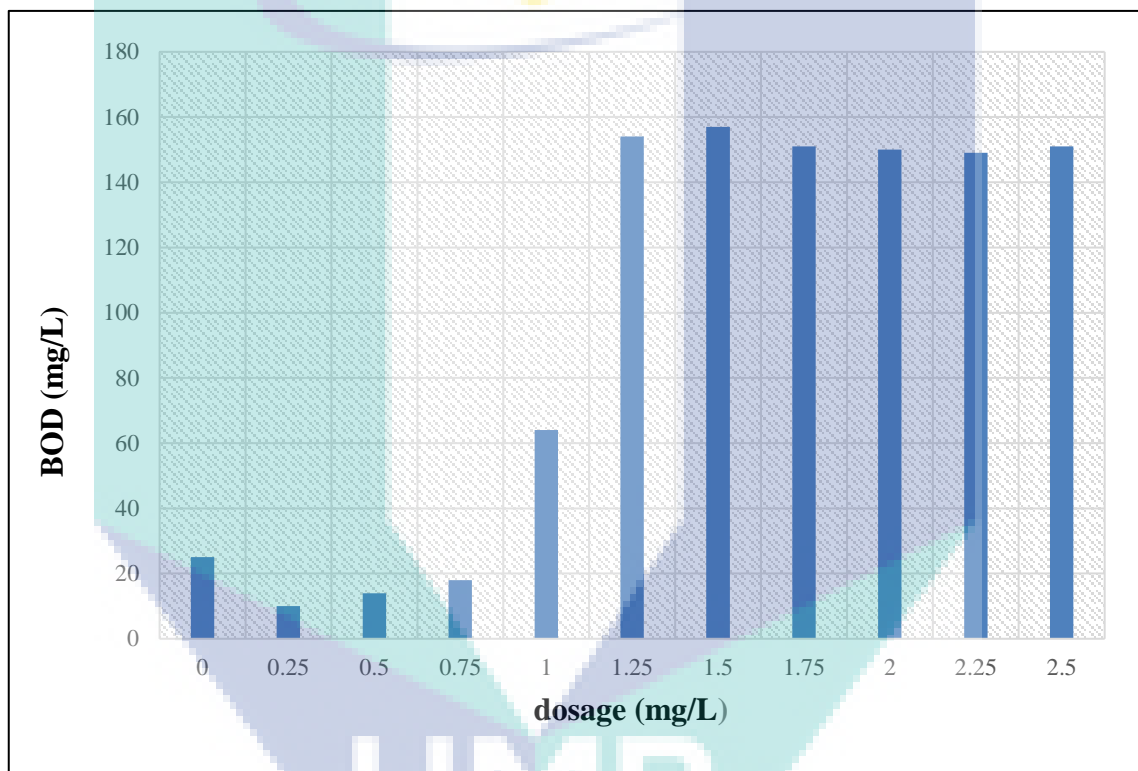


Figure 4-12 BOD Values after Treatment

This is because the demand for oxygen by the bacteria is high and they are taking that oxygen from the oxygen dissolved in the water. If there is no organic waste present in the water, there will not be as many bacteria present to decompose it and thus the BOD will tend to be lower and the DO level will tend to be higher. It should therefore be noted that an increase in the BOD value implies a reduction in the oxygen level and this could be detrimental to the aquatic life and human health as reported by Irenosen et al (2012). According to Malaysian Standard of Water Quality, the BOD value obtained must be less than 100 mg/L in order to categorise the water as clean water (Nasirian, 2007). At BOD

levels of above 100 mg/L, the water supply is considered much polluted with organic waste.

4.7.6 Summary of Water Quality Analysis

Table 4.6 shows the overall result from the analysis of the river water before and after the application of *Moringa Oleifera* as bio-coagulant. In this study, six (6) quality characteristics were considered and this includes the turbidity, TDS, pH conductivity, COD and BOD. The result of the water quality determination revealed that turbidity, TDS, conductivity, COD and BOD with corresponding percentage changes of 99 %, 50 %, 73 %, 75 % and 28 %, respectively. This indicated that only the COD and pH value did not improve to a larger extent the quality of the treated water compared to other analysis conducted. This could be attributed to the presence of extracting solvents in altering the pH and COD value when the bio-coagulants was added (Shan et al., 2017). Based on the result obtained the optimum concentration of the *Moringa oleifera* bio-coagulant is 0.75 mg/L.

Table 4.6 Summary of result for water quality characteristics Using *Moringa oleifera* bio-coagulant

Parameters	Before Treatment	After Treatment	% Change
Turbidity (NTU)	415	4.7	Reduction by 99%
pH	6.4	6.5	-
TDS (ppm)	100	50	Reduction by 50%
Conductivity ($\mu\text{s}/\text{cm}$)	220	60	Reduction by 73%
COD (mg/L)	158	40	Reduction by 75%
BOD (mg/L)	25	18	Reduction by 28%

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Overall Conclusions

In this research, an efficient and cost effective method was developed from *Moringa oleifera* seed for the production of bio-coagulant in the treatment of drinking water. The processed *Moringa oleifera* seed showed a good coagulation activity with different methods towards the turbidity in accordance with the research objectives. The initial quality analysis of the raw water was conducted to determine the turbidity, pH, COD, BOD, TDS and conductivity properties. The result of the *Moringa oleifera* seed Powder (MOSP), investigation showed that 2 mm at dosage of 0.1g is the best the particle size for *Moringa oleifera* seeds powder.

Furthermore, the response surface methodology was applied to determine the parametric effects of mixing speed, time and dosage on the residual turbidity. The optimum condition gave the lowest turbidity at 4.73 NTU when the low speed, low speed time and MO bio-coagulant dosage of 40 rpm, 60 min, and 0.75 mg/L, were respectively used.

The quality characteristics of the treated water was determined at optimum condition using some qualitative tests such as pH, COD, BOD, TDS and conductivity properties. The result of the water quality determination revealed that turbidity, TDS, conductivity, COD and BOD with corresponding percentage changes of 99 %, 50 %, 73 %, 75 % and 28 %, respectively. This study therefore showed the potential of *Moringa oleifera* seed as natural bio-coagulants in the effective treatment of water for drinking purpose. The lower turbidity (<5 NTU) achieved from this study confirmed the potential of this important eco-friendly natural product for the treatment of water.

5.2 Recommendation

Based on the results from this research, it is suggested that the following recommendations be carried out for any future work:

- i. It is recommended that the present result be tested on pilot plant which could later be a potential project for industrial scale. The most important scaling up parameters is usually measured in term of micro-filtration factors which include filter length, membrane area and the pore size.
- ii. It is recommended that the *Moringa oleifera* bio-coagulant be preserved as powder using freeze drying techniques.
- iii. It is strongly recommended to conduct a feasibility study for a proper cost comparison between the existing treatment method price and the use of processed MO bio-coagulants.
- iv. The residual solids can be used for other purpose such as in the production of an environmentally friendly bio-fertilizer.



UMP

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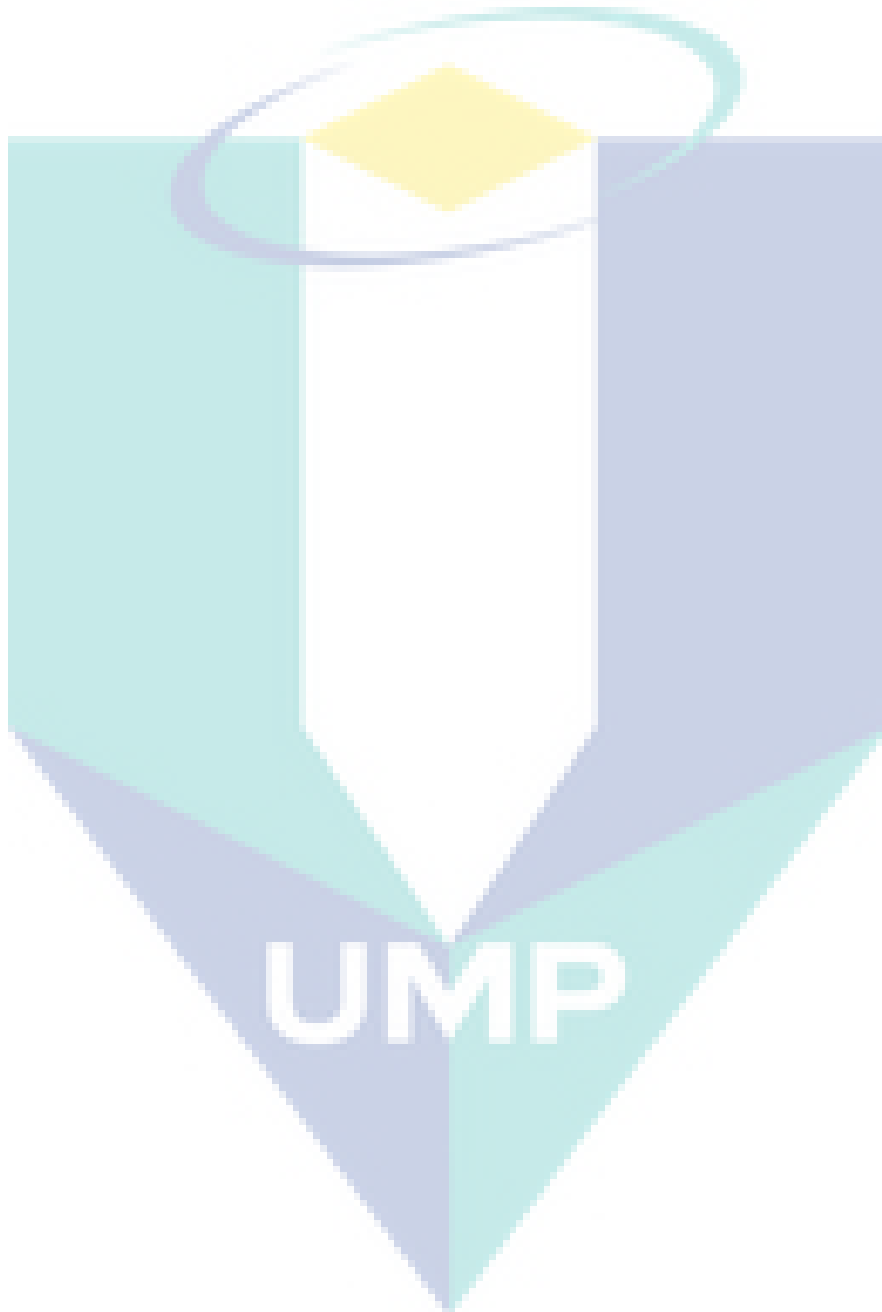
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APPENDICES

Appendix A: RESULTS OF QUALITY CHARACTERISTICS MEASUREMENT (MOSP)

Table A 1 Water turbidity for treated water with different dose of MOSP

Amount of MOSP (mg x 10 ³)	Turbidity(NTU)			
	Sample (2mm MOSP)	Sample (1mm MOSP)	Sample (500μm MOSP)	Sample (250μm MOSP)
0.05	7.43	4.98	14.89	14.65
0.10	4.98	7.42	23.53	13.65
0.25	11.58	7.13	73.74	32.49
0.50	13.50	7.26	101.0	61.34
0.75	12.74	7.26	120.3	89.85
1.00	29.25	17.47	145.6	93.28
2.50	29.3	50.67	130.5	115.7
5.00	45.65	73.33	150.0	137.2
7.50	27.00	83.95	222.1	134.9
10.00	53.99	109.3	157.3	213.3

Table A 2 Conductivity values for treated water with different dose of MOSP

Amount of MOSP (mg x 10 ³)	Conductivity (μs/cm)			
	Sample (2mm MOSP)	Sample (1mm MOSP)	Sample (500μm MOSP)	Sample (250μm MOSP)
0.05	70	130	130	30
0.10	70	160	180	40
0.25	100	190	180	50
0.50	130	210	200	70
0.75	120	230	220	90
1.00	260	260	330	190
2.50	290	300	400	220
5.00	380	390	590	390
7.50	540	580	780	590
10.00	760	720	790	730

Table A 3 COD values for treated water with different dose of MOSP

Amount of MOSP (mg x 10 ³)	COD (mg/L)			
	Sample (2mm MOSP)	Sample (1mm MOSP)	Sample (500µm MOSP)	Sample (250µm MOSP)
0.05	131.33	97.00	89.00	93.00
0.10	100.33	107.33	111.00	92.00
0.25	108.33	110.00	93.67	135.33
0.50	272.33	203.33	116.33	321.33
0.75	277.67	476.67	338.67	368.00
1.00	466.33	346.00	644.67	436.67
2.50	852.33	1140.33	1339.67	1147.00
5.00	1697.33	1635.00	1643.00	1677.67
7.50	2151.00	2182.00	2144.00	2109.67
10.00	2394.00	2240.67	2506.67	2394.67

Table A 4 BOD values for treated water with different dose of MOSP

Amount of MOSP (mg x 10 ³)	BOD (mg/L)			
	Sample (2mm MOSP)	Sample (1mm MOSP)	Sample (500µm MOSP)	Sample (250µm MOSP)
0.05	15.0	11.0	25.0	28.0
0.10	19.0	17.0	40.0	46.0
0.25	18.0	12.0	83.0	75.0
0.50	31.0	72.0	148.0	106.0
0.75	28.0	115.0	158.0	155.0
1.00	51.0	126.0	156.0	160.0
2.50	135.0	133.0	157.0	156.0
5.00	136.0	134.0	157.0	154.0
7.50	135.0	136.0	157.0	152.0
10.00	136.0	142.0	157.0	153.0

Table A 5 TDS values for treated water with different dose of MOSP

Amount of MOSP (mg x 10 ³)	TDS (ppm)			
	Sample (2mm MOSP)	Sample (1mm MOSP)	Sample (500µm MOSP)	Sample (250µm MOSP)
0.05	40	120	70	10
0.10	80	130	130	20
0.25	50	110	160	20
0.50	70	170	150	30
0.75	80	140	120	40
1.00	130	50	240	50
2.50	100	110	260	110
5.00	190	170	290	290
7.50	260	290	420	300
10.00	380	360	450	370

Table A 6 pH values for treated water with different dose of MOSP

Amount of MOSP (mg x 10 ³)	pH			
	Sample (2mm MOSP)	Sample (1mm MOSP)	Sample (500µm MOSP)	Sample (250µm MOSP)
0.05	7.03	7.03	7.13	7.47
0.10	7.03	7.00	7.07	7.40
0.25	6.90	6.97	7.03	7.50
0.50	7.03	6.97	7.00	7.40
0.75	6.93	7.00	6.93	7.30
1.00	6.93	7.10	7.00	7.47
2.50	5.77	5.83	7.03	7.50
5.00	5.23	5.27	6.97	7.33
7.50	5.27	5.10	7.00	7.50
10.00	5.03	5.13	6.90	7.43

Appendix B: RESULTS OF QUALITY CHARACTERISTICS MEASUREMENT (MO BIO-COAGULANT)

Table B 1 Water turbidity for treated water with different dose of MO bio-coagulant

Amount of MO bio-coagulant (mg/L)	Turbidity(NTU)			Average (NTU)
	Reading 1 (NTU)	Reading 2 (NTU)	Reading 3 (NTU)	
0.25	4.8	9.8	9.7	9.9
0.50	4.9	12.2	12.6	12.5
0.75	4.7	4.8	4.6	4.7
1.00	5.0	5.2	5.6	5.3
1.25	4.9	7.2	6.4	6.8
1.50	16.1	15.0	18.8	16.6
1.75	14.9	16.1	14.7	15.2
2.00	17.1	19.1	18.2	18.1
2.25	20.0	20.0	20.0	20.0
2.50	100.0	95.0	103.0	99.3

Table B 2 Conductivity values for treated water with different dose of MO bio-coagulant

Amount of MO bio-coagulant (mg/L)	Conductivity ($\mu\text{s}/\text{cm}$)			Average (NTU)
	Reading 1 (NTU)	Reading 2 (NTU)	Reading 3 (NTU)	
0.25	90	90	90	90
0.50	50	50	50	50
0.75	60	60	60	60
1.00	90	90	90	90
1.25	190	190	190	190
1.50	170	170	170	170
1.75	350	360	360	357
2.00	660	660	650	657
2.25	990	990	990	990
2.50	1220	1220	1220	1220

Table B 3 COD values for treated water with different dose of MO bio-coagulant

Amount of MO bio-coagulant (mg/L)	COD (mg/L)			Average (NTU)
	Reading 1 (NTU)	Reading 2 (NTU)	Reading 3 (NTU)	
0.25	37	36	39	37
0.50	39	41	43	41
0.75	41	40	39	40
1.00	81	85	83	83
1.25	210	210	210	210
1.50	367	367	367	367
1.75	634	634	634	634
2.00	821	821	821	821
2.25	1915	1915	1915	1915
2.50	2371	2371	2371	2371

Table B 4 BOD values for treated water with different dose of MO bio-coagulant

Amount of MO bio-coagulant (mg/L)	BOD (mg/L)			Average (NTU)
	Reading 1 (NTU)	Reading 2 (NTU)	Reading 3 (NTU)	
0.25	10	11	9	10
0.50	14	13	15	14
0.75	19	17	18	18
1.00	64	64	64	64
1.25	154	154	154	154
1.50	157	157	157	157
1.75	151	151	151	151
2.00	150	150	150	150
2.25	149	149	149	149
2.50	151	151	151	151

Table B 5 TDS values for treated water with different dose of MO bio-coagulant

Amount of MO bio-coagulant (mg/L)	TDS (ppm)			Average (NTU)
	Reading 1 (NTU)	Reading 2 (NTU)	Reading 3 (NTU)	
0.25	90	90	90	90
0.50	60	60	60	60
0.75	50	50	50	50
1.00	40	40	40	40
1.25	90	90	90	90
1.50	90	90	90	90
1.75	190	180	180	183
2.00	340	330	330	333
2.25	510	510	510	510
2.50	620	610	620	617

Table B 6 pH values for treated water with different dose of MO bio-coagulant

Amount of MO bio-coagulant (mg/L)	pH			Average (NTU)
	Reading 1 (NTU)	Reading 2 (NTU)	Reading 3 (NTU)	
0.25	7.1	7.0	7.1	7.1
0.50	6.3	6.3	6.3	6.3
0.75	6.5	6.5	6.5	6.5
1.00	5.7	5.8	5.7	5.7
1.25	6.5	6.5	6.5	6.5
1.50	6.6	6.6	6.6	6.6
1.75	6.3	6.3	6.3	6.3
2.00	5.7	5.7	5.7	5.7
2.25	5.3	5.3	5.3	5.3
2.50	5.2	5.2	5.2	5.2

